Rapid Evolutionary Adaptation
DFG SPP1819

Rapid evolutionary adaptation
Potential and constraints

DFG SPP1819 International Meeting

17th – 19th of October 2018
Wissenschaftszentrum Kiel, Germany

#SPP1819Kiel

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Prof. Dr. Hinrich Schulenburg
Dr. Olivia Roth

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C A U
Christian-Albrechts-Universität zu Kiel

KIEL EVOLUTION CENTER

UNIVERSITÄT HOHENHEIM
1818

META ORIGIN AND FUNCTION OF ORGANISMS
COLLABORATIVE RESEARCH CENTRE 1182

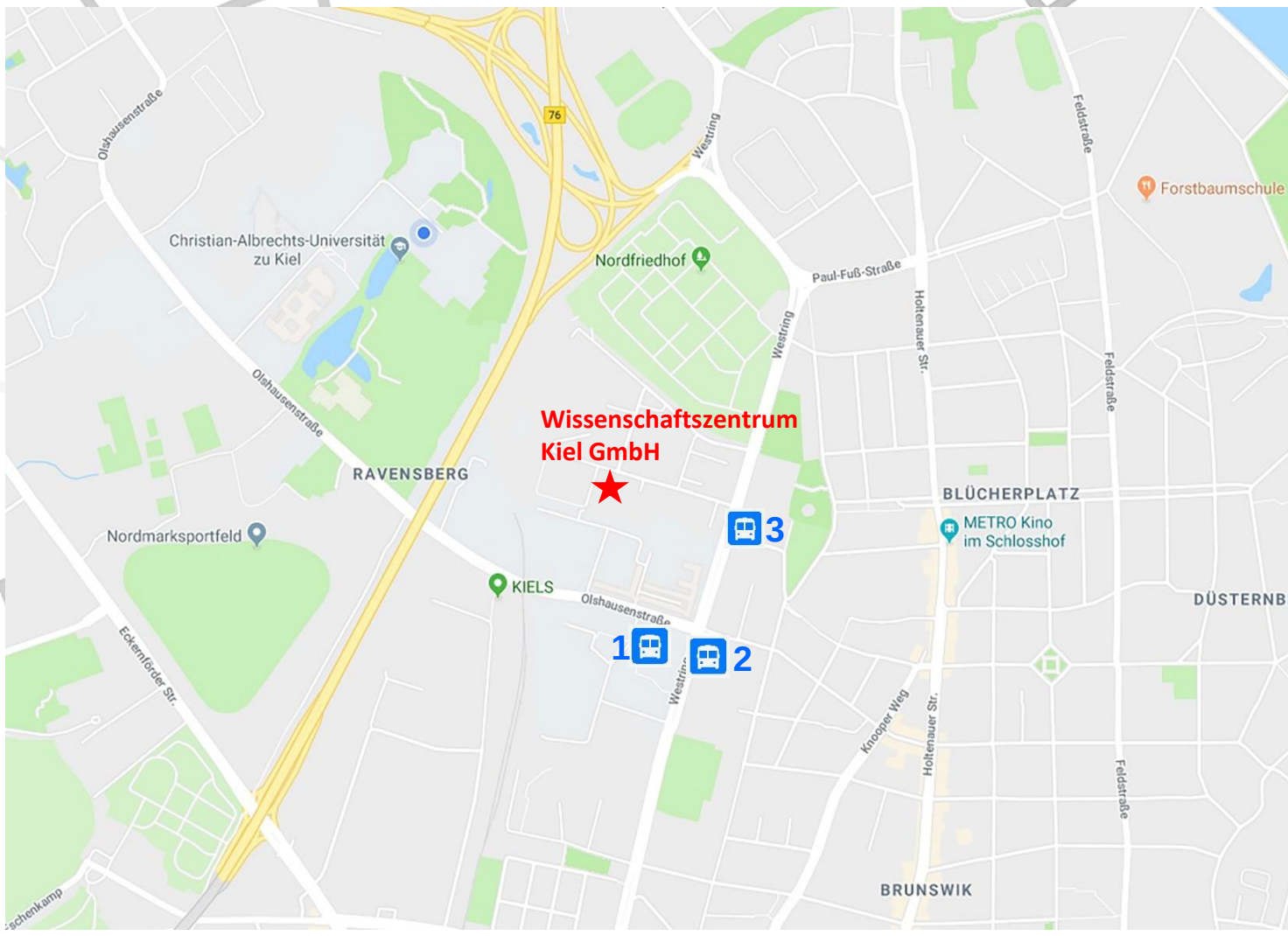
Max-Planck-Institut für Evolutionsbiologie, Plön

Sponsor:

DFG Deutsche Forschungsgemeinschaft

Meeting venue

★ Wissenschaftszentrum Kiel
Fraunhoferstraße 13
24118 Kiel



How to get there

1. Bus lines to “Kiel Universität” 6 50 60S 61 62 81

2. Bus lines to “Kiel Universität/Westring” 6 81 91 92

3. Bus lines to Kiel “Rankestraße” 6 91 92

For more information on how to get around Kiel there is an online bus schedule: https://netzplan-kiel.de/index.php/de/netzplan

What to do in Kiel ?

German Submarine U-995 and War Memorial, Laboe

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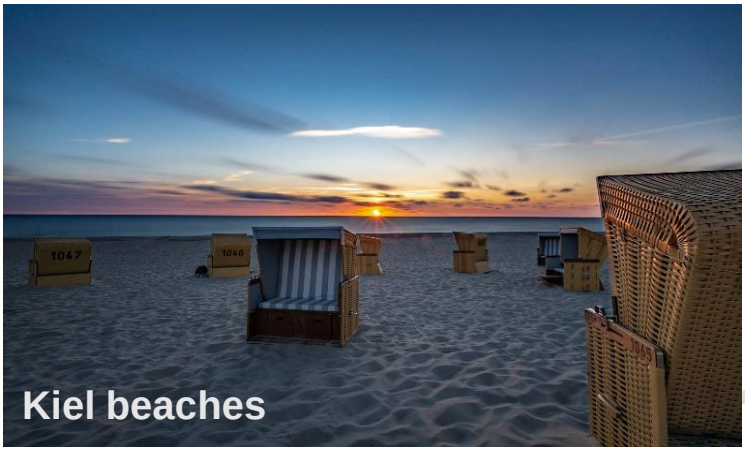

Kiel beaches


City Hall Kiel

Maritime Museum


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Zoological Museum

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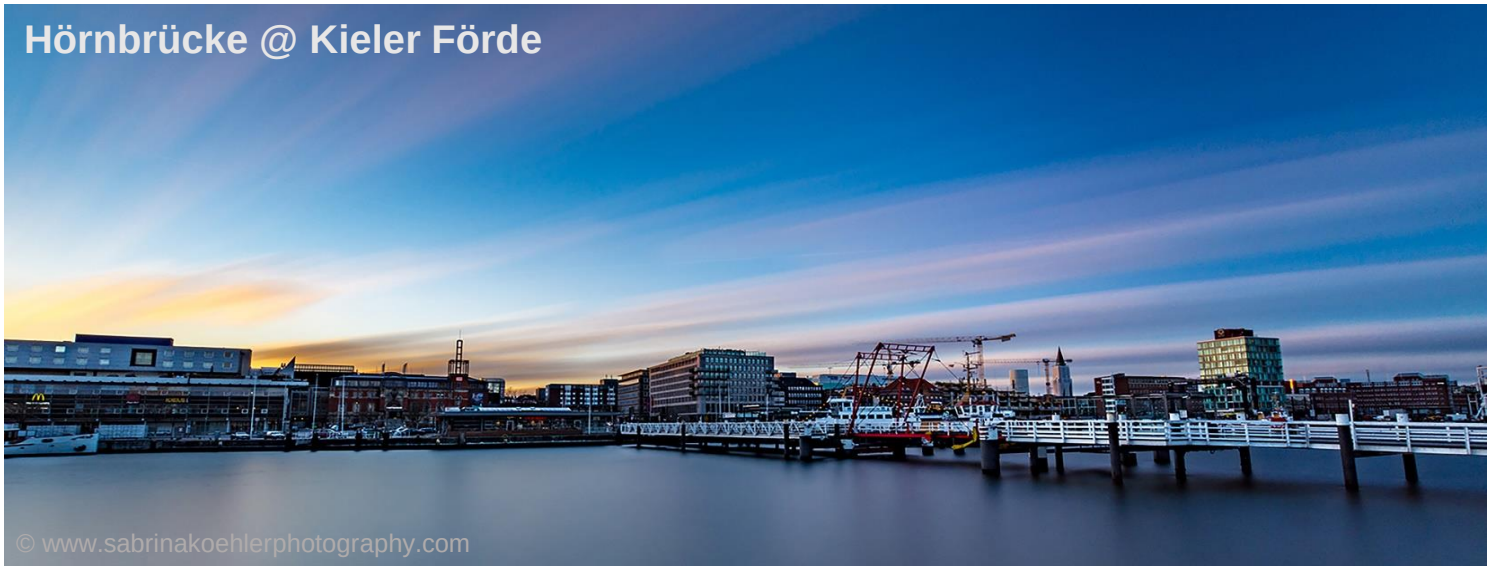
Hörnbrücke @ Kieler Förde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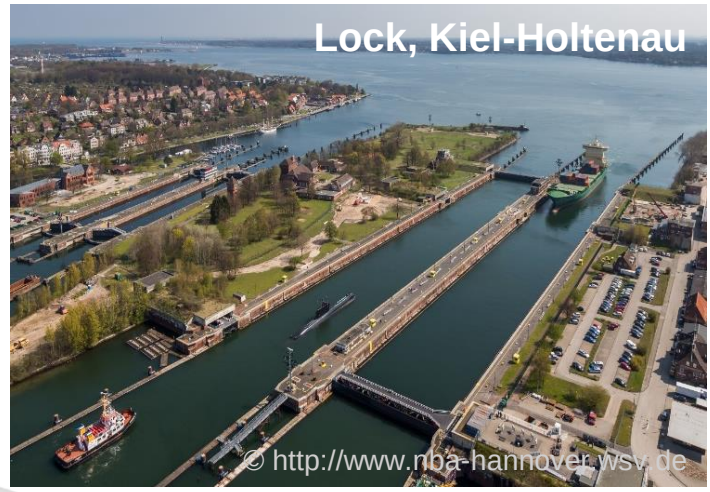
Kiellinie


Art Museum „Kunsthalle“ Kiel
http://www.kunsthalle-kiel.de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SCHILKSEE
Marine - Ehrenmal
Submarine U-995
Laboe
PRIES
FRIEDRICHSORT
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Schönkirchen
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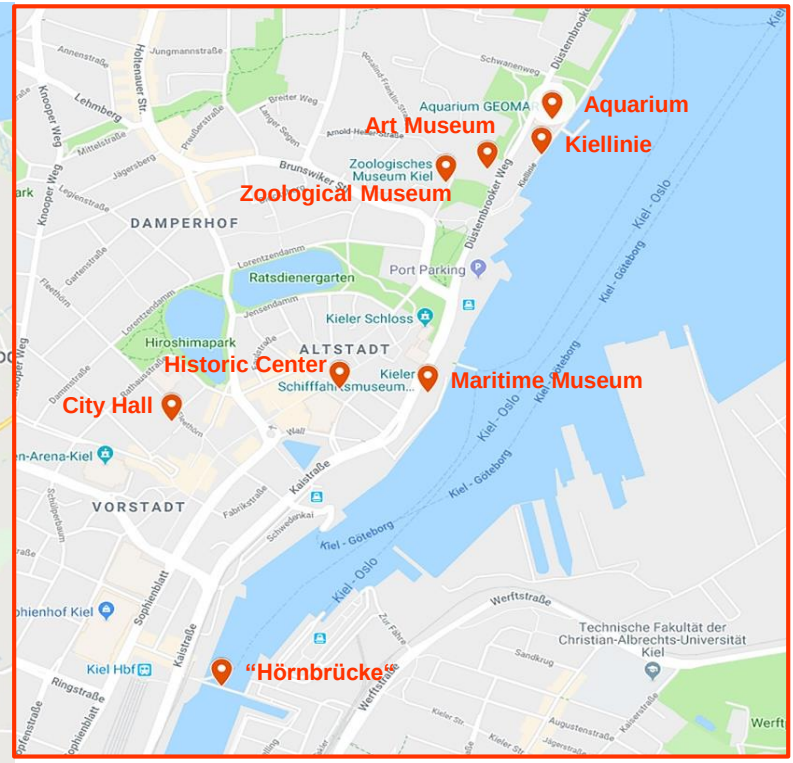
Aquarium GEOMA
Aquarium
Art Museum
Kiellinie
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Technische Fakultät der Christian-Albrechts-Universität Kiel
Kiel - Oslo
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Scientific Program

From 12:00 - Registration and lunch

Wednesday 17th

13:00 Welcome

13:10 *Invited Speaker* Michael Brockhurst (Sheffield University, UK)

14:00 *Break*

14:10 - 17:00 Experimental evolution in studies of rapid evolution

Chair and discussion leader: Tal Dagan

14:10 Introduction by discussion leader

14:15 Niels Mahrt (PI Schulenburg)

14:35 Cas Retel (PIs Becks/Feulner)

14:55 *Coffee break*

15:20 Tanita Wein (PI Dagan)

15:40 Joachim Kurtz (PI Kurtz)

16:00 Henry Göhlich (PIs Wendling/Roth)

16:20 Discussion

16:50 -18:30 Poster Session

18:30 Dinner

Thursday 18th

8:45 - 12:30 Theoretical approaches to study rapid evolution

Chair and discussion leader: Wolfgang Stephan

8:45 Introduction by discussion leader

8:50 *Invited Speaker* Guy Sella (Columbia University, NY)

9:40 Bjarki Eldon (PI Stephan)

10:00 Ana Filipa da Silva Moutinho (MPI Plön)

10:20 *Coffee break*

10:50 *Invited Speaker* Claudia Bank (Gulbenkian Institute, Portugal)

11:40 Hanna Märkle (PI Tellier)

12:00 Discussion

12:30 Lunch

14:00 - 16:30 Plant pathogens as model organisms in studies of rapid evolution

Chair: Olivia Roth

14:00 Janina von Dahlen (PI Rose)

14:20 Emad Albarouki (PI Schirawski)

14:40 Stefan Kusch (PI Panstruga)

15:00 *Coffee break*

15:30 Karl Schmid (PI Schmid)

15:50 Katharina Bersching (PIs Tenzer/Jacob)

16:10 Zoran Nikoloski (PIs Laitinen/Nikoloski)

19:00 Conference Dinner – Forstbaumschule, Kiel

Friday 19th

8:45 - 12:40 Inference of adaptive evolution from population data

Chair and discussion leader: Aurélien Tellier

8:45 Introduction by discussion leader

8:50 *Invited Speaker* Maud Tenaillon (Le Moulon, France)

9:40 Alice Feurtey (PI Stukenbrock)

10:00 Ralf Schneider (PI Meyer)

10:20 *Coffee break*

10:50 Binia De Cahsan (PI Tiedemann)

11:10 Ute Krämer (PI Krämer)

11:30 Jaanus Suurväli (PIs Leptin/Wiehe)

11:50 Amanda Glaser-Schmitt (PI Parsch)

12:10 Discussion

12:40 Final words (Karl Schmid) and lunch

14:00 – 15:00 Mentoring session for female scientists

Discussion leader: Ute Krämer

14:00 Introduction by discussion leader

14:05 Open discussion

14:30 *Networking and coffee break*

14:55 Conclusion

The ecology and evolution of horizontal gene transfer in microbial communities

***Michael Brockhurst**, Sheffield University, UK*

Horizontal gene transfer (HGT) accelerates evolution through the sharing of functional traits between lineages, such as antibiotic resistance. Bacterial comparative genomics reveals extensive horizontal gene transfer facilitated by plasmids but little is known about how the ecology of microbial communities and their environments affects HGT dynamics. Using simple bacterial communities in potting soil microcosms we show that infectious between species transmission is key to the maintenance of costly plasmids in bacterial communities, and that rates of between-species gene mobilization are higher in these environments compared to environments where plasmids are beneficial.

A population genetic interpretation of GWAS findings for human quantitative traits

***Guy Sella**, Columbia University, NY*

One of the central goals of evolutionary genetics is to understand the processes that give rise to phenotypic variation in humans and other taxa. Genome-wide association studies (GWASs) in humans provide an unprecedented opportunity in that regard, revealing the genetic basis of variation in numerous traits. However, exploiting this opportunity requires models that relate genetic and population genetic processes with the discoveries emerging from GWASs. We present such a model and show that it can help explain the results of GWASs for height and body mass index. More generally, our results offer a simple interpretation of the findings emerging from GWASs and suggest how they relate to the evolutionary and genetic forces that give rise to phenotypic variation.

Fitness landscapes and epistasis in adaptation and speciation

***Claudia Bank**, Gulbenkian Institute, Portugal*

Fitness landscapes, which map genotypes or phenotypes to fitness, have developed from a theoretical metaphor into a popular subject of experimental study. The quantification of the shape of fitness landscapes carries the promise to inform us about the nature of adaptation, the probability of speciation, and the predictability of evolution; yet, the true dimensionality of fitness landscapes is so immense that even large data sets can only cover a small area of the total sequence or phenotype space. Thus, the question emerges whether we can use theoretical or statistical approaches to extrapolate from the observed landscape to the surrounding area or even the whole organism's fitness landscape, and what a given experimental landscape can teach us about evolution in natural populations. In the first part of my talk, I will discuss these questions under consideration of experimental data sets, and highlight the challenges when trying to bridge our theoretical and empirical knowledge of fitness landscapes. In the second part of my talk, I will discuss in which conditions a rugged fitness landscape, created through Dobzhansky-Muller incompatibilities, can promote hybrid speciation.

Linking genomic footprints of selection and phenotypic variation in teosintes

***Maud Tenaillon**, Le Moulon, France*

We combined reverse ecology and association mapping to mine the determinants of local adaptation in teosintes, the closest wild relatives of maize. First, we applied methods based on allelic differentiation and correlation with environmental variables to detect signals of past selection in teosinte populations growing along two altitudinal gradients in Mexico. Second, we selected a subset of candidate SNPs to test the association between genotypic and phenotypic variation at 18 traits measured in two common gardens. Phenotypic variation revealed the phenotypic components of an altitudinal “syndrome”, with traits involved in spatially-varying selection. A large majority of candidate SNPs displayed an association with at least one of the traits and shared associated SNPs between traits were common. Interestingly, the higher the correlation between traits the higher the number of shared SNPs, suggesting that phenotypic correlations are in part driven by common genetic determinants.

Periodic bottlenecks in experimental antibiotic resistance evolution

Niels Mahrt, *Camilo Barbosa, Gunther Jansen, Hinrich Schulenburg*

Population bottlenecks frequently occur in nature and play a significant role in the evolutionary history of bacterial populations. Bottlenecks are defined as a strong reduction of population size that can lower the population‘s genetic diversity drastically. After surviving a narrow bottleneck, future adaptation is more likely influenced by selective sweeps and periodic selection, rendering the adaptive paths less predictable. In contrast, higher degrees of parallel evolution and clonal interference are expected in case of a wider bottleneck, as higher genetic diversity is likely maintained. By performing serial transfer evolution experiments, I test the influence of bottleneck size on the adaptability of the pathogenic bacterium *Pseudomonas aeruginosa* PA14 to different levels of sub-inhibitory concentrations (IC) of antibiotics.

While at high IC the highest resistance evolves under large transfer sizes, the highest resistance in low IC populations emerges when the transfer size is small. These different dynamics are reflected by mutational patterns in the evolving bacterial genomes. While the total number of mutations per population for each treatment depends on the treatment drug, the diversity of the most frequent mutations at the final growth season is higher for small transfer sizes than for large transfer sizes. Surprisingly, only few mutations are completely fixed by the final season. This may indicate that clonal interference of de-novo mutations occurs regularly at sub-inhibitory drug concentrations. Overall, my dataset suggests that bottlenecks in combination with antibiotic-induced selective pressure can be a key determinant of resistance evolution and can shape genetic diversity within and also between populations.

Temporal dynamics of molecular evolution in rapidly coevolving host-virus populations

Cas Retel, *Philine Feulner, Lutz Becks*

Dramatic population size fluctuations are often observed in co-evolving systems, but how these affect genomic variation and evolution at the molecular level is less clear. Understanding how demographic processes, selection and their interplay shape genomic variation is particularly interesting when adaptation of one species affects population size of the other and vice versa. We use an experimental setup where host (*Chlorella*) and virus (*Chlorovirus*) populations reciprocally evolve resistance and virulence over 90 days. Previous research has shown that demographic and ecological dynamics closely match across multiple replications of co-evolution. Starting from clonal populations, dynamics initially reflect an "arms race" and later change into a state where both species co-occur at stable densities, but phenotype abundances within populations fluctuate. We now present temporally resolved genomic data for both species. Whole-genome data for 10 time points allowed us to identify and track alleles with frequency changes driven by selection. Using data from three replicates, we identify selective sweeps in both populations and describe the corresponding ecological effects. We further reveal consistent differences in the dynamics of molecular changes between the species, parallel genetic changes and evidence for the importance of genomic background. Hence, both species-specific genome characteristics as well as between-species eco-evolutionary dynamics shape the dynamics of molecular evolution during co-evolution.

An experimental quantification of the association between population size and the fixation of adaptive mutations in bacterial populations

Tanita Wein, *Tal Dagan*

Population size dynamics due to changes in the environmental conditions are frequent in nature. The size of the population is a known determinant of genetic diversity and the rate of genetic adaptation. Population genetics models predict that the frequency of fixed adaptive mutations over time is positively correlated with the population size. However, an empirical validation of the model implications for bacterial population is currently lacking. Here, we quantify experimentally the impact of population size on rapid adaptive evolution of bacterial populations. We evolved large (L) and small (S) *Escherichia coli* populations having a 10-fold difference in the population size (i.e., *NL/NS* = 10/1) during adaptation to cold temperature (20°C). The ratio of fixed adaptive mutations in the two populations is expected to be proportional to their size ratio (i.e., *KL/KS* = 10/1). After 800 generations, we quantified all evolved variants and their allele frequency in the populations. Our results reveal that the ratio
of the fixed adaptive mutations in the evolved populations is *KL/KS* = 2:1. Our results thus demonstrate that rapid adaptation indeed associated with the population size. Nonetheless, the empirical estimate of *KL/KS* does not reach the theoretical expectation. Our study demonstrates that the theoretical prediction overestimate the impact of population size on the rate of adaptation of bacterial populations.

Evolution of immune priming as a form of phenotypic plasticity

Joachim Kurtz

Phenotypic plasticity and host-parasite interactions are thought to be major drivers of fast evolutionary processes. My project aims at elucidating conditions for rapid adaptation by investigating a prime example of phenotypic plasticity, the invertebrate immune memory (i.e. 'priming'). We are using experimental evolution in the red flour beetle *Tribolium castaneum* to investigate under which conditions the degree of phenotypic plasticity (here, priming) constrains evolutionary adaptation or may result in genetic assimilation. As the long-term evolution experiments are currently still ongoing, I will also present relevant data on transgenerational effects of priming and the evolution of priming specificity.

Ocean salinity drop triggers lytic infection of temperate phages

Henry Goehlich, *Oliva Roth, Carolin C. Wendling*

Whereas lytic phages kill their bacterial host, temperate phages can be friend and enemy due to their dual life history. They integrate as prophages into the bacterial genome and provide the bacterial host with beneficial genes enhancing its fitness. However, upon a shift of environmental conditions towards unfavorable for the bacterial host, they switch back to the lytic cycle and thereby kill their host. The Baltic Sea is predicted to decrease in salinity by 5 PSU at the end of this century. We aimed to investigate the consequence of this ocean salinity drop on the interaction between opportunistic *Vibrio* bacteria and temperate phages. We used 32 *Vibrio* strains isolated from the Kiel fjord region and infected them with their inherent temperate phages at ambient salinity conditions (15 PSU) and at future salinity levels (11 and 7 PSU). Under a scenario of future salinity conditions, lytic infections spread and bacterial resistance was diminished. In a subsequent evolution experiment we assessed the impact of a salinity shift on bacterial resistance evolution and propagation of temperate phages in *Vibrio* populations. At future low saline conditions, the evolution and fixation of bacterial resistance against phage infection was slowed down by 50 %. Bacteria carrying the co-evolving phage remained twice as long in the bacterial population at reduced salinity conditions before being outcompeted by phage resistant mutants. A shift in salinity seems to impair bacterial growth and increases the impact of temperate phages on bacterial populations, which may feed back into altered pathogenicity when *Vibrio* bacteria infect their final eukaryotic host in a scenario of environmental change.

Rapid evolution in highly fecund populations

Bjarki Eldon, *Wolfgang Stephan*

Populations can adapt very quickly to changes in the environment; much faster than originally envisioned by Darwin. Examples abound. We consider two statistics: (i) the probability (denoted p) of fixation of the advantageous genetic type(s), and(ii) the conditional expected time (denoted e) to fixation of the advantageous genetic type(s). We compare p and e between populations with or without sweepstakes reproduction. In a diploid population the fitness value of an individual maybe determined by one or many unlinked loci. We consider both directional and balancing selection. Our hypothesis is that (i) highly fecund populations with sweepstakes reproduction can reach the "optimal" state (fixation of the advantageous type(s)) much faster than populations with low fecundity conditional on the event that the optimal state will be reached; (ii) however, the probability of reaching the optimal state may be much smaller in populations with sweepstakes reproduction. Simulation results for a single locus in a haploid population under viability selection support our hypothesis[1].

References:

[1]Bjarki Eldon and Wolfgang Stephan (2018): Evolution in highly fecund haploid populations. Theoretical Population Biology 119:48-56

Does protein architecture impact adaptive evolution?

Ana Filipa da Silva Moutinho

The frequency and nature of adaptive mutations is a long-standing focus of the study of molecular evolution. Here, we address the impact of structural architecture among protein coding regions on the rate of adaptive mutations. We used population genetics to study molecular evolution on a fine scale by analyzing the impact of genetic variants in the different conformations of protein structure. With this, we aimed to understand how protein biophysics and coding sequence evolution influence fitness and adaptation. By using *Drosophila melanogaster* and *Arabidopsis thaliana* population genomics data, we fitted models of distribution of fitness effects and estimated the rate of adaptive amino-acid substitutions both at the protein and amino-acid residue scale, across different categories of gene ontology, cellular localization, chaperone affinity and structural motifs. We found a positive correlation between the rate of adaptive non-synonymous substitutions and protein disorder probability and a higher accumulation of adaptive substitutions at the surface of the proteins. Moreover, we observed that nuclear proteins and genes with binding functions exhibit the highest levels of adaptive evolution. Our results therefore suggest that protein architecture has little impact on adaptive evolution and that most adaptive mutations occur through the formation of new interactions at the network level.

From coevolutionary dynamics to genomics and back: inferring the speed of the Red Queen

Hanna Maerkle, Sona John, Wolfgang Stephan, Daniel Živković,
Aurélien Tellier

It is of general interest to know whether host and pathogen species are coevolving and if so, if we can detect the loci involved in this interaction. I will present the development of an inference method which incorporate the ecological interaction to population genetics, to detect coevolution in host and pathogen genomes.
Two important properties of host-parasite coevolution are 1) changes in allele frequencies at interacting loci, and 2) changes in the population sizes of both species as driven by the epidemiology. Building our models in a discrete as well as continuous setting we make respective use of these two properties for coevolutionary inference and develop an approximate Bayesian computation (ABC) approach tailored for a joint analysis of host and parasite full-genome polymorphism data. We first investigate over which parameter regime the ecological time scale is slow enough to leave a signature of coevolutionary dynamics in the Site Frequency Spectrum of hosts or parasites, and present simulation results demonstrating the power of our method. We implement an estimation of co-demography of hosts and parasites based on full genome SFS at several time points. In addition, we develop an inference method to estimate the parameters of coevolution dynamics. Namely, we estimate the cost for the host of being infected which is crucial to switch dynamics from arms race (selective sweeps) to trench warfare (balancing selection). Our method is accurate to recover this parameter when using jointly host and parasite polymorphism data at loci of interaction.

Evolution and regulation of resistance genes in Solanaceaen by the microRNA family miR482

Janina K. von Dahlen

Matching the rapid evolution and continual adaptation of pathogens is an ongoing challenge for every species. The process of co-evolution between host plants and their pathogens has left an imprint on the genomes of both species (e.g. expanded repertoires of resistance (R)-gene and effectors). These imprints on the side of the plant may be exploited to introduce resistance to certain pathogens in breeding programs of cultivated plants. I am studying the evolution and regulation of disease resistance in Solanaceaen - in particular, how the microRNA (miR482) family regulates R-genes. miR482 negatively regulates R-gene expression by specific degradation of the R-gene transcripts in pathogen-unchallenged circumstances. In the case of a pathogen-challenge, this negative regulation is repressed.

In our studies, we aim to investigate what role miR482 regulation and the co-evolution with their targets has played in the rapid evolutionary responses of hosts to pathogens. To gain an overview of targeting, we mapped the predicted targeting of the miR482 family members onto a phylogenetic tree of the R-genes from potato, wild and cultivated tomatoes. This allows us to directly connect sequence evolution – in both the miR genes and the R-genes – to gains and losses in targeting. In upcoming *in planta* degradome studies, we will verify or refute these inferred evolutionary changes in targeting. To investigate the influence of species-specific gains and losses in targeting relationships, we will use transient expressions to take advantage of the fact that even a single nucleotide change can cause an alteration in the R-gene / miR gene interactions.

Experimental evolution indicates hybridization as a possible rapid-adaptation mechanism of host-adapted *Sporisorium reilianum* to a new host

Emad Albarouki, *Jan Schirawski*

Host jumps have been identified as one of the major sources of newly emerging fungal plant pathogens. During evolution of the plant-pathogenic smut fungi several host jumps have occurred. The head smut fungus *Sporisorium reilianum* is an example of a relatively recent host jump from sorghum to maize leading to the current existence of two host-specific *formae speciales*, *S. reilianum* f. sp. *reilianum* (SRS) and *S. reilianum* f. sp. *zeae* (SRZ) infecting either sorghum or maize, respectively. How host-adapted pathogens succeed in colonizing a new host is currently unknown. Potential mechanisms include hybridization with a related species with different host preference, horizontal gene transfer, gene loss, accumulation of mutations, or epigenetic gene regulation. To unravel possible mechanisms, host-adapted *formae speciales* of *S. reilianum* were exposed to four successive generations of experimental evolution conditions by selection on either one or on both host plants, and either with or without prior hybridization. Immediately after the first hybridization event, the strains were able to infect maize but not sorghum, whereas collected offspring of generations F0, F1, F2 and F3 were able to infect both maize and sorghum hosts simultaneously. Isolated individual strains showed hyper-virulence on sorghum or on maize, and some strains had dual infection ability. Loss of virulence on sorghum has been observed several times during the only four rounds of selection. A genome-wide association analysis of about 200 virulence-phenotyped F0 hybrid strains indicated a linkage of a chromosomal gene cluster from SRS to virulence on sorghum. Several additional SNPs with association to different virulence phenotypes could also be identified. Unexpectedly, most F0 hybrids (96%) contained recombinant mitochondria where an internal part of the SRZ mitochondrial genome had been replaced by the corresponding part of SRS. Apparently, mitochondrial recombination happened at two hotspots and led to the generation of very successful hybrid mitochondria. This short experimental evolution experiment shows that hybridization of host-adapted strains with a related pathogen of another host is a possible mechanism of rapid evolutionary adaptation that explains how host jumps could have occurred.

Genomic variations underlying the rapid evolutionary adaptation of phytopathogenic powdery mildew fungi to highly selective plant environments

Stefan Kusch

The obligate biotrophic ascomycete fungus *Blumeria graminis* causes the powdery mildew disease on grasses including *Triticum aestivum* (wheat) and *Hordeum vulgare* (barley). Different *formae speciales* of *B. graminis* exhibit strict host specificity, e.g. the barley powdery mildew (*B. graminis* f.sp. *hordei*, *Bgh*) can only complete its pathogenic life cycle on barley, but not on other grasses. We hypothesize that the evolution of the fungus is fast enough to detect changes in its virulence spectrum in real time. To identify the nature of genomic alterations underlying such putative rapid evolutionary adaptations, we perform experimental evolution experiments with *Bgh* by exposing the fungal pathogen to normally inaccessible host plant environments. We found that three experimentally evolved *Bgh* isolate K1 derivatives (named SK1-SK3) display variable levels of virulence on otherwise fully resistant barley *mlo* (*Mildew locus O*) mutant plants. By whole transcriptome shotgun sequencing (RNA-Seq) we identified 123 genes that are differentially expressed between SK1 and K1 at 18 hours post inoculation (the time of host cell penetration), suggesting that *mlo*-virulence is polygenic and quantitative. Furthermore, we discovered that a gene coding for a transcription factor known to affect virulence and hyphal development is part of a ~40 kb genomic deletion in the majority of the virulent population. By contrast, we observed no deleterious genomic alterations in genes encoding candidate secreted effectors, which are thought to be key determinants of the fungal virulence spectrum. Nonetheless, we noted copy number variation of candidate secreted effectors between *B. graminis formae speciales* as well as a recent lineage-specific expansion of transposable elements in our newly generated near-chromosome level genome assembly of *Bgh*, which together with the evidence from SK1 indicates that the fungus is capable of rapid evolution and fast adaption towards new host environments. Consequently, our analysis provides evidence that *Bgh* isolates, typically considered to be isogenic, in fact represent genetically heterogeneous populations. This notion is supported by a marked reduction of the standing genetic variation in SK1 compared to the parental isolate K1, indicating that the experimental evolution experiment created a severe population bottleneck.

Genomic analysis of the maize-*Setosphaeria turcicum* pathosystem coevolution

Mireia Vidal Villarejo, Fabian Freund, Bianca Doesselmann,
Karl Schmid

Plants and their pathogens show rapid co-evolution, which is of great importance in agriculture because monogenic race-specific resistances in crop varieties are rapidly overcome by pathogens through evolutionary adaptation. One strategy to slow down the evolution of resistance-breaking alleles is to combine several resistance genes into a single variety ("pyramiding") which is greatly facilitated by the molecular cloning and marker-assisted selection. Another possible approach is to monitor the evolution of pathogen races in real time and to adapt the cultivation of varieties with different resistance genes to reduce the chance of pathogen adaptation. We have begun to explore for the second approach by characterizing the genomic diversity of the fungal maize pathogen *Setosophaeria turcica*, which causes Northern Corn Leaf Blight (NCLB). We sequenced a reference collection of 120 isolates of *S. turcica* from Europe and Kenya and conducted a population genetic analysis. Isolates from Europe reproduce clonally whereas isolates from Kenya provide evidence for more frequent sexual reproduction. Coalescent analyses reveals that the clonal lineages in Europe do not follow a model of sweepstake reproduction as expected under a selection-driven expansion of clonal lineages with a high fitness against modern maize hybrid varieties. Within clonal lineages we found very little genetic variation but observed variation for phenotypically defined races, which suggests that new races originate rapidly. We used k-mer based approaches to identify rapidly evolving genomic regions within clonal lineages and found substantial differences between clonal but not within clonal lineages. Additionally we performed multiple sequence alignments to identify candidate regions for effector genes associated to the different races. To understand the geographic scale of pathogen diversity, we used metagenomic sequencing and found that within single fields different and genetically diverse clonal lineages can be found. Taken together our analyses suggest that different and genetically diverse clonal lineages spread throughout Central and Northern Europe with the recent expansion of maize cultivation to cooler climates. There is little evidence for rapid adaptation by genetic evolution and spatial patterns of distribution of different lineages appear mainly determined by non-selective factors.

Rapid adaptation of signaling networks in the fungal pathogen *Magnaporthe oryzae*

Katharina Bersching, *Stefan Jacob, Stefan Tenzer*

Evolutionary adaptation of living organisms is commonly thought to be the result of processes that acted over long periods of time. The proposed project is motivated by recent observations that microorganisms are able to rapidly adapt to new environments and establish stable phenotypes by natural selection, even within few generations. We found the filamentous rice blast fungus *Magnaporthe oryzae* to rapidly rewire signal transduction required for osmoregulation in several independent “loss-of-function” (lof)-mutants of the High Osmolarity Glycerol (HOG)-pathway upon exposure to salt stress. Adaptation resulted in stable mutants of the model organism being restored in osmoregulation arising as individuals outgrowing from salt-sensitive lof-mutants. The major compatible solute produced upon salt stress by these rapidly “adapted” strains was found to be glycerol in contrast to arabitol in the wildtype strains. These findings lead to the hypothesis that stable adaptation-events under continuously environmental evolutionary pressure enable *Magnaporthe oryzae* to rapidly restore or modify entire signaling networks. To address this hypothesis, we aim to identify the molecular or biochemical mechanisms of this rapid adaption and characterize associated factors and signaling pathways which enable or prevent adaptation. Both project partners will interact synergistically to combine expertise of theoretical approaches to integrate sequencing data from genomics and transcriptomics with modern quantitative (phospho)-proteomics techniques. Furthermore, reversed molecular genetics will be used to validate the candidate genes or even other factors (e.g. phosphorylation patterns) found to putatively promote or constrain rapid evolutionary adaptation.

Plasticity of metabolite concentrations: theory and applications

Zoran Nikoloski

Metabolic functions are shaped by reaction networks whose dynamics are determined by the concentrations of the underlying cellular components. Yet, it is unclear to which extent the structure of the underlying metabolic networks constrains concentrations, since calculation of concentration bounds from large-scale models is not currently possible. I will present a set of approaches which allow us to predict metabolite concentration ranges in large-scale metabolic networks endowed with particular types of kinetics given limited information about well-defined parameters. I will illustrate that the approach can be used for quantitative and qualitative comparison of scenarios (e.g. genetic modifications, environment perturbation) based on plasticity of metabolite concentrations. These results indicate how plasticity of metabolite concentrations can affect growth and other complex phenotypes.

The role of gene flow in rapid adaptive evolution of fungal plant pathogens: a comparative population genomics study

Alice Feurtey, *A. Stevens, C. Eschenbrenner, W. Stephan, E.H. Stukenbrock*

Antagonistic co-evolution between pathogens and their hosts can drive rapid adaptive changes in both partners. Pathogens exert a strong selection pressure on their hosts, in particular on immune defense genes. At the same time, host resistance can be overcome in the pathogen evolving to escape host recognition or to suppress host defenses. The genetic innovations allowing rapid adaptation in this evolutionary “arms-race” can have various origins including mutational events, sexual recombination and gene flow. Genome-based studies of fungal pathogens have revealed a frequent contribution of inter-specific gene exchange in rapid evolution. We used a population genomics approach based on de novo assemblies of genomes and whole genome alignments to characterize the distribution of highly variable regions in the fungal wheat pathogen *Zymoseptoria tritici*. These regions are found throughout the genome, comprise around 5% of the total genome size and overlap with 600 predicted coding sequences. We performed window based phylogenetic analyses on the genome alignment and show that the highly variable regions overlap with regions showing signature of past interspecific hybridization events. We detect a similar pattern in the closely related wild grass pathogen, *Zymoseptoria ardabiliae*, and some hybridization events have involved these two species. Overall, our results demonstrate a significant impact of frequent interspecific hybridization on the genome evolution of this important wheat pathogen. We speculate that gene flow act to fuel arms race evolution of *Z. tritici* with its host.

The molecular basis of phenotypic plasticity and genetic assimilation in rapidly evolving lineages of East African cichlid fishes

Ralf Schneider

Phenotypic plasticity, the ability of a genotype to respond to environmental cues by developing different distinct phenotypes, was long thought to slow down the rate of evolution. Recent theoretical and empirical work, however, suggests that under certain conditions, e.g. during a colonization event, plasticity can facility phenotypic differentiation, ecological segregation and ultimately lineage divergence. The East African Great Lakes' cichlid adaptive radiations comprise some of the most rapid examples of lineage diversification known to science and their founder lineages were likely phenotypically plastic in key traits. In our study, we investigate the degree of plasticity in the pharyngeal jaw in riverine generalist cichlid species resembling (ecologically) the radiations' founders, and specialized lacustrine lineages with different phenotypes from within three of these radiations. We provide insights in the axes of pharyngeal jaw morphological differentiation and convergence within and across lake radiations. Furthermore, we evaluated the degree to which focal species responded morphologically plastic to feeding treatments aimed to elicit such a response. Finally, first results obtained from a transcriptome of the pharyngeal jaws were evaluated in light of these diet treatments. Conducted analyses are the baseline for more comprehensive and in-depth analyses once the full transcriptome set is obtained within the next months.

Does introgression from Austrian toads in Northern Europe distort or promote adaptation? An unintentional experiment of the European fire-bellied toad (*Bombina bombina*) at the edge of its northern distribution

***Binia De Cahsan**, M. Lauritsen, K. Kiemel, H. Drews, G. Gollmann, S. Schweiger, M. Ott, R. Tiedemann*

The European fire-bellied toad (*Bombina bombina*) with its low dispersal capacity is regarded as one of the most threatened species of amphibians in central Europe and is particularly affected by environmental perturbations. During the last decades population numbers in Germany have declined drastically due to pollution, eutrophication and habitat fragmentation. Illegal translocations performed by hobby herpetologists resulted in an introgression from southern genotypes (probably Austrian) into three local *Bombina* populations (Northern Germany and Southern Sweden) belonging to the northern lineage of the species. Interestingly, these populations show high frequencies of allochthonous (non-local) alleles at multiple loci and outperform the autochthonous populations in terms of population growth rate and body condition. Over a time period of ten years, we could show that frequencies of introgressed haplotypes in allochthonous populations do not increase over time. However, the introgression itself expanded towards adjacent populations while the overall haplotype diversity has decreased. In contrast, southern lineage genotypes for a highly polymorphic marker system under selection, the MHC class II gene, occur only at low frequencies in northern populations.

This study is testing two hypotheses: (1) local populations (autochthonous) of *Bombina bombina* are highly adapted to their environments so that introgression of alien genes causes outbreeding depression or (2) local populations of *Bombina bombina* potentially lack adaptive variation so that introgression of alien genes causes genetic rescue and promotes adaptive change.

This scenario is an unintentional experiment as a result of illegal translocations, which imitates introgression of alien genes coming from a southern population (potentially adapted to warmer climate) into a northern lineage (potentially adapted to local pathogens). This scenario provides a unique opportunity to investigate and evaluate introgression as a mechanism for either increasing genetic diversity or disrupting local adaptation in a threatened amphibian species.

Evolutionary adaptation of plants to challenging environments

Lara Syllwasschy, Veronica Preite, Christian Sailer, Justin Anderson, Björn Pietzenuk, Levi Yant, ***Ute Kraemer***

The extremophile Brassicaceae species *Arabidopsis halleri* has colonized heavy metal-contaminated toxic (metalliferous) sites of recent anthropogenic origin multiple times independently across its entire distribution range in Europe and East Asia. By contrast, populations of closely related species on metalliferous soils are either exceedingly rare, such as for *Arabidopsis arenosa* and *Arabidopsis lyrata*, or entirely absent, as in *Arabidopsis thaliana*. We established a large biodiversity resource of *ca*. 800 living *A. halleri* individuals from 165 natural collection sites. All individuals are ionomically and edaphically indexed, which means that we determined leaf and rhizosphere soil mineral composition at their sites of origin in the field[1]. These data provided comprehensive information on the environmental and phenotypic ranges of the species. Genetic diversity and population structure were assessed using genotyping-by-sequencing. Moreover, we conducted genome re-sequencing of multiple individuals from several pairs of geographically neighboring sampling sites on contrasting soils. Multi-metric genome scans identified candidate genes under selection. Our results further support that *A. halleri* is a genetically diverse diploid stoloniferous outcrossing perennial species throughout. We will discuss the roles of exaptation, introgression, novel mutations, natural selection and the genetic architecture of functional networks in repeated evolutionary adaptation to metalliferous soils in *A. halleri*. Using *A. halleri* as a model it is possible to gain general insights into genomic and other properties that underlie the ability of a given plant taxon for repeated evolutionary adaptation to a specific type of challenging environments.

References:

[1]Stein et al. (2017) New Phytol. 213: 1274-86.

Adaptive evolution of immune gene families: origin, diversification and diversity of the NLR genes in zebrafish

Jaanus Suurväli

Zebrafish (*Danio rerio*) is a model animal from the largest known vertebrate family (*Cyprinidae*). Its natural habitat is in the small water bodies of India, Nepal and Bangladesh. Although zebrafish research has been ongoing for decades, most of the focus has been on genes and features with a direct human homolog, allowing the results to be interpreted in the biomedical context. This research is usually performed on inbred fish from lab strains with an unclear origin, as they have been originally obtained from pet stores.

In addition to the biomedical importance, zebrafish also has great potential for the study and modelling of processes that occur in teleost fish. The immune systems of fish contain quite a few unique aspects, e.g. massive duplications of specific immune gene families, an ancient MHC lineage that has been lost in most other vertebrates, etc. In particular, the family of NACHT-LRR receptors (NLRs) has hundreds of copies in fish, but not in other vertebrates. We have proposed duplications and copy number changes of this family in zebrafish to be a recent or possibly even ongoing adaptive event.

Using restriction associated DNA sequencing we have surveyed the population structure and nucleotide diversity of zebrafish populations sampled from their natural habitat. We find clear differences between fish from lab strains, from the wildlife protection areas of Nepal, from India and from the geographically separated Bangladesh. We used this information to select populations for targeted PacBio sequencing of the NLR genes, and for the first time we can estimate the diversity of a hard-to-sequence major immune gene family in wild fish populations. Here we present our first set of results, with observed variation ranging from single nucleotide polymorphisms to previously undescribed genes.

Allele-specific gene regulatory variation in *Drosophila melanogaster*

Amanda Glaser-Schmitt, *John Parsch*

Environmental changes, such as the colonization of new territories or seasonal climate fluctuations, can cause a shift in the optimum of phenotypic traits, leading to rapid allele frequency changes within populations. Gene expression variation is abundant within and among populations, and is thought to be responsible for much of the observed phenotypic variation. Underlying this expression variation are various *cis*- and *trans*-regulatory factors that may be targets of natural selection. The overarching aim of this project is the identification and characterization of gene regulatory polymorphisms in *Drosophila melanogaster* populations as well as the evolutionary mechanisms that maintain them. To characterize gene expression variation and identify *cis* regulatory changes, we perform allele-specific expression analyses for isofemale strains from an ancestral, African population and a derived, European population across several digestive system tissues. *cis*-regulatory variants are known to have tissue specific effects and the examined tissues play important roles in detoxification, excretion, and metabolism. Thus, the genes and regulatory changes we identify are candidates for adaptive evolution associated with the colonization of non-African habitats. In phase one, we performed allele-specific analyses in the Malpighian tubule. Most of the regulatory variation we identified in the Malpighian tubule was *trans*regulatory although we also found ~200 genes with consistent *cis*-regulatory changes between African and European strains, which were enriched for cytochrome P450 genes. To better characterize these regulatory changes, we created a series of transgenic reporter genes to test the regulatory function of the upstream regions of six cytochrome P450 genes. We found that the majority of the tested upstream regions drove differential expression in the expected direction, suggesting that a large proportion of *cis*-regulatory variation lies directly upstream of the affected gene. In most cases the observed differential expression was specific to the Malpighian tubule, underscoring the ability of *cis*-regulatory changes to fine tune expression in a tissue-specific manner. We further found increased genetic differentiation in the majority of the tested upstream regions, suggesting that local adaptation may be occurring.

Signatures of host specialization and a recent transposable element burst in the dynamic one-speed genome of the fungal barley powdery mildew pathogen

Lamprinos Frantzeskakis, Barbara Kracher, Stefan Kusch, Makoto Yoshikawa-Maekawa, Saskia Bauer, Carsten Pedersen, Pietro D Spanu, Takaki Maekawa, Paul Schulze-Lefert, ***Ralph Panstruga***

Powdery mildews are biotrophic pathogenic fungi infecting a number of economically important plants. The grass powdery mildew, *Blumeria graminis*, has become a model organism to study host specialization of obligate biotrophic fungal pathogens. We resolved the large-scale genomic architecture of *B. graminis forma specialis hordei* (*Bgh*) to explore the potential influence of its genome organization on the co-evolutionary process with its host plant, barley (*Hordeum vulgare*). The near-chromosome level assemblies of the *Bgh* reference isolate DH14 and one of the most diversified isolates, RACE1, enabled a comparative analysis of these haploid genomes, which are highly enriched with transposable elements (TEs). We found largely retained genome synteny and gene repertoires, yet detected copy number variation (CNV) of secretion signal peptide-containing protein-coding genes (*SPs*) and locally disrupted synteny blocks. Genes coding for sequence-related SPs are often locally clustered, but neither the *SPs* nor the TEs reside preferentially in genomic regions with unique features. Extended comparative analysis with different host-specific *B. graminis formae speciales* revealed the existence of a core suite of *SPs*, but also isolate-specific *SP* sets as well as congruence of *SP* CNV and phylogenetic relationship. We further detected evidence for a recent, lineage-specific expansion of TEs in the *Bgh* genome. The characteristics of the *Bgh* genome (largely retained synteny, CNV of *SP* genes, recently proliferated TEs and a lack of significant compartmentalization) are consistent with a “one-speed” genome that differs in its architecture and (co-)evolutionary pattern from the “two-speed” genomes reported for several other filamentous phytopathogens.

Convergent evolution of immunological tolerance in male pregnancy

Jamie Parker, *Sissel Jentoft, Olivia Roth*

One of the most significant examples of sex-role reversal and parental investment in the animal kingdom can be attributed to syngnathids (pipefish and seahorses), the fish family responsible for the maternal pregnancy novelty. The extensive study of mammalian pregnancy evolution has uncovered associated evolutionary changes in maternal adaptive immunity. Therefore, understanding the immunological differences between male and female brooders during pregnancy, could help shed some light on evolutionary steps that led to the male pregnancy phenomenon also. One important example supported by recent studies, is the loss or modification of genes in *Syngnathus typhle* and *Hippocampus abdominalis* respectively, involved in the MHC class II pathway. Genetic modifications of this sort may have co-evolved alongside maternal pregnancies or perhaps lead in part to its establishment. This study aims to cross-examine gene expression patterns, including those used in the absence of MHC class II, across various Syngnathiformes with differing brooding methods throughout the pregnancy period. Pregnant fish immunological tolerances and adaptive immune pathways will be tested using allograft fin transplants, while differences in brood pouch morphologies will be analysed with respect to immunological influence. In addition, a comparative genomics approach will be used to highlight discrepancies between syngnathid species and those of other phyla, with regards to pregnancy and adaptive immunity associated genes. This comparative study aims to provide further insight into the convergent evolution of male and female pregnancy, by focusing on the co-adaptation between parental investment and the immune system.

The functional importance of biased codon use in *Pseudomonas fluorescens*

Anuradha Mukherjee

Biological organisms possess 61 DNA codons that code for 20 proteinogenic amino acids. This makes the genetic code redundant – some amino acids are encoded by more than one codon (codon "set"). Codons within a set are not used equally; preferential usage called codon bias, exists across the tree of life. However, there is little information regarding the emergence of codon bias. My research focuses on understanding the functional importance of codon usage in *Pseudomonas fluorescens*. Our laboratory uses three *P. fluorescens* strains (SBW25, A506 and Pf0-1) that show different degrees of evolutionary relatedness, and various differences in codon usage. Using bioinformatics, our group has identified three genes that differ across the three strains *only* at the level of DNA sequence (*glyQ, acpP* and *rpsJ*), while amino acid sequence remains unchanged.

In my PhD, I am using *glyQ* to investigate the effects of altering established codon usage patterns. I am using genetic engineering to alter *glyQ* codon use in two ways: (i) evolutionary alterations whereby each of the three *glyQ* alleles is engineered into its two non-native backgrounds, and (ii) targeted alterations whereby dozens of carefully selected mutations will be engineered into *P. fluorescens* SBW25 *glyQ.*

To date, I have constructed all six strains for the evolutionary alterations set. A battery of phenotypic tests has revealed no phenotypic differences between strains tested so far. I am currently looking for more subtle changes in *glyQ* transcription, translation and function in the evolutionary alteration strains. The second set of mutants – the targeted alterations – have been selected and are under construction. It is expected that these mutants will have varying degrees of effects on fitness. I intend to use the quantitative tests for transcription, translation and function of *glyQ* that are currently under development to unravel the molecular nature of any effects found.

Seasonal Adaptation in *Chironomus riparius*

***Quentin Foucault**, Andreas Wieser, Markus Pfenninger*

Effects of seasonal or daily temperature variation on fitness and physiology of ectothermic organisms, and their ways to cope with such variations have been widely studied. However, the underlying mechanisms of how multivoltine organisms cope with temperature variations from one generation to the next is still poorly understood as it remains complex to identify. To investigate this issue, a common garden approach has been applied using the multivoltine midge *Chironomus riparius* Meigen (1803). Two replicates of 2000 individuals from the same sampling location were raised in the laboratory at three different stable temperatures: 14, 20, 26°C; while two other replicates were raised at fluctuating temperatures, mimicking the expected seasonal variation. During this experiment lasting 2.5 years in total, the lab populations' as well as the natural population's genome were sequenced regularly, as well as their fitness tested allowing us to compare and investigate change in allele frequency from different loci genome wide and the possible modifications they create on the fitness level.

The evolution of tRNA pools in *Pseudomonas fluorescens*

Gökçe Ayan, Frederic Bertels, ***Jenna Gallie***

Transfer RNAs (tRNAs) play a central role in biology: they are the adapter molecules that carry amino acids to the growing peptide chain during protein synthesis. Thus the composition of intracellular tRNA pools heavily influences all cellular processes. We are interested in the evolution of tRNA pools: how and why do particular pools come to exist? Our studies focus on *Pseudomonas*, a diverse genus of bacteria.

Here we present a bioinformatics analysis of tRNA gene pools across *Pseudomonas,* in conjunction with laboratory-based deep sequencing of mature tRNA pools in a subset of species.

The genetic basis of natural variation in Cd hypertolerance in *Arabidopsis halleri*

***Gwonjin Lee**, Justin E. Anderson, Björn Pietzenuk, Arthur Korte, Ute Krämer*

In *Arabidopsis halleri*, evolution has brought about adaptations to highly heavy-metal contaminated soils in the form of metal hypertolerance, as well as the accumulation of very high levels of metals in leaves. Populations of *A. halleri* occur on both metal contaminated (M) and non-contaminated soils (NM), and there is a large extent of within-species variation in both leaf Cd accumulation and Cd hypertolerance. We found that M populations of *A. halleri* exhibit enhanced Cd hypertolerance in comparison to NM populations, which merely show Cd hypertolerance that is a species-wide trait in *A. halleri*. Human activities have caused widespread contamination of soils with heavy metals that has accelerated in the past 175 years. Therefore, the difference in Cd hypertolerance between *A. halleri* populations on M and NM soils could be a result of rapid evolution as a consequence of anthropogenic Cd pollution.

We have a large collection of *A. halleri* germplasm comprising 800 individuals from 165 populations, which were collected from around Europe. In this study, the natural variation in Cd tolerance of a number of *A. halleri* genotypes is compared both within and between populations by quantifying visible toxicity symptoms and EC_{100} (Effective concentration for 100% root growth inhibition) of each genotype upon exposure to sequentially increasing Cd concentrations. These phenotyping data are used in Genome-Wide Association Studies (GWAS) employing Genotyping-By-Sequencing (GBS) data to identify candidate genetic loci contributing to enhanced Cd hypertolerance. A complementary approach includes comparative transcriptome profiling using RNA-Seq alongside a physiological and morphological characterization of extremely Cd-tolerant and Cd-sensitive genotypes. This project will help to understand the physiological basis of natural variation in Cd hypertolerance in *A. halleri* and to uncover the specific gene functions or multi-gene functional networks involved.

Genomic adaptations that allow powdery mildew fungi to specialize on particular host plants

Mirna Barsoum

Members of the species *Blumeria graminis*, *formae speciales*, are to a large part strictly adapted to a particular host species. It has been demonstrated recently that powdery mildews can acquire new host specificities via hybridization. The aim of this project is to define the necessary and sufficient genomic setup to allow a given *forma specialis* of *B. graminis* to colonize a particular plant species and not the others. To address this question, we perform genetic crosses between two *formae speciales* of *B. graminis* (*B. graminis* f.sp. *hordei*, the barley powdery mildew pathogen and *B. graminis* f.sp. *tritici,* the wheat powdery mildew pathogen) and phenotype the infection specificity of the resulting progeny. Mating between compatible *Bgh* and *Bgt* strains on a common host plant (barley SusBgt line) resulted in the emergence of sexual reproductive structures (chasmothecia). For the targeted release of asci and ascospores from the chasmothecia, dried leaves of the SusBgt line harboring chasmothecia, taped on wet filter paper above fresh leaves of both barley and wheat, were placed in plastic containers. In the moist conditions of the closed containers, ascospores are supposed to be released from the chasmothecia and to fall down on the fresh leaves below where they would give rise to new fungal colonies. Indeed, so far two colonies on wheat leaves and five on barley leaves were obtained by this procedure. These colonies, each representing a potential single clone, were recovered and fungal biomass was amplified by propagating each clone individually on fresh barley or wheat leaves (*via* transfer of conidiospores to fresh leaves). Whole genome resequencing will be done to validate the crossing events beyond any doubts. It will be highly interesting to explore the virulence profiles of the progeny strains and to correlate them with the respective genomic setup.

Genetic and epigenetic mechanisms of rapid host adaptation in the aphid parasitoid *Aphidius ervi*

Jürgen Gadau, *Lukas Schrader*

We use the parasitoid wasp Aphidius ervi as a model to understand the molecular mechanisms conferring specialization. In particular, we are interested what role phenotypic plasticity and genetic selection play during this process and whether these two mechanisms interact. For this, we will conduct artificial evolution experiments, where parasites will be forced to either use the same host over at least twenty generations or switch hosts regularly. Aphidius ervi is known to be a generalist that parasitizes pea aphids and grain aphids. Previous experiments with A. ervi have shown that females evolve host preferences rapidly over few generations, but can also quickly lose host preference if both host species are offered (Zepada-Paulo 2013).By comparing specialist lines and generalist lines, we aim to assess whether expression patterns successively diverge over several generations of specialization. We hypothesize that expression differences become more elaborate, suggestive of rapid adaptive changes and incipient stages of host specialization. We furthermore hypothesize that the elaboration of expression differences will at least in part be controlled by alternative methylation as a heritable epigenetic mechanism and that miRNA expression differences will be involved in regulating the downstream effects of epigenetic differences.

Rapid adaptation of signaling networks in the fungal pathogen *Magnaporthe oryzae*

Stefan Jacob, *Stefan Tenzer*

Evolutionary adaptation of living organisms is commonly thought to be the result of processes that acted over long periods of time. The proposed project is motivated by recent observations that microorganisms are able to rapidly adapt to new environments and establish stable phenotypes by natural selection, even within few generations. We found the filamentous rice blast fungus *Magnaporthe oryzae* to rapidly rewire signal transduction required for osmoregulation in several independent “loss-of-function” (lof)-mutants of the High Osmolarity Glycerol (HOG)-pathway upon exposure to salt stress. Adaptation resulted in stable mutants of the model organism being restored in osmoregulation arising as individuals outgrowing from salt-sensitive lof-mutants. The major compatible solute produced upon salt stress by these rapidly “adapted” strains was found to be glycerol in contrast to arabitol in the wildtype strains. These findings lead to the hypothesis that stable adaptation-events under continuously environmental evolutionary pressure enable *Magnaporthe oryzae* to rapidly restore or modify entire signaling networks. To address this hypothesis, we aim to identify the molecular or biochemical mechanisms of this rapid adaption and characterize associated factors and signalling pathways which enable or prevent adaptation. Both project partners will interact synergistically to combine expertise of theoretical approaches to integrate sequencing data from genomics and transcriptomics with modern quantitative (phospho)-proteomics techniques. Furthermore, reversed molecular genetics will be used to validate the candidate genes or even other factors (e.g. phosphorylation patterns) found to putatively promote or constrain rapid evolutionary adaptation.

Genetic basis of metabolic and phenotypic plasticity in *Arabidopsis thaliana*

Prashant Pandey, Jing Yu, ***Neha Vaid****, Zoran Nikoloski, Roosa Laitinen*

Phenotypic plasticity is the ability of an organism to modify its phenotype in response to changes in its environment and is a particularly important trait for sessile plants to adapt to rapid environmental changes. Although plasticity can provide fitness advantage and mitigate negative effects of anthropogenic environmental perturbations, its genetic basis is still largely unknown. We have investigated natural variation in 15 individuals from two different local populations as well as among large panel of global accessions of *A. thaliana* in order to understand the relationship between metabolic and phenotypic plasticity in response to different nitrogen conditions. We chose three nitrogen conditions from which first one is limiting, second is intermediate and third is optimal for the growth of the plant and investigated how increased metabolic plasticity correlates with the plasticity in the analysed complex traits. From the two local populations, one population is highly homogenous and overall showed less plasticity than the other population that shows more genetic diversity. Yet, the genetically less divergent population showed almost 30% less penalty in seed yield across conditions. This indicates that while specific metabolic plasticity might be crucial in optimizing fitness across conditions, higher overall plasticity could have negative effect on fitness in changing conditions. Further, two metabolites namely, ascorbic acid and lysine showed high plasticity in both populations, indicating that these metabolites might act as key mediators of phenotypic plasticity independent of the genetic diversity. The investigation of variability among the global accessions is currently ongoing.

Rapid Evolution of Plant Tolerance to Anthropogenic Copper Pollution

Lara Syllwasschy, *Christian Sailer, Veronica Preite, Levi Yant, Ute Krämer*

Among all essential nutrients, the concentration range permissive of plant growth is particularly narrow for copper (Cu). As a result of Cu toxicity, plants develop visible symptoms, such as reduced biomass, inhibition of root growth, chlorosis, bronzing and necrosis, which can lead to plant death. Copper toxicity in plants is relevant because anthropogenic contamination of soils with Cu has drastically increased in the past 175 years because of intensifying Cu mining and smeltering activities and agricultural Cu application to soils. It is thus possible that growth on Cu-polluted soils required rapid evolution of Cu tolerance in plants.

We have searched for genomic signatures of selection through genome re-sequencing and multi-metric and composite genome scans in local pairs of one Cu-tolerant and one Cu-sensitive population of *A. halleri*. Additionally, these populations were analyzed at the level of genomic copy number variation and loss-of-function variants. One population pair will also be examined at the expression levels using RNA-Seq based expression profiling data grown under two different copper concentrations. At the meeting, chosen candidate Cu tolerance loci from the genomic sequence analysis will be presented and discussed. This project contributes to the fundamental understanding of evolutionary adaptation to rapid or extreme environmental change, provides insights into the re-vegetation and restoration of polluted or disturbed landscapes towards environmental safety, and it may help to establish a knowledge basis for the breeding of Cu-tolerant crops.

Cross-species comparative transcriptomics analysis of heavy metal-related extreme traits in *Arabidopsis halleri*

***Björn Pietzenuk**, Justin Anderson, Vasantika Suryawanshi, Ute Krämer*

A metal hyperaccumulator plant is capable of accumulating extremely high concentrations of a metal or metalloid in above-ground biomass when grown in its natural habitat. To date, more than 700 hyperaccumulator plant taxa have been identified, corresponding to about 0.2% of all vascular plants (http://hyperaccumulators.smi.uq.edu.au/collection/). The central goal of our research is to understand the molecular basis of metal hyperaccumulation and associated metal hypertolerance in plants in an ecological and evolutionary context. To this end, we study the Zn, Cd and Pb hyperaccumulator *Arabidopsis halleri*, a close relative of the classical genetic model plant *A. thaliana*. *A. halleri* is a perennial outcrossing diploid (2*n* = 16), which tolerates about 76-fold higher zinc and 8-fold higher cadmium concentrations in soil than *A. thaliana*[1]. The recently published genome assembly of the Japanese *A. halleri* ssp. *gemmifera* suggests a similar genome size and gene number, as well as a high level of coding sequence similarity to other species of the *Arabidopsis* genus. However, metal hyperaccumulation and tolerance naturally evolved uniquely in *A. halleri* following the divergence from *A. lyrata*. Genome-wide cross-species comparative transcriptomics are thus a promising approach towards uncovering the molecular basis of extreme traits in *A. halleri*. Previous cross-species microarray studies concluded that about 30 metal homeostasis genes are constitutively more highly expressed in *A. halleri* than in *A. thaliana*[1,2]. Here we will present our latest results from a genome-wide comparative sequencing-based transcriptomic study comparing *A. halleri* to its non-metal accumulating relatives *A. thaliana* and *A. lyrata*.

References:
[1]Talke et al. (2006), Plant Physiol 142: 148-67;
[2]Becher et. al. (2004), Plant J 37: 251-68;
[3]Weber et al. (2004), Plant J 37: 269-81

Population Genomics of *Cercospora beticola*

Lizel Potgieter, *Eva H. Stukenbrock*

Cercospora beticola is a fungal pathogen of sugar beet crops resulting in Cercospora Leaf Spot (CLS). Sugar beet is a relatively modern crop with a well documented history. In addition to infecting cultivated sugar beet, this fungus also infects its wild relative, sea beet. This provides an interesting system to study the effect of host domestication of a pathogen since most current plant-pathogen systems have far longer histories of association. With this project we aim to use genomic tools to elucidate genomic regions that may be more dramatically influenced in populations were obtained from infected sugar beet compared to populations that were found on sea beet. Several isolates from sea beet and sugar beet have been assembled in a *de novo* manner and aligned to an existing reference genome. Using the whole genome alignment approach, we were able to determine and compare the diversity statistics of the various regions within the genome. Using the SNPs from the alignment we also clustered the isolates based on their host and region of origin. Our results show that *C. beticola* isolates harbour more variation in the non-coding regions of the genome. The variants determined by this study have also shown that *C. beticola* isolates cluster together based on their location of origin than based on the host they were isolated from. From these data we were also able to infer patterns of natural selection. Results from this study are beginning to shed light on the evolution of the species *C. beticola* relative to its association with sugar beet.

Exploring the relative costs of two antifungal peptides in *Tenebrio molitor* against *Beauveria bassiana*

Caroline Zanchi

The immune system comprises both constitutive and inducible reactions. A good example of this is the antimicrobial defense system of insects, which is composed of mainly strongly inducible antimicrobial peptides (AMPs) and fewer constitutively expressed ones. Evidence suggests that the combination of AMPs within one host organism determines its survival during an infection, which would mean that the whole cocktail of AMPs is under selection rather than individual AMPs.
The relative costs and benefits of both inducible and constitutive AMPs in the case of a natural infection by an entomopathogen however is poorly known. Theory predicts that inducible defenses provide a “moving target” which is harder for pathogens to adapt to, but that inducibility evolved as a way to save their costs when they are not needed. In line with this, we can expect inducible AMPs to bear more fitness costs compared to constitutive ones. We tested this assumption in the mealworm beetle *Tenebrio molitor* towards the entomopathogenic fungus *Beauveria bassiana*.

Domestication-driven Metaorganism Evolution of Wheat

Ezgi Özkurt, *M. Amine Hassani, Uğur Sesiz, Hakan Özkan, Eva H. Stukenbrock*

There is evidence that the compositions of microbial communities of plants are maintained over many plant generations. This observation suggests a conserved mechanism to assemble and maintain microbial communities in spite of changing environments. Furthermore, it points to the presence of an internal reservoir of microbes transmitted vertically from generation to generation. Here, we examine the microbial community composition of seeds of different wheat species as a source for early microbial colonization of plant seedlings. We hypothesize that vertically transmitted microbes represent tightly co-evolving endophytes of the plant. Furthermore, we hypothesize that domestication of wheat has impacted the assembly and maintenance of wheat microbial communities. To address this, we have collected seeds from *Triticum dicoccoides*, *T. boeticum* and *T. urartu* wild wheat species and from domesticated *T. aestivum* in a region of South Turkey located in the Fertile Crescent. Furthermore we included a collection of seeds from cultivated wheat from Schleswig-Holstein, Germany. We characterized bacterial and fungal species by amplification and sequencing of the bacterial 16S locus and the fungal internal transcribed sequence locus (ITS) from seed derived DNA. Our preliminary data show that, although impacted by different agricultural practices and geographically isolated, cultivated wheat from the Fertile Crescent and Germany have highly similar seed-borne microbial communities compared to the wild wheat species. We also find that wild and domesticated wheat share most of their bacterial communities while the domesticated wheat has assembled a specific and diverse community of fungal species that are not found in seeds of the wild wheat. These preliminary results suggest that domestication has greatly impacted the assembly and propagation of the seed-associated microbiota but affecting differently the bacterial and fungal communities. This finding likely reflect different extent of co-evolution between plants and fungal versus bacterial symbionts.

Does introgression from Austrian toads in Northern Europe distort or promote adaptation? An unintentional experiment of the European fire-bellied toad (*Bombina bombina*) at the edge of its northern distribution

***Binia De Cahsan**, M. Lauritsen, K. Kiemel, H. Drews, G. Gollmann, S. Schweiger, M. Ott, R. Tiedemann*

The European fire-bellied toad (*Bombina bombina*) with its low dispersal capacity is regarded as one of the most threatened species of amphibians in central Europe and is particularly affected by environmental perturbations. During the last decades population numbers in Germany have declined drastically due to pollution, eutrophication and habitat fragmentation. Illegal translocations performed by hobby herpetologists resulted in an introgression from southern genotypes (probably Austrian) into three local *Bombina* populations (Northern Germany and Southern Sweden) belonging to the northern lineage of the species. Interestingly, these populations show high frequencies of allochthonous (non-local) alleles at multiple loci and outperform the autochthonous populations in terms of population growth rate and body condition. Over a time period of ten years, we could show that frequencies of introgressed haplotypes in allochthonous populations do not increase over time. However, the introgression itself expanded towards adjacent populations while the overall haplotype diversity has decreased. In contrast, southern lineage genotypes for a highly polymorphic marker system under selection, the MHC class II gene, occur only at low frequencies in northern populations. This study is testing two hypotheses: (1) local populations (autochthonous) of *Bombina bombina* are highly adapted to their environments so that introgression of alien genes causes outbreeding depression or (2) local populations of *Bombina bombina* potentially lack adaptive variation so that introgression of alien genes causes genetic rescue and promotes adaptive change.
This scenario is an unintentional experiment as a result of illegal translocations, which imitates introgression of alien genes coming from a southern population (potentially adapted to warmer climate) into a northern lineage (potentially adapted to local pathogens). This scenario provides a unique opportunity to investigate and evaluate introgression as a mechanism for either increasing genetic diversity or disrupting local adaptation in a threatened amphibian species.

Inferring population differentiation along a latitudinal gradient in *Solidago canadensis* s.l.

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Recent studies suggest that adaptation to local environments may not only have a genetic basis, but is also assisted by heritable epigenetic changes, e.g. in DNA methylation. These epigenetic changes may explain how rapidly spreading invasive species overcome their often limited genetic variation and adapt to new habitats. Both genetic and epigenetic sub-population structuring of invasive species is often investigated through comparisons of two environments only. Here, we inferred the population genetic structure of the invasive species *Solidago canadensis* s.l. along a latitudinal gradient in Mid-Europe from northern Switzerland to northern Germany. Former studies had detected latitudinal clines in morphological and phenological traits in this species, that has been introduced in Europe in the mid-17th century. However, it remains untested whether these clines will be reflected in a genetic and/or epigenetic population structure. For this study, we raised plants from seed material of 25 populations, each with a sample size of up to 10 individuals, in a common garden in Germany (Konstanz) and sampled leaf DNA at the end of the vegetation period in 2015. We expected to find an isolation-by-distance variation of populations analyzing dominant (AFLP) and codominant (microsatellites) markers. Additionally, we expected between-population variation based on DNA methylation levels using an AFLP/MSAP approach. Population differentiation was estimated using a MCMC-based procedure with STRUCTURE assisted by bootstrapping-based procedures of genetic distance and discriminant analysis of principal components (DAPC). In contrast to our expectation, we did not find an isolation-by-distance differentiation in the sampled populations of this invasive species. Outcomes based on STRUCTURE and DAPC suggest a large number of genetic clusters (k = 8 – 14) with a high degree of admixture. Our results point to complementary mechanisms explaining latitudinal clines in *S. canadensis*, such as changes in DNA methylation and/or phenotypic plasticity, but this remains to be tested. Similarly, we are still analyzing methylation-sensitive AFLPs (MSAP) to compare to our AFLP-based results.

Dissecting the evolution of tRNA gene pools in *Pseudomonas fluorescens*

***Zahra Khomarbaghi**, Hye Jin Park, Frederic Bertels, Jenna Gallie*

Transfer RNAs play a central role in protein synthesis. Organisms contain pools of genes that code for different tRNA species, and these pools differ among species and even strains. We are interested in how and why these differences come to exist. We are modeling tRNA gene pool evolution by simulating evolution with a simple theoretical organism. This theoretical organism carries a genome containing (i) a single protein-coding gene consisting of repeats of a single amino acid that is coded for by four distinct codons, and (ii) varying numbers of tRNA genes carrying the four corresponding anticodons. Our results so far show that, in order to reach the fastest rate of protein production, the optimal distribution of tRNA genes involves elimination of two of the four anticodons (via the “wobble” rule of tRNA decoding). This result is consistent with the patterns that we see in many real-life bacterial genomes. We are currently adding other parameters - such as codon usage bias in the protein coding gene, and mutation in tRNA genes - to our model.

Simultaneously in the lab, we are comparing the tRNA gene pools of three *Pseudomonas fluorescens* strains with differing degrees of evolutionary divergence (SBW25, A506 and Pf0-1). We have identified tRNAs present in all three genomes (core tRNAs) and those present in only a subset of the genomes (accessory tRNAs). We have investigated the fitness effects of altering the accessory tRNA pool of individual genomes. So far, our results show that deletion of three accessory tRNA genes from A506 and four accessory tRNA genes from Pf0-1 has no significant fitness effect under the conditions tested. In other words, there appears to be some redundancy in tRNA gene pools. To unravel the advantage of this redundancy, we are currently sequentially deleting multi-copy tRNAs from *the P. fluorescens* SBW25 genome. These engineered strains will provide a basis for future evolution experiments to study how genomes respond to modified tRNA pools.

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