IGC Symposium 2018

Microbial Eco-Evolutionary Dynamics

22-24th October
Instituto Gulbenkian de Ciência
Oeiras, Portugal
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Welcome to The IGC Symposium 2018: Microbial Eco-Evolutionary Dynamics!

The main goal of this symposium is to gather people, from different disciplines and places, that want to understand how the interplay of ecology, evolution, and environmental variation shapes microbial diversity. We believe that this is key for moving forward and expanding the existing knowledge in the field.

While we were preparing this symposium for you, we strove to bring together three themes:
1) What are the impacts of eco-evolutionary dynamics on the adaptive process and diversity of microbial populations?
2) How can adaptation and diversification feed back onto community composition and ecological interactions?
3) How is this eco-evolutionary feedback loop affected by environmental variation?

In order to answer these questions, we welcome every one of you to participate -- not only as the presenter of a poster or talk -- but also to engage in the different activities we will have during the symposium. These activities were put together to foster communication, discussion, and brainstorming during the conference and will be as diverse as the presence of a Wall of Questions, a scavenger hunt, a brainstorming session, and the possibility to use an online platform to discuss the different presentations.

Ultimately, we hope that by joining us in these activities each one of us will be more integrated within the community and that we all together can contribute to a better understanding of the processes that shape microbial eco-evolutionary dynamics.

We wish you a pleasant conference!

The Organizing Committee
Ana-Hermina Ghenu
Hugo Barreto
Inês Fragata
Ricardo Ramiro
Tanja Dapa
All contributed presentations are eligible for the PeerJ Best Talk award. * indicates presentations by post-docs and PhD students. Only these are eligible for the Oeiras Best Talk award.
Evolution in the gut microbiota

Isabel Gordo

Instituto Gulbenkian de Ciência, Oeiras, Portugal.

Hundreds of different bacterial species inhabit our intestines and contribute to our health status. A great deal of variation is however hidden within each microbial species and such intra-specific variation is also key to the proper homeostasis between the host and its microbial inhabitants. Yet our knowledge on this hidden diversity is still limited and an understanding of the evolutionary mechanisms acting on it is extremely reduced. We have been using experimental evolution and NGS to follow the emergence of intra-species diversity in *Escherichia coli*, a common commensal, when it colonizes the mouse gut. The data gathered so far indicates that a strong-mutation-strong selection regime drives the patterns of evolutionary change of this comensal species in this ecosystem. It further shows that *E. coli* evolution is characterized by the spread of similar adaptive mutations across different hosts, under the same dietary regime. Overall these findings strongly suggest that levels of intra-species diversity may be large and highly dynamic, perhaps suggesting that rapid evolutionary change may be important to understand the high diversity levels observed in the mammalian microbiota.
Within a host, malaria parasites face a tradeoff between proliferation and the production of specialized stages required for onward transmission. All else being equal, parasites that invest more into proliferation will exploit resources faster, imposing greater virulence on the host. A multitude of ecological factors within the host lead to selection for proliferation at the expense of transmission investment, including host immunity and within-host competition. Ecology outside of the host, like the abundance of mosquito vectors or susceptible hosts, can change dramatically over the lifespan of a malaria infection, dynamics that could either intensify or oppose within-host selection for proliferation. To understand the relative role of ecology at different scales (and its integrated impact) on transmission investment in malaria parasites, my collaborators and I have developed a mathematical framework that nests within-host infection processes into a model of between-host transmission, with input from a temperature-drive model of mosquito population dynamics. Our results show that ecology outside the host can have an overwhelming influence of the evolution of parasite trait expressed within a host, affecting both disease severity and spread.
Host immunity is a strong selective force on pathogen populations, and systematic differences in their interactions with the immune system partly explain pathogens' divergent epidemiological and evolutionary patterns. In this talk, I contrast these interactions in several common pathogens, dwelling in particular on the complexity of the adaptive immune response to influenza. Influenza appears to experience competing selective pressures in different host subpopulations that are partly defined by their exposure histories. Vaccination can change not only the strength but also the type of immune-mediated selection, as observed in pneumococcus, human papillomavirus, and also influenza. With influenza, the evolutionary dynamics of the adaptive immune response may explain the low effectiveness of the seasonal flu vaccine; nonetheless, the seasonal vaccine might be affecting influenza's evolution. "Rationally managing" adaptive immune responses may be important to manage the evolution of influenza and other pathogens in the long term.
Ecological scaffolding and the evolution of multicellular life

Paul B. Rainey

Max Planck Institute for Evolutionary Biology, Plön, Germany
Ecole Supérieure de Physique et de Chimie Industrielles de la Ville de Paris, Paris, France.

Life is hierarchically structured, with replicating entities nested within higher order self-replicating structures. Take, for example, multicellular life: the multicellular entity replicates, as do the cells that comprise the organism. Inside cells are mitochondria that also have capacity for autonomous replication; the same is true of chromosomes within the nucleus, and of genes that comprise chromosomes. Such hierarchical structure reflects a series of major evolutionary transitions in which lower order self-replicating entities have been subsumed within higher order structures. Typically this involves the lower level entity “giving up its right to autonomous replication” and with this “sacrifice” comes enslavement to the “needs” of the higher order “corporate body”. Posed in these terms it is difficult to see how evolutionary transitions unfold; how selection might shift levels and why life is hierarchically structured. Necessary for progress is clarity concerning what needs to be explained. I will argue that this is the evolution of Darwinian Individuality — the evolution of properties of entities (variation, reproduction and heredity) that ensure participation in the process of evolution by natural selection. There has been a tendency to assume these properties as pre-existing, but they are not: they are derived and require evolutionary explanation. Pressing to the heart of the problem, the challenge is to explain how Darwinian properties emerge from non-Darwinian entities by non-Darwinian means. This challenge permeates each evolutionary transition including the emergence of life from non-life. I will argue that solutions to this seemingly unsolvable problem arise once we consider ecology. Following theoretical presentation of core ideas I will describe experiments that show how scaffolding can be applied. I will pay particular attention to the evolution of reproduction during fraternal transitions and discuss the how millifluidic technologies can be used to study the evolution of heredity during egalitarian transitions.
Most natural environments vary randomly, beyond any trend such as global warming. These stochastic environmental fluctuations often are faster, and of larger magnitude, than environmental trends, making them one the biggest challenges that living organisms have to face in the wild. Furthermore the patterns of these random fluctuations are themselves altered by global change. I will present results from our recent and ongoing research on the impacts of stochastic environmental fluctuations on the eco-evolutionary dynamics of populations. I will start with an overview of results from theoretical investigations of the interplay between phenotypic plasticity, adaptive evolution, and population growth/extinction risk in randomly changing environment. This work highlights the prominent role of temporal autocorrelation in the environment, which determines the time scale of environmental predictability. I will then show how we can try and address similar questions through experimental evolution with the microalgae *Dunaliella salina*. This species is able to tolerate extremely high salinity (up to NaCl saturation) through plastic physiological mechanisms, including glycerol production. Its natural environment includes shallow ponds, where salinity varies through time in connection to climatic conditions (precipitation, evaporation). We experimentally expose a large number of *Dunaliella* populations to random fluctuations in salinity with different levels of autocorrelation, and track their population sizes, tolerance curves, phenotypic plasticity, and (epi)genetic variation. I will use our preliminary results on this system to discuss the challenges and benefits of contrasting broad theoretical predictions with the more specific outcomes of experimental evolution with a given model species.
Predicting Evolution in Life-Giving Slime

Sinnead Collins

University of Edinburgh, Edinburgh, UK

I am interested in predicting changes to biogeochemically important traits in marine microbial primary producers. I will discuss how I've used experimental evolution to answer aspects of this, such as understanding how responses to multiple environmental drivers are determined and understanding limits to cell division rates in improved environments. I will also discuss some of the adventures and misadventures of helping the field of "marine microbial experimental evolution" grow over the past decade.
Eco-evolutionary dynamics and functioning in whole communities

Timothy Barraclough
Imperial College London, London, UK

Many biological processes that humans depend upon – such as global nutrient cycling, plant growth and digestive health – rely on the action of microbial communities with hundreds of species. When the environment changes, ecosystem functioning can change because of shifts in the relative abundances of species or because of evolutionary responses in the traits underlying functioning. I outline theory for predicting how species in a microbial community should evolve when the environment changes and how that response affects metabolic functioning of the whole community. I then present initial evidence tracking evolution and functioning in manipulative experiments of whole gut microbiome communities in vitro.
As they evolve, microbes not only adapt to their abiotic environment but also to other microbes with whom they share their surroundings. In interpreting microbial behavior, then, it is crucial that we move from studying single-species cultures towards approaches that take these interactions and the associated co-evolutionary processes into account. In my lab, we have recently developed a model system consisting of four bacterial species that are capable of degrading pollutants in industrial waste water and can co-exist as a stable community. Our first analysis of the interactions between these species has revealed that all four species grow better in the presence of at least one other member of the community compared to alone. Using a mathematical model and further experiments, we show that these positive interactions are environment-dependent. By changing environmental conditions, we can therefore change the sign and strength of interactions in our synthetic community. Finally, I will also present preliminary data from a co-evolutionary experiment, where we are exploring how these interactions change as the species adapt to each other and to their environment.
It is now widely recognized that substantial evolutionary change can occur on contemporary (or “ecological”) time scales. This is the phenomenon of contemporary (or “rapid”) evolution. What we now need to know is the extent to which contemporary evolution shapes ecological dynamics at the population, community, and ecosystem levels. Corresponding to my recent book, I will outline a conceptual framework for these eco-evolutionary dynamics. I will then illustrate elements of this framework through a series of empirical examples from natural populations. These examples will be used to address several key questions in this emerging synthetic research field. I will also present exciting new experiments in which we are trying to reveal the strength and importance of eco-evolutionary dynamics IN THE REAL WORLD.
Gut microbiota are shaped by ecological and evolutionary forces, yet little is known about how these microbes evolve over time. Here we introduce a model-based framework for quantifying evolutionary dynamics within and across hosts using a panel of metagenomic samples. Within hosts, we find that short-term differences rarely arise from the invasion of distantly related strains. Instead, we more commonly observe evolutionary changes in resident strains, in which nucleotide variants or gene gains or losses rapidly sweep to high frequency. By comparing these mutations with the typical between-host differences, we find evidence that sweeps are driven by introgression from other strains rather than new mutations. Our results suggest that gut bacteria can evolve on human-relevant timescales, and highlight the feedback between short- and long-term evolution across hosts.
Canada Crohn’s disease (CD) has reached alarming levels worldwide. Members of the adherent-invasive *E. coli* (AIEC) pathovar have been recognized as potential risk factors in CD development. Moreover, several AIEC strains have been found to invade the intestinal epithelial cells and induce a pro-inflammatory response, thus exacerbating CD symptoms. However, the mechanisms employed by AIEC to adapt to the gut environment and cause disease are unknown. Within-host evolution is an important driver of bacterial pathogenesis. Selective pressure from the host immune system reshapes bacterial communities via the selection of bacterial lineages with competitive advantage. Our research seeks to understand the dynamics of within-host evolution of AIEC. We developed an AIEC infection-transmission mouse model to select for bacterial lineages with improved fitness in vivo. Whole-genome sequencing was used to capture the population diversity of AIEC within the host, and compare it to the founder population to define the adaptive mutations acquired by AIEC within the host. We detected multiple co-evolving lineages that outcompeted the ancestral strain in the host environment through independent mechanisms. Unlike commensal *E. coli* strains, the in vivo AIEC population exhibited a remarkable variation in motility. The hypermotile isolates displayed higher invasiveness, and an enhanced ability to colonize the host. Inversely, some lineages were less motile than the parent wild type strain; however, they have rewired their metabolic network, including the ability to grow better on short chain fatty acids. Remarkably, we identified recurrent patterns of evolution by screening a library of *E. coli* isolates obtained from CD patients for motility and SCFA metabolism. Our assays show a clear distinction between CD-associated *E. coli* and commensals in motility and metabolic behavior, thus providing new insights into the evolutionary trajectories of the two groups.
O3. Host genotype and gut microbiome interactions drive zooplankton tolerance to toxic cyanobacteria and potentially structure microbial eco-evo dynamics

Shira Houwenhuyse¹, Emilie Macke¹, Martijn Callen² and Ellen Decaestecker¹

¹Kulak, KULeuven, campus Kortrijk, Belgium
²Centre d'Ecologie Fonctionnelle et Evolutive, Montpellier, France

The gut microbiome is not just a random set of microorganisms, but rather a complex community that plays a critical role in host physiology, acclimation and adaptation to continuously changing environments. Here we propose the *Daphnia magna* system to investigate the role of the gut microbiome in the host’s response to the environmental stressor, toxic cyanobacteria. A combination of a metabarcoding with a gut microbiome transplant approach showed that *Daphnia* genotypes interact with the microbiome to increase *Daphnia* tolerance towards toxic cyanobacteria (Macke et al. 2017, Nature Communications). Next, we showed that *Daphnia* genotype, microbiome and diet interact to shape the structure of the surrounding bacterioplankton. These results suggest a selective genotype dependent recruitment of the *Daphnia* gut microbiome which is diet dependent and structures the wider bacterial community. This structuring can occur via a direct effect of strain leakage from the gut microbiome to the bacterioplankton. Other microbiome mediated indirect effects (e.g. metabolite expression of particular microbiome strains, *Daphnia* genotype dependent effects on the algal diet) can further structure the bacterioplankton. Overall, these results indicate strong reciprocal interactions between *Daphnia* genotypes, their gut microbiome and the bacterioplankton and illustrate the potential impact of the host microbiome in eco-evolutionary dynamics and as ecosystem engineer. A follow up on this study will be performed in which different *Daphnia* genotypes will be investigated via a gut transplant experiment and crucial functions will be unraveled via metagenomics. In addition the assembly of the gut microbiome throughout development and which crucial phases lead to increased cyanobacterial tolerance will be investigated more in detail.
Organisms are often host to diverse microbial communities, yet the eco-evolutionary implications for these communities and for the host are largely unknown. In particular, host-microbe interactions can shift along the antagonism-mutualism continuum, with some microbes evolving to confer protection to their hosts from more virulent microbes and hosts reciprocating by favouring more protective microbes. I will discuss recent theoretical and experimental work that provides new insights into the eco-evolutionary dynamics of hosts and microbes along the antagonism-mutualism continuum. Theoretical models reveal how and when microbes are likely to protect their hosts, and in turn, when hosts are most likely to favour protective microbes due to eco-evolutionary feedbacks. Experimental coevolution between nematode hosts (Caenorhabditis elegans) and a mildly parasitic bacterium (Enterococcus faecalis) with host-protective properties against a virulent bacterium (Staphylococcus aureus) show that coinfections with both bacteria drive the evolutionary transition of the C. elegans–E. faecalis relationship toward a reciprocally beneficial interaction. Together, these results highlight the context dependence of host-microbe relationships and the impact of complex communities on eco-evolutionary dynamics.
O5. Parallel evolution of influenza across multiple spatiotemporal scales

Katherine S. Xue¹,², Terry Stevens-Ayers³,⁴,⁵, Angela P. Campbell³,*, Janet Englund⁶, Steven A. Pergam³,⁴,⁵, Michael Boeckh³,⁴,⁵ and Jesse D. Bloom¹,²

¹Department of Genome Sciences, University of Washington, Seattle, USA
²Basic Sciences Division and Computational Biology Program, Fred Hutchinson Cancer Research Center, Seattle, USA
³Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Research Center, Seattle, USA
⁴Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, USA
⁵Department of Medicine, University of Washington, Seattle, USA
⁶Department of Pediatrics, University of Washington, Seattle, WA; Seattle Children’s Research Institute, Seattle, USA

*Present affiliation: Centers for Disease Control and Prevention, Atlanta, USA

Viral variants that arise in the global influenza population begin as de novo mutations in single infected hosts, but the evolutionary dynamics that transform within-host variation to global genetic diversity are poorly understood. Here, we demonstrate that influenza evolution within infected humans recapitulates many evolutionary dynamics observed at the global scale. We deep-sequence longitudinal samples from four immunocompromised patients with long-term H3N2 influenza infections. We find parallel evolution across three scales: within individual patients, in different patients in our study, and in the global influenza population. In hemagglutinin, a small set of mutations arises independently in multiple patients. These same mutations emerge repeatedly within single patients and compete with one another, providing a vivid clinical example of clonal interference. Many of these recurrent within-host mutations also reach a high global frequency in the decade following the patient infections. Our results demonstrate surprising concordance in evolutionary dynamics across multiple spatiotemporal scales.
Multicellularity provides multiple benefits. Nonetheless, unicellular life forms are ubiquitous and there have been multiple cases of evolutionary reversal to a unicellular organization. In this study we evaluated the costs of multicellularity in recently evolved multicellular phenotypes that evolved in the laboratory as a result of selection for increased size. Combining growth assays, competition experiments, computer simulations, and experimental evolution, we showed that costs of resource acquisition and local competition can readily lead to the evolution of reversals to single cells. These costs depend on the size of the multicellular organisms, their ability to disperse and the distribution of resources. In liquid media—where resources are evenly distributed—multicellularity imposes spatial structure and internal cells have less space and/or resources for growth. In contrast, on plates, despite similar growth between multicellular and unicellular isolates, multicellular isolates face a high cost of increased local competition and are rapidly outcompeted by their unicellular ancestor. To evaluate the evolution of reversals to single-cell forms, we propagated recently evolved multicellular isolates of *Saccharomyces cerevisiae* in a spatially structured environment. In this environment all isolates evolved a predominantly unicellular life cycle. However, and despite strong selection and high convergence across lines, the tempo and mode of these reversals was highly contingent on history and chance suggesting epistatic interactions and a rapid reduction of the strength of selection as the frequency of unicellular individuals increases. The evolution of multicellular organization (including biofilms) involves dramatic changes in the ways cells interact in space. In this study we show that this rearrangement can have important consequences for subsequent evolutionary changes.
O7. When increasing population density can promote the evolution of metabolic cooperation

Richard J. Lindsay¹, Bogna J. Pawlowska¹ and Ivana Gudelj¹
¹University of Exeter, UK

Microbial cooperation drives ecological and disease processes including virulence and nutrient cycling. Selective pressure on the success of cooperation can be imposed by social cheats that avoid the cost of cooperation but can benefit from cooperation of others. The success of cooperation can be affected by the ecology and demographic of populations. Population density influences the selection for cooperation, with spatial structure and the type of social dilemma, namely public-goods production or self-restraint, shaping the outcome. While existing theories predict that in spatially structured environments increasing population density can select either for or against cooperation, experimental studies with both public-goods production and self restraint systems have only ever shown that increasing population density favours cheats. We suggest that the discrepancy between theory and empirical studies is because of a mismatch between how theory predicts that the environment will drive selection, and how experimental procedures have previously been conducted. Using model microbial systems involving sugar metabolism by Saccharomyces cerevisiae, our study resolves this issue. We provide the first experimental evidence that high population density can favour cooperation in spatially structured environments, for both self restraint and public-goods production systems. Moreover, cooperative traits often do not act alone. Instead, alteration in one trait can interact to influence the selection on another trait. We examine this using a multi-trait mathematical model supported by laboratory experiments to extend our results to systems where the self-restraint and public-goods social dilemmas interact. We thus provide a systematic understanding of how the strength of interaction between the two social dilemmas and the degree of spatial structure within an environment affect selection for cooperation. These findings help close the current gap between theory and experiments.
Bacteriophages shape microbial communities through predation but also by promoting horizontal gene transfer, including the transmission of pathogenic traits like antibiotic resistance. Here we present a novel individual-based modelling approach to study the evolutionary consequences of bacteria-phage interactions. Our model integrates several important processes involved in the ecological and evolutionary interactions between bacteria and phages, including population dynamics, multiple selective pressures, environmental structure, explicit genome evolution, and phage-mediated horizontal transfer. We simulate dynamics of experimental evolution within diverse ecological conditions, linking these two scales in microbial communities. We recapitulate experimental and theoretical observations of bacteria-phage population and evolutionary dynamics, while providing novel insights. In particular, we find that structured environments, especially if antibiotics are heterogeneously distributed, promote the acquisition of resistance to both phages and antibiotics. We are also able to quantify the relative importance of different mechanisms (e.g., lysogeny and transduction) for the phage-mediated transmission of antibiotic resistance genes. Importantly, the explicit modelling of bacterial and phage genomes allows to temporally follow the genomic composition of microbial populations. We compare these patterns with observations by comparative genomics of thousands of bacteriophage genomes and their hosts, identifying the mechanisms more likely to drive the spread of resistance genes across bacterial species. The mechanistic-based theoretical approach we propose is inspired by results from experimental approaches and comparative genomics, but it can also guide further research in these different areas. Such integrative approaches will be fundamental to disentangle the patterns of adaptation in microbial communities and their consequences for public health.
O9. Sex overrides mutation in *Escherichia coli* colonizing the gut

**Nelson Frazão**¹, Ana Margarida Sousa², Michael Lässig³ and Isabel Gordo¹

¹Evolutionary Biology Group, Instituto Gulbenkian de Ciência, Oeiras, Portugal
²iBiMED, Institute for Biomedicine, Universidade de Aveiro, Aveiro, Portugal
³Statistical Physics and Quantitative Biology, University of Cologne, Cologne, Germany

Free-living bacteria are frequently subjected to environments populated by other microbes that impose selective stress. Under experimental conditions, bacteria respond to stress by accumulating adaptive point mutations, but rates and selective pressures of real-time evolution in natural environments are largely unknown. Here, we developed an antibiotic-free gut animal model and show that the evolution of bacteria invading the mouse intestine is controlled by the microbiota ecological niche. If a resident *E. coli* lineage is present, a new colonizer *E. coli* evolves by rapid, bacteriophage-mediated sex (horizontal gene transfer, HGT), otherwise only by mutation. The invader’s first adaptive step is lysogeny, which promotes coexistence with the resident strain, after which further lysogenic events and mutation accumulation follow. Our results establish bacteriophage-mediated sex (HGT) as a mode of rapid adaptation within a diverse microbiota and show that the dynamics of ecology and evolution in realistic environments are strongly intertwined.
**O10. Plasmid and clonal interference in experimental evolution**

**Stephanie Bedhomme**\(^1\), Danilo Perez-Pantoja\(^2\) and Ignacio G Bravo\(^3\)

\(^1\)Centre d’Ecologie Fonctionnelle et Evolutive, Montpellier, France  
\(^2\)Laboratory MIVEGEC, Montpellier, France  
\(^3\)Programa Institucional de Fomento a la Investigacion, Desarrollo e Innovacion, Universidad Tecnologica Metropolitana, Ignacio Valdivieso, Santiago, Chile

Plasmids are nucleic acid molecules that can drive their own replication in a living cell. Plasmid replication and plasmid gene expression consume cellular resources and carrying plasmids usually incur fitness costs. But many plasmids carry genes that can be beneficial under certain conditions, allowing the cell to endure in the presence of antibiotics, toxins, competitors or parasites. Horizontal transfer of plasmid-encoded genes can thus instantaneously confer differential adaptation to local or transient selection conditions. This conflict between cellular fitness and plasmid spread sets the scene for multilevel selection processes. We have engineered a system to study the short-term evolutionary impact of different synonymous versions of a plasmid-encoded antibiotic resistance gene. Applying experimental evolution under different selection conditions and deep sequencing allowed us to show rapid local adaptation to the presence of antibiotic and to the specific version of the resistance gene transferred. We describe the presence of clonal interference at two different levels: at the within-cell level, because a single cell can carry several plasmids, and at the between-cell level, because a bacterial population may contain several clones carrying different plasmids and displaying different fitness in the presence/absence of antibiotic. Our data suggest that, for a given gene and selection pressure, the localization on a plasmid strongly affects the evolutionary dynamics.
O11. Phenotypic and genome evolution of bacteria

Dennis Vitkup¹
¹Columbia University, New York, USA

For many decades comparative analyses of protein sequences and structures have been used to investigate fundamental principles of molecular evolution. In contrast, relatively little is known about the long-term evolution of species' phenotypic and genetic properties. This represents an important gap in our understanding of evolution, as exactly these properties play key roles in natural selection and adaptation to diverse environments. We performed a comparative analysis of bacterial growth and gene deletion phenotypes using hundreds of genome-scale metabolic models. Overall, bacterial phenotypic evolution can be described by a two-stage process with a rapid initial phenotypic diversification followed by a slow long-term exponential divergence. The observed average divergence trend, with approximately similar fractions of phenotypic properties changing per unit time, continues for billions of years. We experimentally confirm the predicted divergence trend using the phenotypic profiles of 40 diverse bacterial species across more than 60 growth conditions. Our analysis suggests that, at long evolutionary distances, gene essentiality is significantly more conserved than the ability to utilize different nutrients, while synthetic lethality is significantly less conserved. We also find that although a rapid phenotypic evolution is sometimes observed within the same species, a transition from high to low phenotypic similarity occurs primarily at the genus level.
Twelve replicate populations of *Escherichia coli* have been evolving in Lenski’s Long Term Evolution Experiment (LTEE) in flasks for over 67,000 generations in a shared glucose limited environment. The evolved bacteria grow 70% faster than their ancestor, but experience a decrease in numerical yield. These evolved “rapacious” bacteria are more competitive than their ancestor, but less productive, consistent with a tragedy of the commons. We explore if rapacious types are constrained by their previous evolution, and if they can undergo further evolution to become more “prudent” by manipulating structure of the population. If we allow rapacious bacteria to grow in a structured environment, can we select for more efficient resource use and increased numerical yield (i.e., prudence)? We investigate how evolutionary pathways to increased efficiency and yield might be constrained by time spent evolving under conditions favoring rapacity. Water-in-oil emulsions provide a structured environment where millions of nutrient filled droplets are isolated by an oil phase. We manipulate population structure by inoculating droplets with either one bacterial cell (low starting density) or more than two bacterial cells (high starting density). Using multiscale models, we can make predictions about evolutionary prospects for prudent types under different forms of population structure. We test these predictions through experimental evolution using emulsions. To investigate the role of previous evolution under conditions favoring rapacity, we initiate our experiments with isolates from different time points of the LTEE lines. To explore the role of historical contingency, we use isolates from multiple LTEE lines. Generally, in our low starting density treatments, we see evolution towards more efficient resource use and increased productivity (measured by numerical yield). In effect, we have evidence of an evolutionary reversal of the tragedy of the commons.
The evolution of antimicrobial resistance often occurs in a variable environment, as antimicrobial is added and removed from a medium. This environmental variability has a huge impact on the microbes' fitness landscape, and thus on the evolution of resistance. Indeed, mutations conferring resistance often carry a fitness cost in the absence of antimicrobial, which may be compensated by subsequent mutations. As antimicrobial is added or removed, the relevant fitness landscape thus switches from a fitness valley to an ascending landscape or vice-versa.

We have investigated the effect of these time-varying patterns of selection within a stochastic model, focusing on a homogeneous population of fixed size subjected to a periodic alternation of phases of absence and presence of antimicrobial. Combining analytical approaches and stochastic simulations, we quantified how the time necessary for fit resistant bacteria to take over the microbial population depends on the period of the alternations. Fast alternations strongly accelerate the evolution of resistance, and a plateau is reached once the period gets below a fixed threshold. Above this threshold, the time needed for resistance to evolve increases linearly with the period, until it reaches the spontaneous valley-crossing time. Besides, the acceleration of resistance evolution is stronger for larger populations. For asymmetric alternations, featuring a different duration of the phases with and without antimicrobial, a striking result is the existence of a broad minimum of the time taken by the population to fully evolve resistance. This minimum occurs when both phases are longer than fixed thresholds, while having durations of the same order. This realistic situation dramatically accelerates the evolution of resistance. Hence, such parameters should preferably be avoided in clinical situations, while they might be harnessed in evolution experiments focusing on resistance evolution.
014. The emergence of microbial community variability in similar environments

Sylvie Estrela¹, Nanxi Lu¹ and Alvaro Sanchez¹
¹Department of Ecology & Evolutionary biology, Yale University, USA

Microbes live in highly complex and diverse communities, but how such communities form, develop, and are maintained is not well understood. Of particular note is the role of historical contingency, and how it affects the phylogenetic composition of stable communities. Starting from a single source soil microbiome, we show that multiple alternative community states can arise from replicate communities propagated under identical environmental conditions. We visualize the development of these communities through time using a combination of community phenotyping and genotyping. These alternative community states are not only different structurally (species composition) but also exhibit different dynamical properties, including community growth rate, temporal stability, and synchrony. Furthermore, we find that such variability can be reduced either by increasing the size of the founder population or by immigration. Together, our results suggest that community assembly history can lead to both structural and functional variability, even in simple and identical environments.
Evolutionary rescue describes a situation where adaptive evolution prevents the extinction of a population facing a stressful environment. Models of evolutionary rescue could in principle be used to predict the level of stress beyond which extinction becomes likely for species for which conservation is a concern, or conversely the treatment levels most likely to limit the emergence of pests or pathogens. Stress levels are known to affect both the rate of population decline (demographic effect) and the speed of adaptation (evolutionary effect), but the latter aspect has received less attention. We address this issue using Fisher's Geometric Model of adaptation. In this model, the fitness effects of mutations depend both on the genotype and environment in which they arise. In particular, the model introduces a dependence between the proportion of rescue mutants, their cost before the onset of stress, and the level of stress. We obtained analytic results for the evolutionary rescue probability of a large asexual population facing abrupt environmental change, with rescue stemming either (i) from a single mutation of strong effect, or (ii) from a large number of mutations of small effect. In both regimes, we describe a narrow ‘characteristic stress window’ over which the rescue probability drops from very likely to very unlikely as the level of stress increases. This drop is sharper with increasing stress than in previous models, as a result of the decreasing proportion of stress-resistant mutations as stress increases. In regime (i), we show that the effect of the environment on evolutionary rescue can be summarized into a single composite parameter amenable to empirical measurement. In regime (ii), an optimal mutation rate exists for which the evolutionary rescue probability is maximal. We discuss how to test some of these predictions with rescue experiments across gradients of stress.
Recent work, mainly from microbial analysis in aquatic systems, suggests that an important but so far unexplored component of microbial diversity consists of ultra-small bacteria with a cell size smaller than ~0.1μm. Small bacterial cell size is often linked to small genome size and genomic studies suggests that genome streamlining is ubiquitous in bacteria. Recently, we isolated an ultra-small bacterium from soil by applying a novel isolation and culturing method. We identified the isolated ultra-small bacteria as *Hylemonella gracilis*. The whole genome sequencing revealed that the genome size of *H. gracilis* is only 3.8 Mbp. Performing sets of interaction assays we revealed that *H. gracilis* is growing better when interacting with other soil bacteria e.g. *Paenibacillus* sp. (genome size 7.1Mbp) or *Serratia plymuthica* (genome size 5.5Mbp). However, not all interactions were growth stimulating as the growth of *H. gracilis* was found to be inhibited during the interaction with *Burkholderia* sp (genome size 8.2Mbp). These findings indicated that the growth of the ultra small bacteria is dependent on the interacting partner and that they might be metabolically dependent on the neighbouring bacteria. Furthermore, we observed that *H. gracilis* is able to change the behavior of the interacting bacteria without direct cell-cell contact. We will present comparative genomic, transcriptomics, metabolomics and mass spectrometry imaging results revealing the mechanisms of interactions. Our findings indicate that ultra-small soil bacteria may be an overlooked species in soil microbial communities able to induce responses in other microorganisms and therefore have an indirect effect on terrestrial ecosystem functioning.
Metazoans harbour considerable numbers of commensal microorganisms in the gut, named microbiota, which contribute to many aspects of host physiology. One important aspect is host growth. Nevertheless, the molecular mechanisms underlying microbiota’s beneficial influence are still largely undefined. Our project integrates microbial evolution, transcriptomics and the use of model organisms to understand how evolution shapes animal/microbe symbiosis and to identify the bacterial genetic pathways mediating host growth. Specifically, by applying experimental evolution to a well-established model of facultative symbiosis: *Drosophila melanogaster* associated with *Lactobacillus plantarum*, one of its growth promoting symbiont, we showed that the diet, instead of the host, is a predominant driving force in the evolution of this symbiosis. Following these observations, we conducted wide metabolic tests and RNA sequencing on the evolved bacterial symbionts in order to understand how the host nutritional environment shapes microbial adaptation, resulting in the improvement of symbiotic effect. I will report the results about the effects of bacterial adaptation to the host diet on gene expression and metabolism. Such data shed light onto the microbial genetic and metabolic pathways subjected to selection pressure by the host environment and responsible for the emergence and perpetuation of symbiosis.
O18. Evolutionary ecology of root associated microbes

Andrew Matthews\textsuperscript{1,2}, Matt Jones\textsuperscript{2}, Tom Bell\textsuperscript{2} and Ben Raymond\textsuperscript{1,2}
\textsuperscript{1}University of Exeter, UK
\textsuperscript{2}Imperial College London, UK

Our understanding of the evolutionary ecology of microbes in the rhizosphere remains limited, although they are important for plant health. We investigate it here through high-throughput phenotypic and genotypic characterization of culturable microbes from crop species. First we tested if functional traits would be better predictors of host and habitat association than phylogenetic origin. Using a library of 593 bacterial isolates of known host and soil provenance we characterized carbon metabolism, plant associated traits and antibiotic tolerance. Three of the four hosts had phylogenetically diverse, but functionally constrained communities. We also observed significant between trait covariance particularly between metabolic and antibiotic tolerance, potentially indicating recent strong convergent evolution and adaptation of growth strategy to the host. Subsequently we tested how colonization of a plant growth promoting (PGPR) strain was affected by the presence of competing rhizobacteria, in pair-wise colonization experiments in a gnotobiotic culture system. We showed that high bacterial titers on roots tend to reduce shoot mass; and facilitation or inhibition of colonization depends on specific GxG interactions, suggesting that competitive and mutualistic interactions are strong forces shaping microbial communities on roots. Finally, we tested the effect of evolution on colonization ability by experimental evolution. Results indicate stronger colonization potential for PGPR evolved with a community of other putative root mutualists than for PGPR evolved without competing strains. The correlation between root and shoot mass and total observed bacteria suggests that greater colonization by PGPR evolved with a community reduced host mass relative to other treatments, potentially via a host/bacterial growth trade-off in this carbon limited system. In conclusion, symbiont function and eco-evolutionary dynamics all affect community composition and host fitness.
Interactions among microbes within a microbiome and between microbes and their associated hosts are critical for host development, nutrition, and immunity. Despite their importance, these interactions are often difficult to identify and deconvolute due to their high-order complexity. For example, over 90% of cystic fibrosis (CF) patients die due to chronic lung infections. The decline in lung function is greatly accelerated by intermittent and progressively severe pulmonary exacerbations (PEs). Despite their clinical impact, surprisingly few microbiological signals associated with PEs have been identified. Here we introduce an unsupervised, systems-oriented approach to identify key members of the microbiota and apply it to the CF lung microbiome to identify taxa most strongly associated with PEs. Key taxa were defined based on three strategies: relative abundance, prevalence, and ecological importance as defined by co-occurrence network interconnectedness. We measured the association between changes in the relative abundance of the key taxa and changes in patient clinical status over time via change-point detection, and found that taxa with the highest level of network interconnectedness tracked changes in patient health significantly better than the other strategies. We also cross-sectionally stratified all samples into the clinical states and identified key taxa uniquely associated with each state. We found that network interconnectedness most strongly delineated the taxa among clinical states, and that anaerobic bacteria were strongly associated with PEs. These results lend support to the growing consensus that the development of anoxic conditions in the CF lung and the subsequent role of anaerobic microbes is an important factor leading to PEs. The approach also provides a general and robust framework for interrogating complex microbiomes to identify key taxa associated with important environmental or host-associated variables.
O20. Bacterial adaptation is constrained in complex communities

Thomas Scheuerl¹, Meirion Hopkins¹, Reuben W. Nowell¹, Damian W. Rivett¹,², Timothy G. Barraclough¹ and Thomas Bell¹

¹Department of Life Sciences, Imperial College London, Berkshire, UK.
²Division of Biology and Conservation Science, School of Science and the Environment, Manchester Metropolitan University, Manchester, UK.

The evolution of single populations in isolation has been well studied, however, in nature populations are embedded within complex communities. In communities, biotic interactions may either facilitate or constrain evolution depending on whether the interactions expand or contract the range of ecological opportunities. A fundamental challenge is to understand how the surrounding biotic community alters evolutionary trajectories as species adapt to novel environmental conditions. Here we show how community context can dramatically alter the evolutionary dynamics of bacterial populations. We find that evolution of focal bacterial strains depends on properties both of the focal strain and of the surrounding community. In particular, there was a stronger evolutionary response in low-diversity communities, and when the focal species had a larger genome and were initially poorly adapted. The findings demonstrate that adaptation to new environmental conditions can only be understood in the context of interspecific interactions.
P1. Power law fitness landscapes and their ability to predict fitness

Diogo Passagem-Santos¹, Lilia Perfeito¹

¹Instituto Gulbenkian de Ciência, Oeiras, Portugal.

Whether or not evolution by natural selection is predictable depends on the existence of general patterns shaping the way mutations interact with the genetic background. This interaction, also known as epistasis, has been observed both during adaptation (macroscopic epistasis) and from the perspective of individual mutations (microscopic epistasis). Interestingly, a consistent negative correlation between the fitness effect of beneficial mutations and background fitness (known as diminishing returns epistasis) has been observed across different species and conditions. We tested whether the adaptation pattern of an additional species, *Schizosaccharomyces pombe*, followed the same trend. We used strains that differed by the presence of large karyotype differences and yet the same pattern of fitness convergence was observed. Using these data along with published datasets, we measured the ability of different models to describe adaptation rates. We found that a phenotype-fitness landscape shaped like a power law is able to correctly predict adaptation dynamics in a variety of species and conditions. Furthermore we show that this model can provide a link between the observed macroscopic and microscopic epistasis. It may be very useful in the development of algorithms able to predict the adaptation of microorganisms from simply measuring the current phenotypes. Overall, our results suggest that even though adaptation quickly slows down, populations adapting to lab conditions may be quite far from a fitness peak.
Though often dominated by a single pathogen species such as *Pseudomonas aeruginosa*, Cystic Fibrosis (CF) associated chronic bacterial infections are often polymicrobial. Several studies have previously looked at the pairwise interactions between *P. aeruginosa* and either *Staphylococcus aureus* or *Stenotrophomonas maltophilia* in rich lab media or CF sputum-like media to elucidate the bacterial behaviour in these conditions. Here, we investigate in vitro how the composition of communities consisting all three of these CF pathogens are affected by certain factors of the CF lung environment – here a subinhibitory dose of tobramycin antibiotic and increased viscosity – over the course of a full factorial three-week selection experiment. In baseline communities *S. maltophilia* is driven extinct, though tobramycin treatment can shift the dynamics of competitive interactions to favour *S. maltophilia* over *P. aeruginosa*. Increased media viscosity has little impact alone, but the combination of increased viscosity and antibiotic treatment creates a permissive environment where all three species can coexist. We then find that these evolved coexisting communities can continue to coexist outside of the permissive viscous and antibiotic environment, suggesting co-adaptation. Fitness assays show that the fitness of the evolved *P. aeruginosa* is greatly reduced, suggesting that reduced competitive dominance of *P. aeruginosa* is a major factor. Ongoing work will seek to identify whether there is co-adaptation in all species in the community, through sequencing and further experimental assays, and to begin to elucidate possible mechanisms of this coexistence. Understanding the ways in which these species might adapt to each other may help in understanding the course of disease progression and identify ways to lessen the impact of infection on patients.
P3. Modulating mutation mechanisms in gram-positive bacteria

Anne-Marie Veenstra-Skirl¹, Oscar P. Kuipers¹ and G. Sander van Doorn¹

¹University of Groningen, Netherlands

Mutations generate novel, heritable variation in phenotypic traits, thereby supplying the raw material for adaptive evolution. Neo-Darwinian theory posits that mutations occur randomly with respect to their fitness consequences to the organism. Yet, mutations are not necessarily random in other respects as well, given that they carry signatures of the molecular mechanism by which they were generated. Each different mechanism of mutation produces its own characteristic statistical pattern in phenotypic, pleiotropic and epistatic effects, which shape the genetic architecture of traits. Based on this observation, it has been suggested that the mechanisms of mutation can significantly bias microevolutionary processes and influence the future adaptive potential of populations. Evolution experiments with bacterial populations that have differing mutational histories can provide insights into whether the signatures of mutation within the standing genetic variation of a population shape the evolutionary paths or whether the adaptation is dominated by the external selective forces. For this purpose, we have constructed several strains of the lactic acid bacterium *Lactococcus lactis* to to harbour inducible mutational plasmids that are expected to increase either point mutation or intrachromosomal homologous recombination and are supposed to be used for the above mentioned evolution experiments. Here we present results of the characterization of the genetic vectors contained within these strains, regarding the increase in mutant frequencies at different levels of induction, as well as influence on the growth characteristics of the cells. These mutator strains with temporarily elevated mutation rates will provide a useful tool for experiments that will investigate into the influence of different mutation mechanisms on the adaptation of populations in the same selective environment and help in understanding to what extent genetic variation influences the resulting adaptation.
P4. Plasticity and evolution of the expression of the chitinase enzyme, a virulence factor of Bacillus thuringiensis

Ana Lindeza¹, Caroline Zanchi¹ and Joachim Kurtz¹

¹Institute for Evolution and Biodiversity, University of Muenster, Muester, Germany

Gut-infecting bacteria infect their hosts with the help of powerful virulence factors. The production of virulence factors is costly for the bacteria, which may result in trade-offs, but they can also act in synergy. Most strains of Bacillus thuringiensis (Bt) are entomopathogenic bacteria, i.e. natural pathogens of insect species. Until recently, research on the virulence of Bt has mostly focused on the well characterised crystal (Cry) toxin proteins. When spores are ingested, Cry leads to the formation of pores in the midgut epithelial cells. However, for a full understanding of the interactions between host and pathogen it is crucial to also study the importance of other virulence factors, such as chitinases. Chitinases degrade chitin, which is a major component of the peritrophic membrane of the insect gut. Penetration of this chitin-rich barrier facilitates access of Cry toxins to the gut epithelial cells. In this project, we first analyzed the expression pattern of chitinase in Bt tenebrionis, a beetle-infecting strain. In particular, we asked whether chitin expression is modified in response to chitin in the growth medium, and whether this has an effect on Cry toxin expression. Using a serial passage experiment, we will then study whether chitin expression shows microevolutionary change in response to environmental chitin, how this impacts on Cry toxin expression, and finally, whether it affects virulence in its beetle hosts, Tribolium castaneum and Tenebrio molitor. This study thus aims to deepen our understanding of interactions, plasticity and evolution of crucial bacterial virulence factors.
P5. Bacteriophage infections dynamics in the mammalian gut

Marta Lourenço¹,², Luisa De Sordi¹ and Laurent Debarbieux¹

¹Group Interactions Bacteriophages Bacteria in Animals, BMGE Unit, Microbiology Department, Institut Pasteur, Paris, France
²Sorbonne Université, ED-Complexité du Vivant, Paris, France

The mammalian gastrointestinal tract (GIT) is a heterogeneous environment inhabited by diverse microorganisms amongst which the most abundant are bacteria and their specific viruses, bacteriophages. Not only the physical structure of the GIT is variable along its length (small vs. large intestine), but also are the physiological conditions such as pH, nutrients, water and oxygen. Altogether these parameters affect the metabolic state, the ecological network and the evolution of intestinal microorganisms. The characterization of interactions between bacteriophages and bacteria in the GIT environment is key to increase our knowledge on their role and functions in intestinal homeostasis. To investigate the putative role of this environment in the interactions between bacteriophages and bacteria we used a murine model of controlled microbiota composed of a consortium of 12 fully sequenced murine bacterial strains. These Oligo-MM12 mice can be colonized by the *Escherichia coli* strain (Mt1B1) for which we isolated and characterized 3 novel virulent bacteriophages. The overall relative abundance of the 12 strains, assessed by 16S qPCR, was similar between animals exposed or not to bacteriophages administered by oral gavage, despite a significant reduction of the levels of strain Mt1B1 throughout the entire GIT. This confirms that these bacteriophages do not have off targets. Bacteria isolated from all GIT sections never developed resistance to bacteriophages showing that the viral selective pressure does not select for the emergence of resistant clones. Surprisingly, we found that bacteriophages were almost absent in the ileal mucosal section while bacteria were still detected. We hypothesize that bacteriophages could not access the entire bacterial population in the ileum, suggesting that bacteria can survive in reservoirs from which they migrate and recolonize the GIT allowing coexistence between these two antagonistic populations.
Microbial predators are rarely considered as prey themselves. However, the phenotypes that we study are surely influenced by the role of these microbes as prey as well as by their role as predators, and we will not fully understand the evolution of their fascinating traits if we only consider them as the top members of their food chains. *Myxococcus xanthus* is a social soil bacterium and a versatile predator, able to consume both gram-negative and gram-positive prey. Its predatory behavior is being studied, but its ecological role as a mesopredator, its interactions with higher predators, and the ways in which higher predators may have contributed to the evolution of its social life cycle have yet to be investigated. We have developed a *Caenorhabditis elegans* + *M. xanthus* + prey bacterium model food chain which we are using to explore the evolution of *M. xanthus* as a mesopredator, with a focus on its social traits. By observing the predatory behavior exhibited by *M. xanthus* when given a choice of two different prey species and in the presence and absence of *C. elegans*, we assess the effect of a model top predator on *M. xanthus’* prey choice and consider how two motile micro-predators compete for food.
P7. The effect of mismatch in codon usage preference on horizontally transferred antibiotic resistance

Caroline Rose¹ and Stéphanie Bedhomme¹

¹Centre d'Écologie Fonctionnelle et Évolutive (CEFE), CNRS, Montpellier, France

Horizontal gene transfer can instigate the rapid acquisition and evolution of antibiotic resistance in multispecies communities. Genetic transfer between species with diverse codon usage preferences (CUP) may however result in a mismatch in codon usage between a recently transferred gene and its recipient genome. Here we assess the impact of, and sensitivity to, maladapted CUP in a multispecies context. We have synthesized a set of synonymous versions of the Gentamicin resistance gene aacC1 to be expressed in several bacterial species representing a broad range of CUP. The synonymous gene versions were designed such that they encompass a wide range of codon usage mismatch within each species, and the degree of this mismatch varies for each version across host species. We evaluate the cost of codon usage mismatch with regards to fitness and level of resistance conferred by the transferred synonymous genes across multiple bacterial species. Understanding the consequences of codon usage mismatch within multispecies communities may be instrumental for predicting the evolutionary dynamics of antibiotic resistance gene spread.
Horizontal gene transfer, a central trait shaping microbial adaptation and diversity, is mainly associated with the spread of mobile genetic elements like plasmids. Rates of plasmid transfer can evolve in response to selective pressures acting on both plasmids and their bacterial hosts. Previously I showed that investment in transfer is an altruistic act for host bacteria. When transferred plasmids are beneficial, kin selection can promote altruistic transfer if it happens preferentially towards kin, which could occur through kin discrimination mechanisms ensuring more efficient transfer among related donors and recipients. Here, to investigate variation in transfer rates at an ecologically relevant scale, I characterized rates of plasmid transfer among isolates from natural populations of *E. coli* with a known history of co-existence in the field. Transfer rates are highly variable, spanning several orders of magnitude even within local populations and at a small phylogenetic scale. Overall, transfer of both narrow and broad host range plasmids is strongly biased towards kin. Furthermore, the bias is not a function of average genetic distance but relies, at least partially, on shared restriction-modification systems between donor and recipient genotypes. These results suggest that the conditions for kin selection of transfer are met in natural environments, promoting increased investment in transfer by host bacteria. Moreover, genetic exchange will mostly take place within lineages, and might be overlooked by studies detecting only gene movement among divergent genotypes. The eco-evolutionary dynamics described here will also affect how transfer shapes microbial diversity, as it will promote increased variation in mobile gene content among lineages.
Chronic bacterial respiratory infections are the leading cause of morbidity and mortality among cystic fibrosis (CF) patients. The thick mucus covering the CF airway is an ideal environment for a polymicrobial community, including opportunistic pathogens of the *Burkholderia cepacia* complex species, to develop. Tracing bacterial evolution during these long-term infections can provide insights into how host selection pressures, such as antimicrobial therapies and the immune system, shape bacterial genomes. We performed genomic and phenotypic analysis of 128 longitudinally collected *Burkholderia multivorans* isolates from nine CF patients spanning a period of 7-20 years. Genome analysis within each patient showed coexisting clades with distinct evolutionary dynamics. Evidence of clonal lineages shared by some patients was observed suggesting inter-patient transmission. We also observed recurrent genome reduction, with deletions ranging from few nucleotides to 200 kb, including plasmid losses. Several loci, mostly involved in gene expression regulation, lipid metabolism, and cell wall biosynthesis were identified as likely targets of selection. Further, a broad range of phenotypes changed in association with the evolved mutations; they included antimicrobial resistance, biofilm regulation, motility, and presentation of lipopolysaccharide O-antigen repeats. This study provides the first comprehensive genome-phenome analyses of *Burkholderia multivorans* infections in CF lungs and defines the “chronic infection phenotype” as slow growth, higher adhesion to surfaces, reduced motility, greater antibiotic resistance, and reduced virulence. Identifying traits under strong selection during chronic infection not only sheds new light onto *Burkholderia* evolution, but also sets the stage for tailored therapeutics targeting prevailing lineages associated with disease progression.
Bacteria must survive and compete in complex environments, including inside hosts. We have leveraged laboratory work alongside naturally-occurring evolution experiments within patients to study one of the most dramatic of competitive phenotypes: the type VI secretion system. This molecular spear is used by bacteria to poison neighbouring cells in mixed communities. Theory predicts that such an interaction between an actor and recipient is favoured depending on its cost, benefit and the relative relatedness between actor and recipient. We perform experiments on the bacterium *Pseudomonas aeruginosa* to determine the costs and benefits of T6SS-mediated killing. Our data indicates that bacterial killing has a direct benefit for the attacking strain and affects the spatial organization within a bacterial community. A long-term collection of clinical *P. aeruginosa* isolates enables us to analyze T6SS-mediated killing in natural evolution experiments that occur in complex environments. Bioinformatics on the clinical isolates show the potential for T6SS-mediated interference competition in patients and suggests competition to be a dynamic trait during evolution in a changing environment.
P11. The population genetics of the heat-shock protein groEL sheds light on the population structure of the pneumococcus

José Lourenço¹, Uri Obolski¹, Martin Maiden¹ and Sunetra Gupta¹

¹Department of Zoology, University of Oxford, Oxford, UK

Recently we have used whole genome multi-locus sequence typing (wgMLST) together with machine learning to decipher what appear to be universal signatures within the genetic population structure of the pneumococcus. We discovered that alleles within the groESL operon are highly predictive of sequence cluster (lineage); and offered an evolutionary rationale, based on the current literature, on how selective pressures on the heat-shock protein groEL may have led to this universal population structuring. Here, we expand on those findings and explore in detail the population genetics of groEL across major pneumococcal datasets, covering wide time and spatial ranges. The findings elucidate further on the types of selection that have acted on groEL in the recent history of the pneumococcus and help understand how vaccination may been changing its population genetics.
P12. Single cell imaging uncovers a new subcellular distribution pattern of the iron-scavenging molecule pyoverdin in Pseudomonas

Clara Moreno-Fenoll$^{1,2}$, Maxime Ardré$^3$ and Paul B. Rainey$^{1,2}$

$^1$Department of Microbial Population Biology, Max Planck Institute for Evolutionary Biology, Germany
$^2$Laboratoire Génétique de L’Évolution, École Supérieure de Physique et de Chimie Industrielles de la Ville de Paris (ESPCI Paris-Tech), France
$^3$New Zealand Institute for Advanced Study, Massey University, New Zealand

Rigorous study of the role of diffusible extracellular products in microbes necessitates consideration of their ecological and evolutionary context. Biophysical constraints on systems based on extracellular resources can directly impact on ecology and evolution – for instance, limited sugar diffusion after hydrolysis by invertase in Saccharomyces cerevisiae enables co-existence of producers and non-producers of the enzyme. Another widely-studied extracellular product is pyoverdin, a metal chelator produced by Pseudomonas spp. which scavenges iron from the medium and releases it in the bacterial periplasm. It has been shown that pyoverdin producers retain a fitness advantage over non-producers in conditions where this siderophore is necessary – and where avoiding the cost of its synthesis should otherwise benefit non-producers. Furthermore, in a microcolony, this molecule preferentially diffuses between adjacent cells, thus reducing its loss into the environment. Together these results point to the importance of spatial control of pyoverdin, and suggest the possibility of personalization. Here we describe a novel distribution pattern of pyoverdin that adds evidence supporting this notion. Single-cell imaging of Pseudomonas fluorescens SBW25 revealed that, while pyoverdin is usually homogeneously disseminated in the periplasm, it can transiently accumulate at the cell pole. We characterize some of the ecological variables influencing the appearance of this asymmetry (by varying the stringency of the environment and the presence of other bacterial strains) and the underlying molecular mechanism. We discuss its potential functional implications for the dynamics of a community that relies on pyoverdin.
What is the time delay between the occurrence of a genetic mutation and the manifestation of the corresponding phenotypic effect? Given that a mutation appearing in the DNA has to first transcend the RNA and protein level the phenotypic effect cannot appear instantaneously. We used genetic recombineering to introduce antibiotic resistance mutations into E. coli within a narrow time interval and followed the appearance of resistance by replica plating. We observed a phenotypic delay of 3-4 generations until 50% of the population harboring the resistance mutations expressed the resistant phenotype. Using a LacZ based reporter system and single cell observation we provide evidence that the mechanism causing phenotypic delay is multiple copies of the target gene are produced by multifork replication resulting effectively in polyploidy. Multiple cell division are required until the cell becomes homozygous for the resistance allele causing a multigenerational phenotypic delay. Using mathematical models we explore the consequences of phenotypic delay for the estimation of mutation rate based on fluctuation tests and the for the evolution of antibiotic resistance.
P14. Interferon as a social “public bad” for a virus

Pilar Domingo-Calap, Ernesto Segredo-Otero, María Durán-Moreno and Rafael Sanjuán

Institute for Integrative Systems Biology (I2SysBio), Consejo Superior de Investigaciones Científicas-Universitat de València, València, Spain

Social evolution is a key aspect in the evolution of complex ecological systems, and is an important framework to understand the interactions between individuals of the same or different species. However, the principles of social evolution have seldom been validated in viruses. In particular, whereas infection and immunity have been investigated extensively from the point of view virus-cell interactions, the role of virus-virus interactions remains largely unexplored. Here we show that shutdown of interferon (IFN)-mediated innate immunity is a viral social game in which IFN operates as a “public bad” or “pollutant”, that is, a virus-induced, diffusible product with a negative impact on viral fitness, as opposed to typical public goods. We show that the condition for IFN shutdown to evolve is akin to Hamilton’s rule: \( rb - c > 0 \), where \( c \) is the fitness effect of IFN production on the actor, \( b \) the fitness effect on social partners, and \( r \) measures the level of assortment between IFN-blocking and IFN-stimulating virus variants, as determined by spatial structure. We apply this model to the prototypic vesicular stomatitis virus (VSV) by comparing the ability of an IFN-stimulating mutant (\( \Delta 51 \)) and an isogenic wild type (WT) to infect mouse embryonic fibroblasts. Whereas the WT was vastly superior in isolation, \( \Delta 51 \) severely interfered with WT fitness in mixed infections, and took over the population in the absence of spatial structure or IFN. Therefore, the WT VSV functions as an altruistic virus (\( c > 0 \)), whereas \( \Delta 51 \) is a social cheater that reduces the total fitness of the population by stimulating IFN production.
Microbial communities are characterized by a multitude of interspecific interactions, which can range from mutualism to competition. The overall sign and strength of interspecific interactions have important consequences for the emergent properties at the community level, but it is currently unclear whether and how these interactions change over evolutionary time scales. I study the evolution of species interactions in a model microbial community from the ocean. This community plays an important role in global carbon cycling, and as such has broad direct relevance. Extending from previous theory, I developed a set of specific hypotheses for the evolution of interspecies interactions in such complex systems. To address these hypotheses, I will experimentally evolve communities of five bacterial species in either well-mixed or spatially structured environments, and investigate how each of the constituent species, as well as their interactions, changes over time using a combination of phenotypic assays, whole-genome sequencing, and metabolic profiling. Moreover, I will investigate whether adaptations in a community context are typically selected because of their direct or indirect fitness effects by studying the growth of single cells in precisely controlled environments, where their effect on the—local and global—environment can be manipulated. I anticipate that evolution in well-mixed environments will proceed exclusively via the selection of directly beneficial traits. Conversely, evolution in spatially structured environments may proceed via the selection of both directly and indirectly beneficial traits, and which of these two predominates should depend on the availability and effect size of mutations affecting both types of traits. Selection of indirectly beneficial traits is predicted to result in an increase in interaction strength over time, while selection of directly beneficial traits is not predicted to have such a systematic effect.
Phylosimbiosis is an ecoevolutionary pattern in which evolutionary changes in host associate with ecological changes in microbiota. *Pacifigorgia seafans* are gorgonian corals (Octocorallia: Haloxonia: Gorgoniidae) that are considered habitat-forming species providing nutritional substrate and protection for many species in coral reefs. Previous studies have found endosymbiont bacterial communities on gorgonian contributing to metabolism, adaptation and acclimatization processes, as well as fitness and resilience on the coral holobiont. These bacterial communities are often host-specific moreover hypothesized to co-evolve with their host. In the Colombian Tropical Eastern Pacific, the azooxanthellate corals of the genus *Pacifigorgia spp.* are highly diverse. The species present different mesh-like morphologies with diverse metabolic demands, presumably achievable through different assemblies of bacterial communities. However, their microbiota and the relationships among native microbiota, host fitness and host evolution are still unknown. We are studying bacterial metabarcoding (16s rRNA) to assess the structure, composition and diversity of bacterial communities of ten sympatric species of *Pacifigorgia* corals. This is the first study on assemblages of bacterial communities from *Pacifigorgia* octocorals, which are presumably undergoing an adaptive radiation.
P17. Evolution destabilizes pair-wise interactions in microbial communities exposed to fluctuating environments

Alejandra Rodriguez-Verdugo and Martin Ackermann

1Department of Environmental Microbiology, Eawag, Dübendorf, Switzerland

Positive species interactions underlie the health and functioning of many ecosystems - e.g. mutualistic interactions in corals and commensal interactions in metabolically dependent bacterial communities. Given their importance, it is crucial to understand how stable are positive interactions over evolutionary time-scale, not only in constant environments but also in fluctuating environments where the interaction between species changes in function of the environmental conditions (e.g. changes from positive to negative interactions). We addressed this question using two-species microbial consortia in which we could change species interactions from commensalism to competition depending on the nutrient provided. To test how stable were these interactions over the long-term we evolved four consortia for 200 generations in a constant environment and in a fluctuating environment with daily changes between commensalism and competition. We observed that coexistence persisted over 200 generations in the constant commensal environment, but broke down around 100 generations in two out of four cases in the fluctuating environment. To explore the underlying genetic changes associated with the occasional extinction of one of the community members, we sequenced the full genome of clones isolated before and after the collapse. In the two consortia in which species coexisted until the end of the experiment, both species fixed a mutation after 92 generations. In the other two consortia, only one of the species fixed a mutation (the species that persisted until the end of the experiment) while the other species did not fix any mutations and went extinct. Our results suggest that commensal interactions are stable over evolutionary time-scales in constant environments, but can be destabilized by evolutionary responses in fluctuating environments.
Human activity is severely modifying global environments and accelerating climate and biotic changes. Studying how organisms responded to environmental changes in the past can be useful for anticipating their responses to current and future changes, and thus aid the development of effective conservation strategies. However, organism-environment interactions are rarely preserved in historical and fossil records. Dental calculus is one of the few biological substrates that preserves information about the host, its associated microorganisms and its environment in a virtually unchanged form through time. Dental calculus is a calcified microbial biofilm that forms on mammalian teeth. In addition to the oral microbiome, dental calculus captures pathogens, dietary particles and host molecules. Although dental calculus is a powerful source of temporal information on organism-environment interactions, it has received little attention outside of human studies, where it has revealed shifts in the microbiome composition as result of cultural transitions, and uncovered dietary and pathogenic components in extinct and extant hominins. Although many mammals produce dental calculus, it remains unclear how far it can be used to study evolutionary responses of these species to environmental change. Here, we apply ancient DNA techniques to dental calculus from museum specimens of several mammals, with the aim to characterise changes in the oral microbiome composition, diet and pathogen exposure through time. As the first step towards establishing dental calculus research in non-human animals, we demonstrate that the oral microbiome signature can be retrieved from species as diverse as brown bear and reindeer. We also test for associations of microbiome profiles with known oral pathologies, such as caries and periodontitis, and for signature of dietary composition. Taken together, our study demonstrates the usefulness of dental calculus for studies of organism-environment interactions.
Microbial communities shape our lives at many different levels. Consequently, explaining how these communities evolve and adapt is crucial to better understand, benefit and manipulate them towards greater utility and reduced harm. One of the most important mechanisms of microbial adaptation is Horizontal Gene Transfer (HGT). Although the mechanisms enhancing and limiting HGT have been widely studied in vitro in isolated organisms, little is known about their impact in nature. This lack of knowledge ultimately limits our understanding on how HGT affects a microbial community evolution and co-evolution. However, the higher rate of HGT in multispecies biofilms and the capacity of phylogenetically diverse bacteria to recognise similar methylation patterns (important in self and non-self DNA recognition) suggest that HGT might have an important role in community co-evolution. To address this question we have assembled and evolved a synthetic community of 4 different bacteria, and analyzed the patterns of HGT between them. Our results suggest that HGT rates are higher than previously expected, specially among mobile genetic elements, but their fixation rate is extremely low. This result suggests important evolutionary constraints associated to the genome size and architecture.
P20. Spread and evolution of plasmid pOXA-48 in a hospital setting

Javier de la Fuente¹, Jerónimo Rodríguez-Beltrán¹, Ricardo León-Sampedro¹, Marta Hernández¹, Rafael Cantón¹, Maria Morosini¹ and Alvaro San Millán¹

¹Hospital Universitario Ramon y Cajal (IRYCIS), Spain

Plasmids mediate the horizontal transmission of genetic information between bacteria by conjugation, playing a pivotal role in the evolution of antibiotic resistance in pathogenic bacteria. Associations between enterobacteria and antibiotic resistance plasmids are not random. Certain plasmid-bacterium associations become particularly successful, creating multi-drug resistant bacteria that spread in clinical settings. Good examples of these events are the associations between the carbapenemase-coding plasmid pOXA-48 and specific clones of Klebsiella pneumoniae. In this study we investigate the spread and the evolution of plasmid pOXA-48 in the Hospital Universitario Ramon y Cajal (Madrid, Spain) since it was first reported in 2012. We used a collection of clinical enterobacteria recovered from fecal samples of hospitalized patients as part of the European funded project R-GNOSIS (http://www.r-gnosis.eu). We performed Whole Genome Sequencing of 244 clones carrying plasmid pOXA-48 from 135 patients during a period of 5 years. Combining epidemiological and genomic data, we were able to follow and reconstruct the dissemination and the evolution of plasmid pOXA-48 over a period of five years. We detected a high conserved plasmid sequence and multiple events of inter-patient spread of pOXA-48-carrying enterobacteria, which were mainly mediated by clones of K. pneumoniae (ST11) of high prevalence in our hospital. Our results strongly suggest that K. pneumoniae clones drive the dissemination of pOXA-48 among patients in the hospital. Interestingly, once K. pneumoniae/pOXA-48 colonizes the gut of a new patient, pOXA-48 spreads horizontally towards other resident enterobacteria in the gut microbiome of the patient.
P21. Diversity and composition of the microbiome in the lower respiratory tract of patients with pulmonary tuberculosis are associated with lung damage

Monica Ticlla\textsuperscript{1,2,3}, Jerry Hella\textsuperscript{1,2,4}, Hellen Hiza\textsuperscript{4}, Mohamed Sasamalo\textsuperscript{4}, Francis Mhimbira\textsuperscript{1,2,4}, Sara Droz\textsuperscript{5}, Sara Schaller\textsuperscript{5}, Sebastien Gagneux\textsuperscript{1,2}, Markus Hilty\textsuperscript{5}, Iñaki Comas\textsuperscript{6}, Christoph D. Schmid\textsuperscript{1} and Lukas Fenner\textsuperscript{7}

\textsuperscript{1}Swiss Tropical and Public Health Institute, Basel, Switzerland
\textsuperscript{2}University of Basel, Switzerland
\textsuperscript{3}Swiss Institute of Bioinformatics, Lausanne, Switzerland
\textsuperscript{4}Ifakara Health Institute, Dar es Salaam, Tanzania
\textsuperscript{5}Institute for infectious Diseases, University of Bern, Switzerland
\textsuperscript{6}Tuberculosis Genomics Unit, Biomedicine Institute of Valencia, Spain
\textsuperscript{7}Institute of Social and Preventive Medicine, University of Bern, Switzerland

Tuberculosis (TB) is currently the deadliest bacterial infectious disease worldwide, primarily caused by \textit{Mycobacterium tuberculosis} (Mtb) which invades the lungs. When reaching the lungs, Mtb has not only to face an army of immune cells but also an even more numerous army of commensal microorganisms inhabiting the lower respiratory tract (LRT), known as the LRT microbiome. How Mtb interacts with the LRT microbiome is currently unknown. Previous studies, limited by their small sample sizes, have reported conflicting results on the association of the LRT microbiome with pulmonary TB. We assess here the association of TB disease severity and Mtb burden with the diversity and composition of the LRT microbiome in a large cross-sectional case-control study. We collected sputum samples from 224 newly diagnosed cases of pulmonary TB and 191 household contact controls from Tanzania. TB cases were stratified by Mtb burden, and by the degree of lung damage assessed by chest radiography. To characterize the microbial community composition, we performed 16S rRNA gene sequencing. After controlling for potential confounding factors (sex, age, BMI, anemia, smoking, co-infections with HIV and viral respiratory infections, season), our analysis revealed a shift in the composition of the LRT microbiome from TB cases compared to controls. More interestingly, variation in the diversity and composition of the LRT microbiome of TB cases was associated to the severity of lung damage but not Mtb burden. Species of the bacterial genera \textit{Fusobacterium}, \textit{Neisseria}, \textit{Streptococcus}, \textit{Veillonella}, \textit{Leptotrichia}, \textit{Campylobacter}, TG5 and \textit{Lautropia} are among the most associated species with TB and lung damage. This is the largest study providing evidence about the association of the LRT microbiome with TB.
P22. The microbiome and its role in conferring resistance towards parasites in *Daphnia*

**Lore Bulteel**¹, Shira Houwenhuyse¹, Steven Declerck², Emilie Macke¹ and Ellen Decaestecker¹

¹KU Leuven, KULAK, Campus Kortrijk, Belgium
²Netherlands institute for Ecology (NIOO - KNAW), Wageningen, The Netherlands

Recently, the contribution of the microbiome in host-parasite interactions has received attention as research indicates that this complex community of microorganisms plays a crucial role in host physiology and pathogen resistance (colonization resistance). By consuming common limited resources in competitive microbe-microbe interactions and providing the host of extra food resources, the indigenous gut microbiota is assumed to contribute to limit the attachment, growth and survival of introduced pathogenic bacteria. In our study, we utilized gut microbiota transplants combined with metagenetics to examine the role of the microbiome in host-parasite interactions. *Daphnia magna* and its parasites is our model system of choice. Firstly, *Daphnia* individuals (donors) were exposed to one of two different (or no=control) parasite communities (White Fat Cell Disease - *Binucleata daphnia* community & *Pasteuria ramosa* - *Colligata ordospora* - *Mitosporidium daphnia* community), after which the *Daphnia* gut was dissected. We assumed that parasite exposure would induce a shift in the gut microbiota in parasite-infected populations and that microbiota beneficial for protection against parasites would be selected. Secondly, *Daphnia* individuals (recipients) from three clones were made axenic and received the donor microbiome which were pre-exposed to one or no parasite community. We assumed that there was an interaction between the gut microbiome and the host genotype, that mediates the *Daphnia* tolerance towards infection. Thirdly, we exposed the recipients of each donor microbiome to one or none of the parasite communities. The same parasite communities of the donor exposures were here used to expose the recipients. By doing so, we investigated whether a specific microbiome confers resistance towards a community of particular parasites.
Community-level migration is widespread in microbial ecology. Random events (such as immigration) and deterministic ecological interactions (such as competition and predation) combine to shape community dynamics, together with abiotic conditions. How immigration shapes community functions and composition is still poorly understood. We have used consumer-resource models to top-down assemble a community with hundreds of species competing for a single supplied resource, and coexisting by cross-feeding metabolic secretions. Communities are periodically perturbed by immigration events involving entire invasive communities. The model explicitly incorporates various functional groups, which are used to examine the change in community structures brought forth by migration. We report three main results. First, frequent immigration drives community to be more efficient in converting resources into biomass and distributes secreted resources more evenly at equilibrium. Second, and consistent with recent experimental results from our lab, we found that immigration increases species richness and divergent composition across replicates, while at the same time producing functionally more convergent and less diverse communities. Third, the probability of resident species to survive migration is higher (and the probability of invasive species to invade is lower) for communities that have experienced many previous migration events. Overall, our model suggests that high rate of community-level migration enhances community efficiency and species-level diversity while at the same time reducing community resilience. Our results may provide strategies for designing more efficient microbial communities, with applications for bioremediation, health, and bioengineering.

Anshuman Swain¹ and William F. Fagan¹

¹Department of Biology, University of Maryland, College Park, USA

The Warburg effect refers to a curious behavior observed in many organisms and cell types including cancer cells, yeast and bacteria, wherein both the efficient aerobic pathway and the inefficient fermentation pathway are utilized for respiration, despite the presence of ample oxygen. Also termed as overflow metabolism in bacteria, this phenomena has remained an enigmatic and poorly understood phenomenon despite years of experimental work. Here, we focus on bacterial cells and build a model of three trade offs involved in the utilization of aerobic and anaerobic respiration pathways (rate versus yield, surface area versus volume, and fast versus slow biomass production) to explain the observed behavior in cellular systems. The model so constructed also predicts changes in the relative usage of both pathways in terms of size and shape constraints of the cell, and identifies how substrate availability influences growth rate. Additionally, we use the model to explain certain complex phenomena in modern- and paleo-ecosystems, via the concept of overflow metabolism.
P25. Evolutionary training of bacteriophages to enhance the biological control of diversified bacteria pathogen

Pilar Puentes-Tellez¹ and Alexandre Jousset¹

¹Ecology and Biodiversity Group, Faculty of Science, Utrecht University, The Netherlands

*Ralstonia solanacearum* is a highly diverse pathogenic species that can infect more than 200 crops. It can rapidly disseminate or adapt to different ecological niches such as soil, water and plants. The resulting high diversity of life history strategies in this pathogen make it very difficult to control. Here we train bacteriophages to consume multiple co-occurring strains of this pathogen. We first performed a genetic and phenotypic characterization of isolates obtained from Rosa sp. cultivars across The Netherlands. We used whole-genome sequencing to obtain high-resolution detection of genetic variation among the isolates as well as the tracking of pathogen dissemination within the country. We link resistance patterns to genomic and life-history adaptations. Genetic and phenotypic examination allowed us to distinguish strains to be used as individual hosts in a one-sided experimental evolution setup. We then produced multiple virulence-enhanced and specialized bacteriophages which as a consortium are able to effectively suppress genetically and phenotypically diverse *R. solanacearum*. 

Jake L Weissman¹, Rohan Laljani¹, William F. Fagan¹ and Philip Johnson¹

¹University of Maryland College Park, USA

Bacteria and archaea are locked in a near-constant battle with their viral pathogens. Despite previous mechanistic characterization of numerous prokaryotic defense strategies, the underlying ecological and environmental drivers of different strategies remain largely unknown and predicting which species will take which strategies remains a challenge. Here, we focus on the CRISPR immune strategy and develop a phylogenetically-corrected machine learning approach to build a predictive model of CRISPR incidence using data on over 100 traits across over 2600 species. We discover a strong but hitherto-unknown negative interaction between CRISPR and aerobicity, which we hypothesize may result from interference between CRISPR associated proteins and DNA repair due to oxidative stress. Our predictive model also quantitatively confirms previous observations of an association between CRISPR and temperature. Finally, we contrast the environmental associations of different CRISPR system types (I, II, III) and restriction modification systems, all of which act as intracellular immune systems.
P27. Plasticity evolution in temporally autocorrelated environments: tolerance curve and glycerol reaction norms of Dunaliella salina exposed to fluctuating salinities

Marie Rescan¹, Nicolas Leurs¹, Daphné Grulois¹ and Luis-Miguel Chevin¹

¹Centre d'écologie fonctionnelle et évolutive - CEFE, Equipe Génétique & Ecologie évolutive – GEE, Montpellier, France

Most natural environments vary in a random way, beyond any trend like global change, and such stochasticity has important consequences for evolutionary demography. In fluctuating environments, phenotypic plasticity is beneficial and may prevent extinction in populations exposed to abrupt environmental changes. However, plasticity could also be detrimental in unpredictable environment: if individuals phenotype responds to early environmental cues that are not correlated with their environment of selection, being responsive decreases fitness. The optimal level of plasticity should therefore depends on the temporal autocorrelation of the environment.

We measured tolerance curves and reaction norms of glycerol production in 25 populations of Dunaliella salina evolved for 200 generations in fluctuating levels of salinities, with four autocorrelation treatments, from negative to strongly positive. Fitness was measured across a range of salinity, in population acclimated for seven days in one of the tested salinities. The shape of this 3D tolerance curve evolved differently in populations exposed to different autocorrelation treatments. Surprisingly, we show that the major shift in the tolerance curve did not imply growth response to the salinity of the assay, but growth response to the acclimation salinity, with a larger range of acclimation salinities tolerated after evolution in autocorrelated environment. Tolerance curve from the negative autocorrelation treatment was similar to positive autocorrelation treatment, implying that environmental predictability (negative or positive) might have driven this evolution. While population evolved in stochastic environment displayed lower mortality when exposed to high from low salinity, we show no difference in the equilibrium intracellular content of glycerol nor in its production rate, suggesting that other mechanisms should be responsible for the difference in tolerance curves.
P28. Low diversity in the highly polymorphic Irgb2-b1 gene in South American mice: a role in resistance to local Toxoplasma gondii strains?

Catalina Alvarez1,2, Luis Teixeira2 and Jonathan Howard1

1Host-Pathogen Co-Evolution Laboratory, Instituto Gulbenkian de Ciência, Oeiras, Portugal
2Host-Microorganism Interactions Laboratory, Instituto Gulbenkian de Ciência, Oeiras, Portugal

Immunity-related GTPases (IRGs) are IFNγ-inducible genes of a cell-autonomous resistance system that protects the mouse host from avirulent Toxoplasma gondii strains. However, virulent strains can inactivate IRG proteins and kill mice within days. The evolution of virulence in T. gondii seems paradoxical because mouse death before encystment interrupts the parasite life cycle. Specific alleles of the Irgb2-b1 gene found in wild mice can confer resistance against virulent parasite strains. The interaction between South American (SA) T. gondii strains and Eurasian Mus musculus species in the wild is only 500 years old and virtually all SA T. gondii tested are virulent for laboratory mice. Nevertheless, host-pathogen interactions between SA house mice and T. gondii strains are still uncharacterized. We have found that mice obtained from different locations across Brazil carry Irgb2-b1 alleles already characterized in European populations. However, we detected an exceptionally high prevalence of one specific Irgb2-b1 allele in the Brazilian populations. These mice show polymorphisms at other nuclear and mitochondrial loci, indicating multiple geographic origins; a founder effect is thus unlikely to account for the high frequency of this allele. A hypothesis raised by these findings is that the highly prevalent allele provides a resistance advantage against SA Toxoplasma strains.
P29. High-order interactions dominate the functional landscape of microbial consortia

Alicia Sanchez-Gorostiaga\textsuperscript{1,2,*}, Djordje Bajić\textsuperscript{1,2,*}, Melisa L. Osborne\textsuperscript{3,4}, Juan F. Poyatos\textsuperscript{3,5} and Alvaro Sanchez\textsuperscript{1,2,3}

\textsuperscript{1}Department of Ecology & Evolutionary Biology, Yale University, New Haven CT, USA
\textsuperscript{2}Microbial Sciences Institute, Yale University, West Haven CT, USA
\textsuperscript{3}The Rowland Institute at Harvard, Harvard University, Cambridge MA, USA
\textsuperscript{4}Department of Biology, Boston University, Boston MA, USA
\textsuperscript{5}Logic of Genomic Systems Laboratory, Centro Nacional de Biotecnología (CNB-CSIC), Madrid, Spain.
* These authors contributed equally.

Understanding the link between community composition (structure) and function is a major challenge in microbial ecology, as it has implications for the management of natural microbiomes and the design of synthetic consortia. Borrowing approaches from the study of complex genetic interactions and fitness landscapes, we have examined how the amylolytic function of combinatorial assemblages of seven starch-degrading soil bacteria depends on the contributions from each species and their interactions. Filtering our experimental results through the theory of enzyme kinetics, we show that high-order functional interactions dominate the amylolytic rate of our consortia, even though this function is biochemically simple, redundantly distributed in the community, and additive in the absence of inter-species interactions. As the community grows in size, the contribution of high-order functional interactions grows too, making the community function increasingly unpredictable. We can explain the prevalence of high order effects and their sign from the redundancy of ecological interactions in the network, in particular from redundant facilitation towards a high-performing community member. Our results suggest that even simple functions can be dominated by complex interactions, posing challenges for the predictability and bottom-up engineering of ecosystem function in complex multi-species communities.
Bacterial conjugation is an important process in microbial evolution, and plays a significant role in the spread of antibiotic resistance genes through horizontal gene transfer. Models have shown how plasmid persistence and prevalence is the result of a combination of competing parameters, but these parameters are not fixed and are subject to evolution through selection. While further models have been constructed to show how some of these parameters can evolve (e.g. the amelioration of plasmid cost), the selective pressures on the rate of plasmid transfer remain largely unexplored. Adaptive dynamics is used here to identify the direction of evolution on the plasmid and a host population under different selective conditions. Plasmids and hosts were found to have conflicting evolutionary trajectories where the plasmid was costly. Intermediate levels of plasmid prevalence commonly found in the environment are, in part, likely to be the result of this conflict. In addition, the role of the recipient in controlling the evolution of plasmid transfer rate is emphasized.
P31. Genetic exchanges are more frequent in bacteria encoding capsules

Olaya Rendueles¹,² and Eduardo P.C. Rocha¹,²

¹Institut Pasteur, Paris, France
²CNRS, France

Bacterial adaptation is accelerated by the exchange of genetic information between cells by either homologous recombination (HR) or by horizontal gene transfer (HGT). HGT is regarded as a key process in shaping the genetic repertoires of bacteria because it can result in the acquisition of different genes leading to the gain of novel functions and contributes greatly to genome plasticity and allows the acquisition of radically novel traits. However, little is known on how the bacterial cell envelope shapes these transfer events and what are the eco-evolutionary consequences. We had previously shown that one extracellular structure, the bacterial capsule, a major virulence factor present in ca. 50% of the bacterial genomes across all major phyla, allowed bacteria to be better colonizers and to numerically dominate most environments. This suggested a positive role for capsules in adaptation and genetic diversification of bacteria. Yet, capsules are recurrently believed to decrease the rate and efficiency of gene exchange. This raises two conundrums. First, capsulated species should exhibit slower adaptation rates. Second, capsules themselves are known to evolve fast by horizontal gene transfer and recombination. We thus analyzed over 5000 bacterial genomes for the evidence of an association between genetic exchanges (or lack thereof) and the presence of a capsule system. Surprisingly, we find that bacteria encoding capsules have larger pan-genomes, higher rates of horizontal gene transfer, and higher rates of recombination in their core genomes. Further, genomes encoding capsules have more plasmids, conjugative elements, prophages, and integrons. We also found that capsules themselves are encoded in MGE, most notably in plasmids, but also in prophages. We conclude that bacteria with capsule systems are more genetically diverse and have fast-evolving gene repertoires, which may further contribute for their success in colonizing novel niches.
Tuberculosis (TB) affects humans and other animals and is caused by bacteria from the *Mycobacterium tuberculosis* complex (MTBC). Previous studies have shown that there are at least nine members of the MTBC infecting animals other than humans; these have also been referred to as ecotypes. However, the ecology and the evolution of these animal-adapted MTBC ecotypes are poorly characterized. Here we analyzed 12'886 public MTBC samples and additional newly sequenced 17 animal-adapted MTBC strains, gathering a total of 529 genomes of animal-adapted MTBC strains. Phylogenomic and comparative analysis confirm that the animal MTBC members are paraphyletic with some members more closely related to the human adapted *Mycobacterium africanum* Lineage 6 than to other animal strains. Furthermore, we identified four main animal-MTBC clades which could correspond to four main host shifts; two of them associated with independent cattle domestication events. Contrary to what would be expected from an obligate pathogen, MTBC nucleotide diversity is not positively correlated with host genetic distances suggesting that host tropism in animal MTBC seems to be driven more by contact rates and demographic aspects of the host population, than host relatedness. This also emphasizes that the animal-adapted MTBC provide a remarkable example of host range diversification while undergoing genome erosion. By combining phylogenomics with ecological data we propose an evolutionary scenario in which the ancestor of Lineage 6 and all animal-adapted MTBC ecotypes was a generalist pathogen that adapted to different host species. This study provides a new phylogenetic framework for better understanding the evolution of the different ecotypes of the MTBC and guide future work aimed at elucidating the molecular mechanisms underlying host specificity.
P33. Does metabolic cross-feeding correlate with phylogeny in bacteria?

Samir Giri$^{1,2}$ and Christian Kost$^{1,2}$

$^1$University of Osnabrück, Osnabrück, Germany  
$^2$Max Planck Institute for Chemical Ecology, Jena, Germany

Metabolic cross-feeding, wherein one microorganism consumes metabolites provided by another, is a ubiquitous feature of microbial communities. Empirical studies have shown several benefits of cross-feeding such as, (i) promoting diversity in nutrient-poor environments, (ii) degradation of toxic compounds and, (iii) increased growth. By exchanging metabolites, bacteria can save metabolite biosynthesis cost and hence enhance growth as a consortium. Evolutionary theory predicts that organisms should favour related individuals over distant ones when carrying out beneficial tasks. However, cells from the same species are in biochemical conflict with regards to the metabolic processes carried out during growth on a given substrate. Hence there is a high competition for the same essential metabolites with species as compared to different species wherein the metabolic/niche overlap is reduced. Interestingly, it has never been experimentally verified whether and to which extent the phylogenetic relatedness among two bacterial lineages promotes cross-feeding. In order to test whether two more closely related genotypes are more likely to engage in a cross-feeding interaction than two more distantly related strains, I combine different wild-type (Donor) strains with phylogenetically close and distant auxotrophs (recipient). Even though many donors clearly supported the growth of the cocultured auxotrophs, the results of these experiments did not reveal a statistically significant correlation with the phylogeny of donor genotypes tested. The same was true when auxotrophic genotypes were grown in the culture supernatant of donor genotypes. Thus, parameters other than phylogeny like metabolic distance may influence donor and recipient cross-feeding. The results from this study provide unique insights into the rules that govern cross-feeding within microbial communities and may thus help to explain the widespread distribution of such interactions.
P34. Assaying growth traits for understanding antibiotic resistance across environments

Ana-Hermina Ghenu¹, Isabel Gordo¹,*; Claudia Bank¹,*

¹Instituto Gulbenkian de Ciência, Oeiras, Portugal.
*Equal contributors

Antibiotic agents inhibit the growth of bacteria by interfering with essential cell processes, like protein synthesis, DNA replication, and cell wall maintenance. Commonly, mutations that allow bacteria to grow in the presence of an antibiotic are functionally characterized only in terms of the minimum antibiotic concentration necessary to inhibit visible growth of bacteria (MIC). The phenotypic effects of antibiotic resistance (ABR) mutations on bacterial growth traits (e.g. duration of lag phase, density at stationary phase, and maximum growth rate) have largely been ignored. These growth traits are components of bacterial fitness that potentially determine the population dynamics, evolution, and maintenance of ABR mutations in a given environment. Studying bacterial life history traits may explain why ABR can evolve in lab environments with low concentrations or even an absence of antibiotics. Furthermore, human associated bacteria, such as Escherichia coli, encounter a variety of environmental conditions throughout their lifecycle, which may impact ABR evolution.

In order to understand how ABR is maintained in different natural environments, we investigated how bacterial growth traits vary across different environments and genotypes. We characterized bacterial growth traits across batch culture conditions with varied concentrations of the RNA polymerase-inhibiting antibiotic, rifampicin, for susceptible and ABR genotypes grown alone. Using a model of bacterial growth with a finite limiting substrate, we describe the effects of environmental variation on the measured growth traits and predict the outcome of competition at different time points. Our work demonstrates that environmental variables change the response of bacteria to antibiotic treatment.
P35. Hosts can use most pathogens as biological weapons

Francisco Dionisio\textsuperscript{1,2} and João A. Gama\textsuperscript{1,2,3}

\textsuperscript{1}cE3c—Centre for Ecology, Evolution and Environmental Changes, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal
\textsuperscript{2}Instituto Gulbenkian de Ciência, Oeiras, Portugal
\textsuperscript{3}Department of Pharmacy, Faculty of Health Sciences, UiT The Arctic University of Norway, Tromsø, Norway

Pathogens have been used by humans in warfare for centuries. Arguably, the most (in)famous example is the effect of European human pathogens (smallpox and many others) on the Amerindians since the beginning of the XVI century. Recently, the hypothesis that hosts may use pathogenic agents as biological weapons has been demonstrated, but only with viruses that confer immunity to their bacterial hosts upon integration into hosts’ chromosomes (which is not the case, for example, of the variola virus, the causative agent of small-pox). Here, with mathematical models, thousands of computer simulations with randomly generated parameters, and experiments with \textit{Escherichia coli} bacterial cells and their lambdoid viruses, we show that hosts can use most pathogens as biological weapons, not necessarily only temperate viruses that are able to integrate in the host’s chromosome. This represents an important generalization of the hypothesis and may have strong consequences to hosts’ population biology. Moreover, because playing the role of weapons is also advantageous to pathogenic agents, it is possible that their pathogenicity has evolved taking that into account.
Cooperative behaviors are ubiquitous in nature, occurring in many taxa across the tree of life. Bacteria engage in many types of these behaviors, a common one being the sharing of secreted siderophores, public goods used to scavenge iron from the environment. An enormous diversity of siderophore systems exists, differing in the chemical structure of both the siderophore and its cognate receptor. For instance, different fluorescent pseudomonad strains typically produce different variants of their primary siderophore pyoverdine, together with its specific receptor. Here, we test if and how different social environments impact the diversification of this public good and its receptor. We conducted experimental evolution starting with a pyoverdine-producing Pseudomonas aeruginosa strain, subjected to nine different environments varying in their levels of spatial structure and iron limitation. We hypothesize that pyoverdine-receptor diversification could be promoted for two main reasons. First, when both the costs and benefits of cooperation are high, cooperation can be undermined by cheating mutants, which exploit but no longer contribute to siderophore production. Thus, diversification of a cooperative trait could be a powerful way to escape cheating. Alternatively, selection could favor the evolution of modified siderophores with increased efficiency, which would allow evolved strains to gain a competitive advantage over ancestral siderophore producers. Following the experimental evolution, we used a combination of phenotypic screens and sequencing analysis to identify pyoverdine-receptor diversification and its underlying genetic basis.
P37. Quantifying the impact of treatment history on plasmid-mediated resistance evolution in human gut microbiota

Burcu Tepekule¹, Roger Kouyos²,³ and Sebastian Bonhoeffer¹

¹Institute of Integrative Biology, ETH Zurich, Zurich, Switzerland
²Division of Infectious Diseases and Hospital Epidemiology, University Hospital Zurich, Zurich, Switzerland
³Institute of Medical Virology, University of Zurich, Zurich, Switzerland

Prior antibiotic exposure is considered to be a risk factor for treatment failure, and mostly investigated in healthcare settings. Although a positive correlation between antibiotic usage and prevalence of resistance is observed on a population level, risk for an individual cannot be properly quantified due to the diversity in each patients treatment history. In this work, we aim to quantify the impact of personal treatment history on antibiotic resistance evolution by developing an ecological model of the population dynamics of the human gut microbiota. Metagenomic data in the absence and presence of antibiotic treatment is used to parameterize the gut microbiota model. Resistance is assumed to be plasmid-mediated, and transferred via horizontal gene transfer among the commensals in the human gut, where a subpopulation is assumed to be opportunistic pathogens. Using this model, we considered the effect of a number of treatment schemes with uniformly sampled timing and duration, and quantified the chance of treatment failure in case of a resistant commensal infection. Our results suggest that, on average, the probability of treatment failure increases 3.5-fold with every additional treatment. Moreover, the intensity of the treatment plays a crucial role in determining the chance of treatment failure. We observed that doubling the overall treatment duration for the same amount of drug exposure decreases the chance of treatment failure by 99%. Overall, our model highlights the key importance of both the amount and the temporal distribution of previous drug exposure for treatment success at the level of individual patients.
P38. Tribolium castaneum and E. coli Nissle - a novel experimental system for studying probiotics and host-parasite interactions

Ana Korsa\textsuperscript{1}, Ulrich Dobrindt\textsuperscript{2} and Joachim Kurtz\textsuperscript{1}

\textsuperscript{1}Institute for Evolution and Biodiversity, University of Münster, Germany
\textsuperscript{2}Institute for Hygiene, University of Münster, Germany

Experimental studies of the interactions of probiotic bacteria with their hosts and the resulting fitness effects can provide important insight into host-microbiome evolution. \textit{Escherichia coli} Nissle 1917 (EcN) is a nonpathogenic strain of \textit{E. coli} and it is used as a probiotic for treatment of various intestinal disorders in humans. This strain does not carry pathogenic adhesion factors and does not produce any toxins. It is not invasive and not uropathogenic, it is rapidly killed by non-specific defense factors of blood serum and has antagonistic activities against other microorganisms. It also provides some health benefits to a host like anti-inflammatory activity, strengthening of the intestinal epithelial barrier, promoting colonic motility and induction of the gut immune system. For large-scale experimental approaches, the availability of a simple invertebrate model host would be ideal, but is currently not available. We here introduce the red flour beetle \textit{Tribolium castaneum} as a well established model organism for studies of evolution and infections, as an experimental host for the probiotic strain EcN. Our preliminary experiments have shown that EcN can colonize the gut of the beetles when added to the diet. We will analyze effects of EcN on the fitness and life span of the beetles after successful colonization of their gut. Importantly, making use of an established oral infection protocol with the entomopathogen \textit{Bacillus thuringiensis}, we will investigate the evolution of this pathogen in interaction with the probiotic bacterium in the gut. This novel experimental system will also enable to further investigate evolution of EcN and give insight into its behavior in different host organisms.
P39. Diversity and Stability of the Microbiome: an intertwining Eco-Evolutionary Dynamics

M. Amicone\textsuperscript{1}, C. Bank\textsuperscript{1} and I. Gordo\textsuperscript{1}

\textsuperscript{1}Instituto Gulbenkian de Ciência, Portugal

The mammalian gut microbiomes represent complex ecosystems where hundreds of diverse species interact with each other and with the host. Their stability and diversity have been correlated with different host conditions but yet poorly understood. Thus a unified theory to explain the underlying microbial dynamics is needed to understand how the host’s health is maintained and to comprehend and manipulate the microbiome. Contrary to the competitive exclusion principle, the gut shows persistent species-rich communities. This discrepancy, often referred to as the “paradox of the plankton”, has been extensively studied from an ecological perspective. Recent experiments indicate that substantial evolutionary change can occur within species of the microbiota. However not much evolutionary theory has been developed in this regard and little is known about the ecological and evolutionary interplay. The framework of Adaptive Dynamics has provided insights into eco-evolutionary patterns, but in these models mutations are assumed to be rare. Therefore, ecological and evolutionary time scales are separated and processes such as clonal interference, which has been demonstrated to occur in the gut, are ignored. To resolve this gap, we here propose a mathematical framework to study microbiome dynamics in a system in which the two time scales meet and mutant lineages can appear at non-steady states. We extend classical ecological models to study the dynamics ranging from the phenotypic to the population level. We obtain the conditions for coexistence analytically, and study eco-evolutionary trajectories and their properties through numerical simulations. Our work aims at providing a mechanistic explanation of how diversity is affected by evolutionary processes and how these changes in diversity feed back on evolutionary dynamics and shape the fitness landscape during community assembly. It targets microbiota-specific phenomena but can be applied to various types of scenarios.
P40. Epigenetic gene silencing alters the mechanisms and rate of evolutionary adaptation

Dragan Stajic1, Lilia Perfeito1 and Lars Jansen1

1Instituto Gulbenkian de Ciência, Oeiras, Portugal

Natural selection acts upon heritable variation in fitness-related traits. In principle, epigenetic, non-DNA sequence based inheritance can potentially contribute to adaptation. Whether this is the case is largely unknown. To address this, we placed a URA3 reporter gene at different positions within subtelomeric, chromatin-silenced regions in the otherwise isogenic Sacharomyces cerevisiae strains, causing variable degrees of URA3 repression. We placed populations of cells under negative URA3 selection to determine mechanisms of adaptation and the role of heritable gene silencing in this process. We show that populations in which heritable gene silencing occurs have a higher rate of survival and an increased probability of producing genetic mutations. Adaptive mutations appear and fix in populations faster in strains with intermediate level of epigenetic silencing, suggesting that there is an optimal frequency of epigenetic switching that enables genetic assimilation. Genome sequencing of evolved strains indicates that epigenetic silencing of genes under selection allow cells to adapt, not only by inactivation of the URA3 pathway, but also by changing gene expression or by changing the rate of epigenetic switching itself. This work, for the first time, experimentally demonstrates the impact and mechanisms of how epigenetic forms of inheritance shape the evolutionary outcome of a population.
**P41. Multicopy plasmids as drivers of bacterial evolution**

**Jerónimo Rodriguez-Beltran¹, Javier DelaFuente¹, Craig MacLean², Rafael Peña-Miller³ and Alvaro San Millan¹**

¹Ramon y Cajal Institute for Health Research, Madrid, Spain  
²Department of Zoology, University of Oxford, Oxford, UK  
³Centro de Ciencias Genómicas, Universidad Nacional Autónoma de México, Morelos, Mexico.

The role of plasmids in bacterial evolution has been extensively documented, because they include large conjugative plasmids, which are key drivers of horizontal gene transfer. Small, multicopy plasmids (MCP) are ubiquitous in bacteria, yet their role in evolution remains largely unexplored. A salient feature of MCP is that their multicopy nature provides a hotspot of polyploidy in an otherwise haploid bacterial genome. In other words, MCP stockpile tens of copies of the same genes whereas chromosomal genes are held at nearly single copy. In this work, we investigated the fate of beneficial mutations emerging in MCP-encoded genes using a tractable plasmid system and the evolution of blaTEM-1 β-lactamase as experimental model. Our results show that, thanks to polyploidy, plasmids carrying mutated blaTEM-1 versions co-exist in the same cell with plasmids encoding the ancestral allele under heterozygosis during hundreds of bacterial generations. Hence, MCP allow bacteria to transiently store copies of different alleles of the same gene at different proportions, increasing genetic diversity. We demonstrate that genetic diversity, in turn, facilitates the adaption of bacterial populations to a range of constant and fluctuating antibiotic selective pressures. Additionally, theory predicts that in polyploid systems, such as MCP, dominant mutations have more chances to reach fixation than recessive mutations (Haldane’s sieve). We experimentally validate this prediction using common antibiotic resistance mutations, demonstrating that MCP-encoded genes explore different adaptive landscapes than chromosomal genes. In summary, our results demonstrate that MCP promote the evolution of resident genes by increasing standing genetic variation and opening new adaptive avenues to their host bacteria.
P42. The distribution of fitness effects of an antibiotic resistance plasmid

Aida Alonso¹, Javier de la Fuente¹, Jerónimo Rodríguez-Beltrán¹, Ricardo León-Sampedro¹, Marta Hernández¹, Rafael Cantón¹, Maria Morosini¹ and Alvaro San Millán¹

¹Ramón y Cajal Institute of Health Research, Spain

Plasmids are circular DNA molecules that are able to transfer horizontally between bacteria by conjugation, playing a key role in the evolution of antibiotic resistance (AR) in bacteria. Notably, certain associations between AR plasmids and bacteria become particularly successful, creating “superbugs” that disseminate uncontrollably in clinical settings. Despite the potential advantages conferred by plasmids, in the absence of selection for plasmid-encoded traits, they usually impose a fitness cost in the host bacterium, which limits the spread of the plasmid-carrying clone in the population. Interestingly, this cost can be alleviated over time through compensatory mutations. We argue that that the strong associations observed between AR plasmids and clinically important bacteria could be determined by plasmid fitness costs and compensatory evolution. To test this hypothesis, we are building an experimental system based in a prominent example of a clinically relevant AR plasmid-bacterium association, which frequently colonizes the gut of hospitalized patients: Klebsiella pneumoniae and plasmid pOXA-48 (which carries the carbapenemase gene blaOXA-48). Our first goal is to obtain an accurate estimate of the distribution of fitness effects of pOXA-48 in ecologically relevant bacterial hosts. To this end, we have constructed a GFP-labelled version of the plasmid (pOXA-48-GFP) that allows us to perform flow cytometry-based competition assays. Using this tool we are studying pOXA-48 fitness effects both (i) in wild-type pOXA-48-carrying clones from the gut microbiota of hospitalized patients and (ii) in potential new enterobacteria hosts (other enterobacteria clones form the gut of these same patients). Our results will help to understand the “fitness compatibility” between pOXA-48 and enterobacteria clones, and will help to predict which plasmid-bacterium associations are likely to arise in the future.
Prokaryote genome evolution is characterized by the frequent gain of genes through horizontal gene transfer (HGT). When a gene is transferred from one species to another, it arrives in a genomic context different from its original one. Consequently, efficient expression and integration of this new gene can be limited due to mismatch between the transferred gene and the expression machinery and network of the receiving organism. Mismatch in codon usage between the transferred gene and the receiving genome is known to impair efficient gene expression after HGT. Because of the redundant nature of the genetic code, the same amino acid can be coded by different codons, and it is commonly observed that different species use their own preferred subset of codons, giving rise to species-specific codon usage preferences (CUP). When the CUP of a transferred gene strongly diverges from the CUP of the receiving organism, the speed and accuracy of protein translation can be strongly affected. One mechanism to compensate for this is codon usage amelioration, whereby the transferred gene evolves towards a CUP similar to that of the receiving organism. We are investigating codon usage amelioration on a phylogenetic timescale in *Pseudomonas aeruginosa*. To obtain estimates of the timing of HGT events, we use a reconciliation approach to place gene trees of genes from the dispensable genome on a core genome based strain phylogeny. This allows us to investigate the relation between the residence time of a gene within the species and its CUP, which could give us clues about the magnitude of amelioration processes in post-HGT evolution. Furthermore, CUP is known to be related to the gene copy number of tRNA’s matching specific codons. We are therefore also investigating the importance of tRNA gene pool evolution in *P. aeruginosa* as an alternative way of providing post-HGT compensatory evolution.
P44. Local adaptation and thermal specialization by loss of developmental plasticity of the cyanobacterial heterocyst

Scott Miller¹ and Thorsten Bauersachs²

¹Division of Biological Sciences, University of Montana, USA
²Department of Organic Geochemistry, Christian-Albrecht-University, Kiel, Germany

The ‘plasticity-first’ hypothesis proposes that the environmental induction of alternative phenotypes precedes and promotes evolutionary adaptation. This idea that natural selection can improve upon an ancestrally plastic trait is controversial due to limited compelling support from natural populations. Here, we show that adaptation to high temperature (55 °C) by the multicellular cyanobacterium Fischerella thermalis has involved the loss of plasticity for glycolipid composition of the heterocyst, the nitrogen-fixing cell produced by this bacterium in the absence of a preferred nitrogen source. The glycolipid layer of the heterocyst envelope provides a barrier to gas diffusion that protects the oxygen-sensitive nitrogenase complex, and cyanobacteria exhibit temperature-induced changes in glycolipids that modulate heterocyst permeability. Most members of a Yellowstone F. thermalis population exhibit a typical plastic response under heterocyst-forming conditions, yet still grow poorly and exhibit symptoms of nitrogen starvation at high temperature. By contrast, recently evolved high-temperature specialists in the population constitutively over-produce high-temperature glycolipid isomers, which results in a less permeable heterocyst. This maintains heterocyst function at high temperature at the cost of a decrease in performance at lower temperature. Our study illustrates how the adaptive innovation of a novel cell architecture by the genetic assimilation of developmental variation can be a source of population divergence, ecological specialization and biological diversification.
P45. Environmental change rate and dispersal modulate the dynamics of evolutionary rescue in the cyanobacterium Microcystis aeruginosa

Ignacio José Melero-Jiménez1, Elena Martín-Clemente1, Andreas Reul2, Elena Bañares-Españo1, María Jesús García- Sánchez1 and Antonio Flores-Moya1

1Departamento de Biología Vegetal, Facultad de Ciencias, Universidad de Málaga, Málaga, Spain
2Departamento de Ecología y Geología, Facultad de Ciencias, Universidad de Málaga, Málaga, Spain

The rate of biodiversity loss is so high that some scientists affirm that we are being witnesses of the sixth mass extinction. In this situation, it is necessary to ask the following question: can the organisms be able to resist the environmental changes that are taking place? Recent studies have shown the possibility of a population recovering from a stress situation through evolutionary rescue (ER) events. These events depend on the size of the population, its previous history and the rate of the environmental change. The aim of this work is to add more knowledge about the ER dynamics creating stress situations with selective agents (sulphur and salinity) and using the toxic cyanobacterium Microcystis aeruginosa as a model organism. The experiments are based on exposing populations to severe stress and analyze the effect of previous dispersal events and deterioration rates on the occurrence of ER events among populations. The model consists in three different rates of environmental change (constant, slow and fast; under salinity stress we only used the first two treatments) and three dispersal models (isolated, local or global). In total, 324 and 720 populations were exposed to stressful conditions caused by sulphur and salinity, respectively. The results showed that the dispersal modes and the environmental deterioration rates modulated the occurrence of ER events. It has been observed that dispersal favours ER events for both selective agents. Regarding the rate of environmental change, we observed an increase of ER events under constant changes in the populations exposed to sulphur stress. However, ER events were higher when there was previous deterioration (i.e., slow environmental change rate) under saline stress. As a conclusion, ER events in M. aeruginosa depend on selective agent, being the probability higher for salinity than for sulphur. Thus, it could be hypothesized that general conclusions in ER studies must take into account the selective agent.
Our arms race against pathogens is challenged by the spread of bacteria that are resistant to antibiotic treatment. Environmental pollution with human waste carrying antibiotic resistant bacteria and antibiotics in sublethal doses favors the development and persistence of antibiotic resistance in natural habitats and wildlife. Using bulk RNA-sequencing (meta-transcriptomics) we assessed the functionally active resistance genes in the microbiome of wild birds. We found clinically relevant resistance genes in birds from all localities, including in metropolitan regions in Australia and in supposedly pristine habitats in Antarctica. There was a strong link between the carriage of resistant bacteria by birds and the impact of human activities on natural habitats. Notably, ducks living at a wastewater treatment plant harboured the highest resistance gene load, including genes typically associated with multidrug resistance plasmids. Our results suggest that anthropogenic impact poses a selective pressure that is strong enough to maintain antibiotic-resistant bacterial populations in wild animals.
Bacterial adaptation to diet-induced gut dysbiosis

Tanja Dapa¹, Karina B. Xavier¹

¹Instituto Gulbenkian de Ciência, Oeiras, Portugal.

Misbalances in the gastrointestinal microbiota (gut dysbiosis) lead to inflammatory processes with strong consequences for the health of the host. These perturbations can be caused by antibiotics, infections and diverse environmental factors, such as diet. A common reason for gut dysbiosis in Western societies is the nutritional switch from a diet rich in fibres and plant polysaccharides to the so-called Western-style (WS) diet, rich in calories from saturated fat and simple sugars. This switch affects the microbiota composition, causing an increase in Firmicutes and a decrease in Bacteroidetes. The absence of plant polysaccharides from the diet can prompt a prevalent species of Bacteroides, Bacteroides thetaiotaomicron (Bt), to start consuming host mucus glycans. This causes a decrease in the thickness of the mucosal layer, generating further misbalance in the microbiota and higher sensitivity to colonization by pathogens. The ecological consequences of a diet switch are well documented, whereas the evolutionary processes involved are largely unexplored. Understanding both processes is essential to determine the mechanisms underlying the maintenance of the functional diversity in the gut microbiota. The evolutionary adaptation of commensal bacteria during the diet switch can reveal key factors involved in such functional homeostasis. By using a multidisciplinary approach involving experimental evolution, next generation sequencing, phenotyping, metagenomics and metabolomics, we are dissecting the evolutionary adaptation of Bt to the gut under different nutritional regimes, as well as its consequences for the microbial community and the host. Whole-genome sequences (WGS) of populations sequenced after three months evolutionary experiment showed diet-specific adaptation, with different group-specific parallel mutations appearing in mice undergoing different nutritional diets. Thus we plan to unravel key mechanisms underlying the genetic and functional composition of the gut microbiota under different nutritional diets, and provide a theoretical framework for the development of novel strategies to promote and maintain a stable and healthy gut microbiota.
Feeding the growing human population is a pressing issue; crop plants are limited in their ability to grow largely by a lack of bio-available nitrogen in the soil. Large quantities of nitrogenous fertilizer are added: a practice that is costly both financially and environmentally. Leguminous plants enter a symbiotic relationship with nitrogen fixing bacteria, Diazotrophs, where they provide the bacteria with a carbon source in exchange for bio-available nitrogen, allowing growth in nitrogen poor soil. A biochemical model has been built of a Diazotroph that simulates its total nitrogen balance. The goals from analysis of this model are to understand the mathematics governing this symbiosis, determine strategies to maximize nitrogen output of the system within constraints, and inform experiments to replicate this symbiosis in other plants. There is a trade-off between the yield of fixed nitrogen and the growth of the population such that any increase in nitrogen output must be accompanied by a corresponding increase in basal metabolic rate of the bacteria.

Experiments to inform an equation which will simulate the production and use of amino acids in the cell is ongoing in collaboration with other groups. Once finished, this model will represent all major nitrogen pools in the cell and how they interact. Future work will place this biochemical model into its ecological context so that conditions necessary for a evolutionarily stable plant/Diazotroph symbiosis can be explored. This model will provide information regarding which parameters have most impact on stability of the symbiosis by allowing us to test conditions which are not be viable in vivo. The model will be extended to simulate the free living stage of the Diazotroph in its soil dwelling state. It is expected that there are many differences between optimizing the bacteria for symbiosis and free living states, thus this part of the model seeks to mathematically explore the balance of the trade-offs that occur.
Recombination is both an agent of change and of stability in the microbial world. It permits the integration of genetic innovations acquired by horizontal gene transfer, but it is also essential to many mechanisms of DNA repair and thus to the maintenance of genome integrity. At an intermediate time scale between evolution and ecology, integron systems based on site specific recombination also permit a fast response to environmental challenges by shuffling existing gene cassettes. The natural life style of a bacterial taxon and its biotic (social) environment are likely to be key drivers of the evolution and maintenance of recombination functions. Uptake and integration of foreign DNA can only be beneficial in a sufficiently diverse environment, which may on the other hand be a source of biotic stress. The challenges associated with the maintenance of genome integrity strongly depend on the lifestyle of the organism and for example on its effective population size. However, the study of the genetic mechanisms of recombination is usually separated from any consideration regarding the natural environment of a bacteria. In this work, we study recombination in bacterial communities by bringing together three approaches: (1) detection of macro-molecular recombination systems from fully sequenced genomes, (2) estimation of the rates of recombination and horizontal gene transfer from phylogenies of the species for which we have several full genomes, and (3) 16S amplicon sequences from public metagenomic datasets. This integration of metagenomic and genomic data permits a finer understanding of the selective pressures acting on the evolution and maintenance of recombination systems by taking into account the biotic environment.
P50. M1CR0B1AL1Z3R - A user-friendly webserver for the analysis of microbial genomics data

Oren Avram¹, Shir Portugez¹, Dana Rapoport¹ and T. Pupko¹

¹George S. Wise Faculty of Life Sciences, School of Molecular Cell Biology and Biotechnology, Tel Aviv University, Tel Aviv, Israel

The technological advances in the last decade brought with them opportunities for large-scale mining and analysis of pathogenic bacterial species in an unprecedented resolution. Studying large-scale bacterial evolutionary dynamics poses many challenges. These include data-mining steps, such as gene annotation and orthologs detection, sequence alignment and accurate phylogenetic tree reconstruction. These steps as well as additional analysis-specific computations require the execution of multiple bioinformatics tools, making this process cumbersome, tedious and prone to errors due to manual handling. This motivated us to develop an automatic easy-to-use pipeline that integrates basic and advanced analysis components. We are developing a user-friendly software tool (written in Python) called M1CR0B1AL1Z3R. The M1CR0B1AL1Z3R tool is a “one-stop shop” for conducting such microbial genomics data analyses via a simple graphical user interface without any installations, making this processes efficient and easy. An example of features which are implemented in M1CR0B1AL1Z3R include: (1) Extracting orthologous sets from input genomes; (2) Analyzing presence-absence patterns of genes and rates of gene gain and loss events on each branch; (3) Reconstructing a phylogenetic tree based on the extracted orthologous set; (4) Identifying selective sweeps events (Avram et al., in final preparation); (5) Inferring GC content variation among species lineages. This should allow scientists to analyze hundreds of bacterial genomes, with a click of a button.
P51. The percolation of metabolism and bacterial lifestyle and evolvability

German Plata¹ and Dennis Vitkup¹

¹Columbia University in the City of New York, USA

How the structures of biological networks constrain their function and evolutionary plasticity, and how they reflect the lifestyle and phenotypic properties of species, are central questions of systems biology. Graph-based comparisons of biological networks have previously shown structural differences between groups of organisms; however, how these differences correspond to specific molecular and evolutionary mechanisms remains poorly understood. In this work, we used more than a thousand genome-scale bacterial metabolic reconstructions to investigate the interaction between metabolic network structure, function, and bacterial lifestyle and evolvability. Specifically, we describe the occurrence of a percolation transition as a function of bacterial metabolic network size. We found that networks below a certain number of reactions (i.e. a critical genome size) display a sharp decrease in the fraction of metabolites that they can interconvert at steady state. Below this critical size, the nutritional fastidiousness of microbes increases, and metabolic networks become less likely to acquire new phenotypes through the horizontal gain of additional reactions. The transition also coincides with marked differences in the fraction of obligate symbiotic and parasitic lifestyles, the complexity of known growth media, and the probability of metabolic cross-feeding among pairs of bacteria. Additionally, the fraction of genes devoted to transcriptional regulation, signaling, and metabolism drop more rapidly with decreasing genome sizes below the transition. Altogether, our results suggest that adaptation to stable and rich nutrient environments facilitates a physical transition in metabolic network architecture distinguishing bacteria with different lifestyles, regulatory complexity, and phenotypic and evolutionary plasticity.
P52. Interactions between an obligate killer pathogen and the microbiota of its hosts: a metabarcoding approach

Marine Cambon¹,², Jean-Claude Ogier², Sylvie Pagès², Marie Frayssinet², Pierre Lafont¹, Jean-Baptiste Ferdy¹ and Sophie Gaudriault²

¹Lab. Evolution et Diversité Biologique, Toulouse, France
²Lab. Diversité Génomique et Interactions Microorganismes Insectes, Montpellier, France

The understanding of pathogens evolution has for long relied on studying their interactions with their host. However, it is now well known that multicellular species are associated with a lot of microorganisms, their microbiota. This can turn the classical pairwise interaction between a host and a pathogen interactions into a complex, multi-species, host-pathogen-microbiota interaction network. Here, we surveyed how the bacterial community inside an insect host may impact the success of the bacterial insect pathogen *Xenorhabdus*. This pathogen co-infests its host with its nematode vector (*Steinernema* sp.) and has to wait for several days, until the nematode has reproduced, before it can be transmitted. This long incubation time opens the possibility of many interactions with the microbial community present in the host cadaver. In order to survey these interactions, we first developed a method by which we could obtain insect hosts reared in controlled conditions, but which microbiota mimics that of soil sampled in two different natural environment. We then infected these insects with the *Xenorhabdus*-Steinernema pair. We show that *Xenorhabdus* does not dominate the microbial community inside cadavers. Numerous other bacteria originating in part from the host microbiota are still present in the infection several days after host death. Nevertheless, in addition to *Xenorhabdus*, successful infections always harbor a few abundant taxa that were not in the insect but have been brought by the nematode vector. Our results also suggest that the host microbiota seems to determine in part which bacteria are present after the host has been killed. We indeed found that insects raised on soil coming from different locations have infections which composition differ. Overall, our results suggest that despite the large amount of antimicrobial compounds it produces, *Xenorhabdus* interacts with a very diverse microbiota inside the host it has killed.
Chromosomal mutations that confer resistance to antibiotics often involve a cost in the absence of the drug. However, the cost of a resistance can vary when bacteria grow in different resources, reflecting a change in the selective pressures. We studied resistant *Escherichia coli* carrying either a mutation that confers resistance to streptomycin, a mutation that confers resistance to rifampicin or both when competing against the sensitive strain in its natural environment - the mammalian gut. We first performed competitions in germ-free mice, to ask about the cost of single and double resistance in the absence of other commensal species. We found the costs of the two mutations to be additive in this environment, indicating lack of epistasis between the two mutations in the germ-free gut. When performing competitions in mice with microbiota, a wide variation of fitness effects between hosts was found. Remarkably, in some mice the resistant strains showed no cost. Consistent with the observed personalized cost of resistance, the resistant mutants also showed variation across mice for the acquisition of compensatory mutations. Overall, these results suggest that the cost of resistance in vivo can be dependent on the gut microbiota composition.
P54. Insisting on the error: On the emergence and maintenance of high mutation rates within the gut microbiota

Ricardo S. Ramiro¹, Paulo Durão¹, Daniela Güleresi¹, Claudia Bank¹ and Isabel Gordo¹

¹Instituto Gulbenkian de Ciência, Portugal

DNA replication generally occurs with high fidelity because of a sophisticated cellular machinery that avoids or corrects the majority of mutations. However, these correcting mechanisms can themselves be targets of mutation. Thus, mutation rates can vary and be shaped by natural selection. As mutation is the ultimate source of genetic variation, understanding the evolution of mutation rates is key for evolutionary biology. Mutation rate variation is often found in the wild, in populations of virus, bacteria or human cells. However, the dynamics of the emergence and maintenance of mutation rate polymorphism are rarely studied. Recently, while studying *Escherichia coli* adaptation to the mouse gut, we observed the emergence of multiple lineages exhibiting mutation rate polymorphism during colonization by commensal *E. coli* of the mouse gut. The observed polymorphism spans three orders of magnitude, with mutator lineages with strength higher than 1000-fold being maintained over hundreds of generations. We determine the causes of the mutator phenotypes and from their molecular evolution infer that the distribution of fitness effects in the microbiota has a large mode of very slightly deleterious mutations.
P55. Understanding the metabolic processes that shape the adaptation of Escherichia coli to the mouse gut

Miguel Pedro¹, Catarina Pinto², Joana Dias³, João Barroso-Batista⁴, Isabel Gordo¹, Karina Xavier¹

¹Instituto Gulbenkian de Ciência, Oeiras, Portugal
²Faculdade de Ciências, Lisbon, Portugal
³Instituto de Tecnologia Química e Biomédica, Oeiras, Portugal
⁴University of Montreal, Canada

Mammalian gut is colonised by many microbes establishing interactions with their host and the nutritional environment. Studies on their genetics and metabolism brought about the drive and potential to engineer communities to promote health and improve industrial processes. However, structuring artificial communities in a predictable way is underdeveloped. We studied genetic targets and physiological mechanisms in Escherichia coli for adaptation and colonisation and how these are shaped by the metabolic environment/microbiota complexity. Previously, we studied adaptation of E.coli K-12 in mice Germ-free or with polymicrobial community. Whole Genome Sequencing identified potential adaptive targets. Here, we established phenotypic assays to characterise effects of key mutations and metabolomics was performed with 1H-NMR of intestinal contents. We previously identified targets in presence of complex microbiota (Barroso-Batista et al 2014) and here, those selected in absence of microbiota. Genes for sugar alcohol metabolism (gat) was the only target common to both mouse models, evidencing specificity. Facing complex microbiota E.coli targeted use of sugars (srlR, kdgR) and anaerobic respiration (dcuB, focA) (Barroso-Batista et al 2014) whereas alone, we observed instead mutations pointing to increased ability for amino acid use (lrp, dtpB, alaA). Mutations selected correlated with metabolomics: a working hypothesis is that other microbiota members deplete oxygen and breakdown complex sugars limiting E.coli to anaerobically respire simple by-product carbon sources. In the opposing scenario, their absence and amino acid excess are favoured colonisation factors. Through experimental evolution we gained insight on shaping metabolic traits of E.coli through genetic engineering to colonise specific host environments. This work also highlights the versatility of E.coli as potential biotic sensor.
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<td>Brites</td>
<td><a href="mailto:d.brites@swisstph.ch">d.brites@swisstph.ch</a></td>
</tr>
<tr>
<td>Lore</td>
<td>Bulteel</td>
<td><a href="mailto:lore.bulteel@kuleuven.be">lore.bulteel@kuleuven.be</a></td>
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<tr>
<td>María Belén</td>
<td>Carbonetto</td>
<td><a href="mailto:mbcarbonetto@igc.gulbenkian.pt">mbcarbonetto@igc.gulbenkian.pt</a></td>
</tr>
<tr>
<td>Luis</td>
<td>Cardoso</td>
<td><a href="mailto:lcardoso@igc.gulbenkian.pt">lcardoso@igc.gulbenkian.pt</a></td>
</tr>
<tr>
<td>Inês</td>
<td>Carvalho</td>
<td><a href="mailto:icarvalho@igc.gulbenkian.pt">icarvalho@igc.gulbenkian.pt</a></td>
</tr>
<tr>
<td>Chang-Yu</td>
<td>Chang</td>
<td><a href="mailto:chang-yu.chang@yale.edu">chang-yu.chang@yale.edu</a></td>
</tr>
<tr>
<td>Luis Miguel</td>
<td>Chevin</td>
<td><a href="mailto:luis-miguel.chevin@cefe.cnrs.fr">luis-miguel.chevin@cefe.cnrs.fr</a></td>
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<td>Lounes</td>
<td>Chikhi</td>
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<td>Cobey</td>
<td><a href="mailto:cobey@uchicago.edu">cobey@uchicago.edu</a></td>
</tr>
<tr>
<td>Sinead</td>
<td>Collins</td>
<td><a href="mailto:s.collins@ed.ac.uk">s.collins@ed.ac.uk</a></td>
</tr>
<tr>
<td>Tanja</td>
<td>Dapa</td>
<td><a href="mailto:tdapa@igc.gulbenkian.pt">tdapa@igc.gulbenkian.pt</a></td>
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<tr>
<td>Javier</td>
<td>de la Fuente</td>
<td><a href="mailto:fuentehidalgo91@gmail.com">fuentehidalgo91@gmail.com</a></td>
</tr>
<tr>
<td>Tatiana</td>
<td>Dimitriu</td>
<td><a href="mailto:t.dimitriu@exeter.ac.uk">t.dimitriu@exeter.ac.uk</a></td>
</tr>
<tr>
<td>Francisco</td>
<td>Dionísio</td>
<td><a href="mailto:francisco.dionisio@gmail.com">francisco.dionisio@gmail.com</a></td>
</tr>
<tr>
<td>Paulo</td>
<td>Durão</td>
<td><a href="mailto:pdurao@igc.gulbenkian.pt">pdurao@igc.gulbenkian.pt</a></td>
</tr>
<tr>
<td>Wael</td>
<td>Elhenawy</td>
<td><a href="mailto:elhenaww@mcmaster.ca">elhenaww@mcmaster.ca</a></td>
</tr>
<tr>
<td>Sylvie</td>
<td>Estrela</td>
<td><a href="mailto:sylvie.estrela@yale.edu">sylvie.estrela@yale.edu</a></td>
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<tr>
<td>Alexandre</td>
<td>Figueiredo</td>
<td><a href="mailto:alexandre.figueiredo@botinst.uzh.ch">alexandre.figueiredo@botinst.uzh.ch</a></td>
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<tr>
<td>Antonio</td>
<td>Flores-Moya</td>
<td><a href="mailto:floresa@uma.es">floresa@uma.es</a></td>
</tr>
<tr>
<td>Inês</td>
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<td><a href="mailto:irfragata@gmail.com">irfragata@gmail.com</a></td>
</tr>
<tr>
<td>Nelson</td>
<td>Frazão</td>
<td><a href="mailto:nfrazao@igc.gulbenkian.pt">nfrazao@igc.gulbenkian.pt</a></td>
</tr>
<tr>
<td>Antoine</td>
<td>Frenoy</td>
<td><a href="mailto:antoine.frenoy@pasteur.fr">antoine.frenoy@pasteur.fr</a></td>
</tr>
<tr>
<td>Paolina</td>
<td>Garbeva</td>
<td><a href="mailto:p.garbeva@nioo.knaw.nl">p.garbeva@nioo.knaw.nl</a></td>
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<tr>
<td>Marc</td>
<td>Garcia-Garcera</td>
<td>marc.garcia@<a href="mailto:garcera@unil.ch">garcera@unil.ch</a></td>
</tr>
<tr>
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<td>Garud</td>
<td><a href="mailto:Nandita.garud@gmail.com">Nandita.garud@gmail.com</a></td>
</tr>
<tr>
<td>Ana-Hermina</td>
<td>Ghenu</td>
<td><a href="mailto:aghenu@igc.gulbenkian.pt">aghenu@igc.gulbenkian.pt</a></td>
</tr>
<tr>
<td>Samir</td>
<td>Giri</td>
<td><a href="mailto:samirgiri2809@gmail.com">samirgiri2809@gmail.com</a></td>
</tr>
<tr>
<td>Isabel</td>
<td>Gordo</td>
<td><a href="mailto:igordo@igc.gulbenkian.pt">igordo@igc.gulbenkian.pt</a></td>
</tr>
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<td>Florien</td>
<td>Gorter</td>
<td><a href="mailto:floriengorter@gmail.com">floriengorter@gmail.com</a></td>
</tr>
<tr>
<td>Daniela</td>
<td>Guleresi</td>
<td><a href="mailto:dzwerschke@igc.gulbenkian.pt">dzwerschke@igc.gulbenkian.pt</a></td>
</tr>
<tr>
<td>David</td>
<td>Guttman</td>
<td><a href="mailto:david.guttman@utoronto.ca">david.guttman@utoronto.ca</a></td>
</tr>
<tr>
<td>Andrew</td>
<td>Hendry</td>
<td><a href="mailto:andrew.hendry@mcgill.ca">andrew.hendry@mcgill.ca</a></td>
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<tr>
<td>Shira</td>
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</tr>
<tr>
<td>Ana</td>
<td>Korsa</td>
<td><a href="mailto:korsa@uni-muenster.de">korsa@uni-muenster.de</a></td>
</tr>
<tr>
<td>Yunyoung</td>
<td>Kwak</td>
<td><a href="mailto:yun@knu.ac.kr">yun@knu.ac.kr</a></td>
</tr>
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<td>Signe</td>
<td>Lagercrantz</td>
<td><a href="mailto:signe.lagercrantz@linacre.ox.ac.uk">signe.lagercrantz@linacre.ox.ac.uk</a></td>
</tr>
<tr>
<td>Jake</td>
<td>Law</td>
<td><a href="mailto:jl1227@york.ac.uk">jl1227@york.ac.uk</a></td>
</tr>
<tr>
<td>Ana Sofia</td>
<td>Lindeza</td>
<td><a href="mailto:lindeza@uni-muenster.de">lindeza@uni-muenster.de</a></td>
</tr>
<tr>
<td>Richard</td>
<td>Lindsay</td>
<td><a href="mailto:R.J.Lindsay@exeter.ac.uk">R.J.Lindsay@exeter.ac.uk</a></td>
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<tr>
<td>José</td>
<td>Lourenço</td>
<td><a href="mailto:jose.lourenco@zoo.ox.ac.uk">jose.lourenco@zoo.ox.ac.uk</a></td>
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<tr>
<td>Marta</td>
<td>Mansos Lourenço</td>
<td><a href="mailto:marta.mansos-lourenco@pasteur.fr">marta.mansos-lourenco@pasteur.fr</a></td>
</tr>
<tr>
<td>Adam</td>
<td>Marques</td>
<td><a href="mailto:ajmarques@igc.gulbenkian.pt">ajmarques@igc.gulbenkian.pt</a></td>
</tr>
<tr>
<td>Loïc</td>
<td>Marrec</td>
<td><a href="mailto:loic.marrec@upmc.fr">loic.marrec@upmc.fr</a></td>
</tr>
<tr>
<td>Maria Elena</td>
<td>Martino</td>
<td><a href="mailto:mariaelena.martino@unipd.it">mariaelena.martino@unipd.it</a></td>
</tr>
<tr>
<td>Leanne</td>
<td>Massie</td>
<td><a href="mailto:leanne.massie15@imperial.ac.uk">leanne.massie15@imperial.ac.uk</a></td>
</tr>
<tr>
<td>Andrew</td>
<td>Matthews</td>
<td><a href="mailto:a.matthews3@exeter.ac.uk">a.matthews3@exeter.ac.uk</a></td>
</tr>
<tr>
<td>Ignacio José</td>
<td>Melero Jiménez</td>
<td><a href="mailto:imelero@uma.es">imelero@uma.es</a></td>
</tr>
<tr>
<td>Ana Portugal</td>
<td>Melo</td>
<td><a href="mailto:ammelo@igc.gulbenkian.pt">ammelo@igc.gulbenkian.pt</a></td>
</tr>
<tr>
<td>Nicole</td>
<td>Mideo</td>
<td><a href="mailto:nicole.mideo@utoronto.ca">nicole.mideo@utoronto.ca</a></td>
</tr>
<tr>
<td>Scott</td>
<td>Miller</td>
<td><a href="mailto:scott.miller@umontana.edu">scott.miller@umontana.edu</a></td>
</tr>
<tr>
<td>Sara</td>
<td>Mitri</td>
<td><a href="mailto:sara.mitri@unil.ch">sara.mitri@unil.ch</a></td>
</tr>
<tr>
<td>Sandra Milena</td>
<td>Montaño</td>
<td><a href="mailto:smmontanos@unal.edu.co">smmontanos@unal.edu.co</a></td>
</tr>
<tr>
<td>Leonilde M.</td>
<td>Moreira</td>
<td><a href="mailto:lmoreira@tecnico.ulisboa.pt">lmoreira@tecnico.ulisboa.pt</a></td>
</tr>
<tr>
<td>Clara</td>
<td>Moreno-Fenoll</td>
<td><a href="mailto:cmorenofenoll@evolbio.mpg.de">cmorenofenoll@evolbio.mpg.de</a></td>
</tr>
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<td>Barbara</td>
<td>Parreira</td>
<td><a href="mailto:bparreira@igc.gulbenkian.pt">bparreira@igc.gulbenkian.pt</a></td>
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<td>Paupério</td>
<td><a href="mailto:frpauperio@gmail.com">frpauperio@gmail.com</a></td>
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<td>Pedro</td>
<td><a href="mailto:mfpedro@igc.gulbenkian.pt">mfpedro@igc.gulbenkian.pt</a></td>
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<td>Pinto</td>
<td><a href="mailto:frpinto@fc.ul.pt">frpinto@fc.ul.pt</a></td>
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<tr>
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<td>Plata</td>
<td><a href="mailto:gap2118@columbia.edu">gap2118@columbia.edu</a></td>
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<td>Pilar</td>
<td>Puentes-Tellez</td>
<td><a href="mailto:p.e.puentestellez@uu.nl">p.e.puentestellez@uu.nl</a></td>
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<td>Paul</td>
<td>Rainey</td>
<td><a href="mailto:rainey@evolbio.mpg.de">rainey@evolbio.mpg.de</a></td>
</tr>
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<td>Ricardo</td>
<td>Ramiro</td>
<td><a href="mailto:rramiro@igc.gulbenkian.pt">rramiro@igc.gulbenkian.pt</a></td>
</tr>
<tr>
<td>María</td>
<td>Rebolleda-Gómez</td>
<td><a href="mailto:mrebolleda@pitt.edu">mrebolleda@pitt.edu</a></td>
</tr>
<tr>
<td>Artur</td>
<td>Rego-Costa</td>
<td><a href="mailto:rego-costa@g.harvard.edu">rego-costa@g.harvard.edu</a></td>
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<td><a href="mailto:olaya.rendueles-garcia@pasteur.fr">olaya.rendueles-garcia@pasteur.fr</a></td>
</tr>
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<td>Rescan</td>
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<td>Alejandra</td>
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<td><a href="mailto:alejandra.rodriguez@eawag.ch">alejandra.rodriguez@eawag.ch</a></td>
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<td>Jeronimo</td>
<td>Rodriguez-Beltran</td>
<td><a href="mailto:jeronimo.rodriguez.beltran@gmail.com">jeronimo.rodriguez.beltran@gmail.com</a></td>
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<tr>
<td>Vanessa</td>
<td>Rossetto Marcelino</td>
<td><a href="mailto:vanessa.marcelino@sydney.edu.au">vanessa.marcelino@sydney.edu.au</a></td>
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<tr>
<td>Alicia</td>
<td>Sanchez-Gorostiaga</td>
<td><a href="mailto:alicia.sanchezgorostiaga@yale.edu">alicia.sanchezgorostiaga@yale.edu</a></td>
</tr>
<tr>
<td>Oloketuyi</td>
<td>Sandra Folarin</td>
<td><a href="mailto:oloketuyisandra@gmail.com">oloketuyisandra@gmail.com</a></td>
</tr>
<tr>
<td>Guillem</td>
<td>Santamaria Aguilar</td>
<td><a href="mailto:guillem.santamaria.aguilar@gmail.com">guillem.santamaria.aguilar@gmail.com</a></td>
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<td>Diogo</td>
<td>Santos</td>
<td><a href="mailto:dsantos@igc.gulbenkian.pt">dsantos@igc.gulbenkian.pt</a></td>
</tr>
<tr>
<td>Kaitlin</td>
<td>Schaal</td>
<td><a href="mailto:kaitlin.schaal@env.ethz.ch">kaitlin.schaal@env.ethz.ch</a></td>
</tr>
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<td>Thomas</td>
<td>Scheuerm</td>
<td><a href="mailto:tscheuer@ic.ac.uk">tscheuer@ic.ac.uk</a></td>
</tr>
<tr>
<td>Ernesto Alejandro</td>
<td>Segredo-Otero</td>
<td><a href="mailto:eraseo@uv.es">eraseo@uv.es</a></td>
</tr>
<tr>
<td>Richard</td>
<td>Sheppard</td>
<td><a href="mailto:rjs11@ic.ac.uk">rjs11@ic.ac.uk</a></td>
</tr>
<tr>
<td>Jorge</td>
<td>Sousa</td>
<td><a href="mailto:jorge-andre.sousa@pasteur.fr">jorge-andre.sousa@pasteur.fr</a></td>
</tr>
<tr>
<td>Ana Margarida</td>
<td>Sousa</td>
<td><a href="mailto:amsousa2@gmail.com">amsousa2@gmail.com</a></td>
</tr>
<tr>
<td>Dragan</td>
<td>Stajic</td>
<td><a href="mailto:dstajic@igc.gulbenkian.pt">dstajic@igc.gulbenkian.pt</a></td>
</tr>
<tr>
<td>Rike</td>
<td>Stelkens</td>
<td><a href="mailto:rike.stelkens@zoologi.su.se">rike.stelkens@zoologi.su.se</a></td>
</tr>
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<td>Élio</td>
<td>Sucena</td>
<td><a href="mailto:esucena@igc.gulbenkian.pt">esucena@igc.gulbenkian.pt</a></td>
</tr>
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<td>Anshuman</td>
<td>Swain</td>
<td><a href="mailto:answain@terpmail.umd.edu">answain@terpmail.umd.edu</a></td>
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<td><a href="mailto:burcu.tepekule@env.ethz.ch">burcu.tepekule@env.ethz.ch</a></td>
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<tr>
<td>Monica</td>
<td>Ticlla</td>
<td><a href="mailto:monicaroxana.ticllaaccenhua@unibas.ch">monicaroxana.ticllaaccenhua@unibas.ch</a></td>
</tr>
<tr>
<td>Daniel</td>
<td>Unterweger</td>
<td><a href="mailto:daniel.unterweger@zoo.ox.ac.uk">daniel.unterweger@zoo.ox.ac.uk</a></td>
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<td>Katrina</td>
<td>van Raay</td>
<td><a href="mailto:kvanraay@uw.edu">kvanraay@uw.edu</a></td>
</tr>
<tr>
<td>Anne-Marie</td>
<td>Veenstra-Skirl</td>
<td><a href="mailto:a.m.veenstra-skirl@rug.nl">a.m.veenstra-skirl@rug.nl</a></td>
</tr>
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<td>Vitkup</td>
<td><a href="mailto:dvitkup@yahoo.com">dvitkup@yahoo.com</a></td>
</tr>
<tr>
<td>Jake</td>
<td>Weissman</td>
<td><a href="mailto:jw4336@terpmail.umd.edu">jw4336@terpmail.umd.edu</a></td>
</tr>
<tr>
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<td>Xue</td>
<td><a href="mailto:ksxue@uw.edu">ksxue@uw.edu</a></td>
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