MÜNSTER GRADUATE SCHOOL OF EVOLUTION

3rd Annual MGSE Symposium

MÜNSTER GRADUATE SCHOOL OF EVOLUTION

ABSTRACT BOOK 2013

Edited by Joachim Kurtz and Rebecca Heiming

Institute for Evolution and Biodiversity

University of Münster

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Münster Graduate School of Evolution (MGSE)

Modern evolutionary thinking can provide a unifying conceptual framework and is thus particularly suited as a topic for an interdisciplinary graduate school. The *Münster Graduate School of Evolution* (MGSE) is based on biology, medicine, geosciences, philosophy, and mathematics. MGSE students benefit from one another because similar general principles act across disciplines, thus allowing common theoretical approaches and experimental testing at different levels.

The 3rd MGSE symposium will provide the first cohort of MGSE doctoral students the opportunity to present and discuss their recently started PhD projects. Their presentations and posters will be embedded into contributions from scientists of the MGSE research groups and invited speakers. We will especially focus on current topics in evolutionary medicine and the interface of biology and geosciences. Moreover, on the occasion of the symposium, the *Kavaliershäuschen* will be inaugurated as the new center of the MGSE.

We are looking forward to lively discussions and fruitful exchange in this atmospheric building in the heart of Münster.

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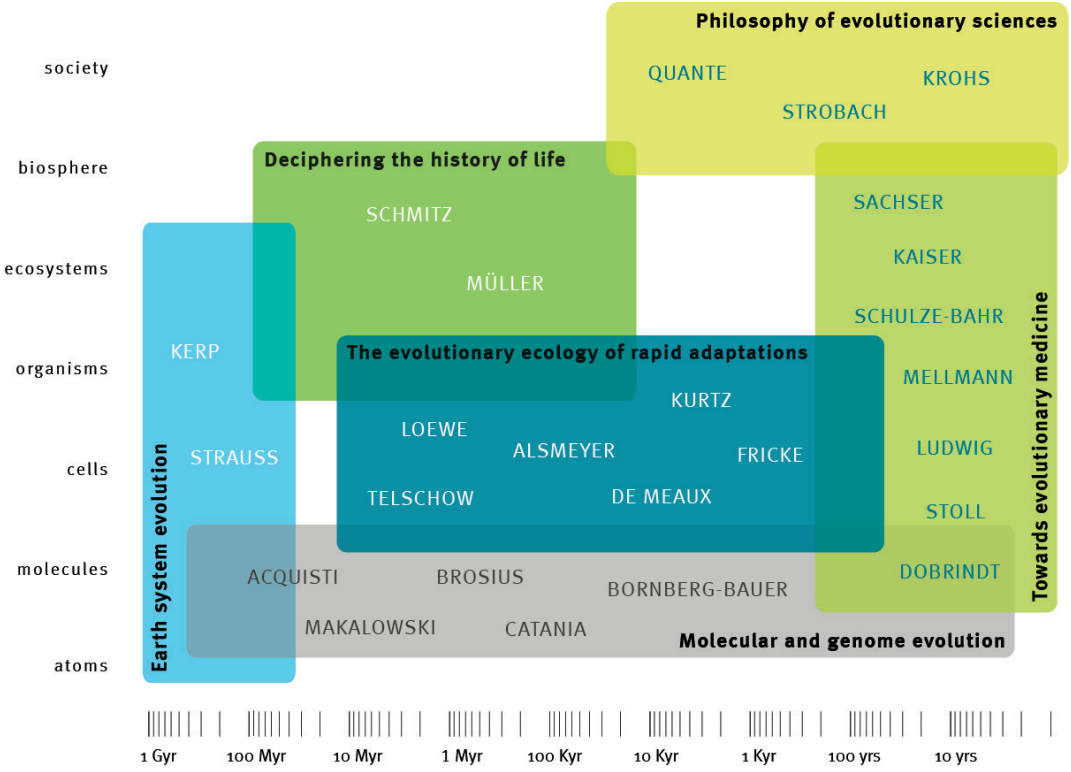
Name, Title	Research Topic	MGSE Research Area
Acquisti, Jun. Prof. Claudia	Evolutionary Functional Genomics	Molecular and genome evolution
Alsmeyer, Prof. Gerold	Mathematical Statistics	The ecology of rapid adaptations
Bornberg-Bauer, Prof. Erich	Evolutionary Bioinformatics	Molecular and genome evolution
Brosius, Prof. Jürgen	Experimental Pathology	Molecular and genome evolution
Catania, Dr. Francesco	Evolutionary Cell Biology	Molecular and genome evolution
De Meaux, Prof. Juliette	Plant Molecular Evolution	The evolutionary ecology of rapid adaptations
Dobrindt, Prof. Ulrich	Microbial Genome Plasticity	Towards evolutionary medicine
Fricke, Dr. Claudia	Evolution and Sexual Conflict	The evolutionary ecology of rapid adaptations
Kaiser, Prof. Sylvia	Behavioural Biology	Towards evolutionary medicine
Kerp, Prof. Johannes	Paleobotany	Earth system evolution
Krohs, Prof. Ulrich	Philosophy of Science and of Nature	Philosophy of evolutionary sciences
Kurtz, Prof. Joachim	Animal Evolutionary Ecology	The evolutionary ecology of rapid adaptations
Löwe, Prof. Matthias	Mathematical Statistics	The evolutionary ecology of rapid adaptations
Ludwig, Prof. Stephan	Molecular Virology	Towards evolutionary medicine
Makalowski, Prof. Wojciech	Bioinformatics	Molecular and genome evolution
Mellmann, PD Alexander	Hospital and Environmental Hygiene	Towards evolutionary medicine
Müller, Prof. Kai	Evolution of Biodiversity of PLants	Deciphering the history of life
Quante, Prof. Michael	Philosophy of Ethics and Practical Phil.	Philosophy of evolution and education research
Sachser, Prof. Norbert	Behavioural Biology	Towards evolutionary medicine
Schmitz, PD Jürgen	Experimental Pathology	Deciphering the history of life
Schulze-Bahr, Prof. Eric	Cardiology and Angiology	Towards evolutionary medicine
Stoll, Prof. Monika	Genetic Epidemiology of Vascular Disorder	Towards evolutionary medicine
Strauss, Prof. Harald	Historical and Regional Geology	Earth system evolution
Strobach, Prof. Niko	Philosophy of Logic and Language	Philosophy of evolution and education research

MGSE - Research Areas



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Why „Kavaliershäuschen/Kavaliershaus"(cavalier house)?
by Angela Noll

What first comes to mind when reading the name of this building is: why is it called *Kavaliershaus*? Originally, this building and its chiral twin were used by horsemen to guard the castle. Since French was a fancy and modern language in Baroque times, horsemen were called *chévaliers*. Quickly, these two satellite buildings of the castle were called accordingly.

Building History

After overcoming several obstacles, *Johann Conrad Schlaun* could finally begin construction of the castle in 1767 on the site of the former citadel. Six years later *Schlaun* died, but in this time he managed to finish the entire sleeve of the castle as well as the southern Kavaliershaus by 1773. *Schlaun* issued the plans for both guardhouses in 1769, calling the southern one *la grande garde et le logment du valet de la residence* and the northern one *logement du concierge avec un quartier pour un étrange*.

(This symposium takes place there.)

Both terms show the future functions of the buildings. The one in the south would accommodate the guard, which can still be seen in the different roof designs of the two houses:

The southern house has a protrusion on the roof windows in order to protect the guards on watch from the rain. The caretaker of the castle was to reside within the northern *Kavaliershaus*. Demolition material from the stables of the former citadel was employed to build the guardhouses in order to save costs. Nevertheless, the guardhouses with their bare brick walls are evocative of the late Baroque because of their rectangular layout and the typical Mansard roof. *Wilhelm Ferdinand*

Lipper was the successor to *Schlaun* and compiled a draft for the interior design of the northern guardhouse which was finished in 1779. Officially, construction of the new castle in Münster was finished on May 14th of 1787. In 1802 the city fell to Prussia. The twin houses were most likely still used by the caretaker and the guard, respectively, until the Second World War.

The Second World War

Ninety-one percent of the city of Münster were destroyed during the war. The last and worst bombing was on Palm Sunday 1945. The castle was hit by two bombs, as a consequence it burned out completely. The same happened with both guardhouses.

After 1945

After the Second World War, the administrative structure of Germany was changed fundamentally so that Münster lost its function as provincial capital and the castle lost its use as the seat of government. To prevent demolition, curator *Wilhelm Rave* initiated a campaign to rebuild the ruins of the castle as the main building of the *University of Münster*. Like many other historic buildings in Münster, only the exterior of the castle was reconstructed true to the original. The interior featured the simple architecture of the fifties.

The chief architect was *Hans Malwitz*. The Kavaliershäuschen were reconstructed as homes for employees of the district government, so that by the 1950s eight families could live inside both houses. The university tried to acquire the buildings in 1954. Five years later, an empty flat became the home for the student union AStA. Soon, the seminar for musicology followed. In April 1960, both buildings became the property of the university, the last tenants moved out by 1962. The southern *Kavaliershaus* was first occupied by AstA and the intenational office, later only by AStA. Musicology used the entire northern building until 2012. During the 1980s, now famous German musician and TV-show host *Goetz Alsmann* did his PhD on American Pop music here with *Maria Elisabeth Brockhoff*. In 2008, following the invitation of *Prof. Andreas Jacob* to give a talk in his seminar on Pop and Ideology inside the *Kavaliershaus*, *Alsmann* remarked that the seminar room had changed since his PhD time, but the smell was still the same. In 2011 he became an honorary professor of the University of Münster.

In 2013, after a fundamental renovation, the northern guardhouse became the seat of the Münster Graduate School of Evolution, as well as the Graduate Centre, University Marketing and University Fundraising. The northern *Kavaliershäuschen* has become the perfect location for students of different disciplines to discuss and understand important aspects of contemporary research and to further their knowledge in evolutionary thinking.

Ringbeck B (1993) Das Schloss zu Münster. Westfälische Kunststätten, 65; Geisberg M (1932) Die Bau- und Kunstdenkmäler von Westfalen. Band 41 (1. Teilband): Die Stadt Münster; http://www.wn.de/Muenster/2008/06/Nachrichten-Muenster-Goetz-Alsmann-Ein-Jazz-Experte-packt-aus; http://ieb.uni-muenster.de/mgse/

14.15-14.45: Introduction // Lecture hall "Badestraße"

14.15: Joachim Kurtz
Welcome address

14.30: Stephan Ludwig
Address from the Rector's Office

14.45-16.15: Public lectures 1 // Lecture hall "Badestraße"
Chair: Erich Bornberg-Bauer

14.45: **Sarah Teichmann** (p. 23)
EMBL-EBI and Wellcome Trust Sanger Institute, Cambridge
From protein interactions to gene expression distributions

15.30: **Ulrich Krohs** (p. 22)
Department of Philosophy, Münster
Function and dysfunction:
The evolution of normative differences

16.30-17.00: Coffee break // First floor "Kavaliershäuschen"

17.00 - 18.15: Session 1: "Biology meets Geology" // Ground floor "Kavaliershäuschen"
Chair: Francesco Catania

17.00: Claudia Acquisti & Harald Strauß (p. 26)
Biogeochemistry nurturing metagenomics: nitrogen flows from ecosystems to genes

17.15: Max Halama (p. 31)
Variations in marine nitrate concentration, Tyrrhenian Sea

17.30: John Vollmers (p. 38)
Comparative metatranscriptomic analyses along a depth gradient in the marine water column

17.45: Hannes Dittberner & Niklas Ohlmann (p. 28)
Stoichiogenomics of the Global Ocean Survey metagenomes in a biogeography context

18.00: Stefanie Henze (p. 32)
An experimental approach to directly quantify the impact of nitrogen limitation on genome

18.15-19.00 : Poster Session // First floor "Kavaliershäuschen

19.00-open end: Dinner buffet // Ground floor "Kavaliershäuschen

09.00 - 10.15: Session 2: "Evolution of Disease I" // Ground floor "Kavaliershäuschen"
Chair: Manuel Talarico

09.00: Neele Meyer (p. 33)
The effects of genotype and social experience during adolescence on anxiety-like behaviour later in life: pathology, constraint or adaptation?

09.15: Shirin Glander (p. 29)
How are flowering time and immune defense related in *Arabidopsis thaliana*?

09.30: Barbara Milutinovic (p. 34)
Coevolution between the red flour beetle *Tribolium castaneum* and the bacterium *Bacillus thuringiensis*

09.45: Hans-Dieter Görtz (p. 30)
Bacteria in Paramecium - what kind of symbionts?

10.00: Angela Noll (p. 35)
The generation and distribution of tailless retropseudogenes - News from an old acquaintance

10.15-10.45 : Coffee break // First floor "Kavaliershäuschen"

11.00-12.30: Public lectures 2 // Lecture hall "Badestraße"
Chair: Joachim Kurtz

11.00: **Ulrich Dobrindt** (p. 21)
Institute of Hygiene, Münster
The good, the bad and the ugly: Extraintestinal pathogenic or commensal *Escherichia coli* - what makes the difference?

11.45: **Thomas Bosch** (p. 20)
Zoological Institute, Kiel
The holobiont revolution: why bacteria will change the way you think about evolution and development

12.45-14.00: Lunch break// First floor "Kavaliershäuschen"

14.00 - 14.45: Session 3: "Evolution of Disease II" // Ground floor "Kavaliershäuschen"
Chair: Angela Noll

14.00: Monika Stoll (p. 37)
B3GAT2 - a novel susceptibility locus for pediatric thrombosis subject to recent selection

14.15: Christoph Preuss (p. 36)
Detection of rare genetic variants involved in congenital heart disease in the Quebec founder population

14.30: Michael Berger (p. 27)
Bacterial Virulence Gene Regulation: Just new pearls on a string or a new loop for the string?

14.45-15.30: Coffee break // First floor "Kavaliershäuschen"

PUBLIC LECTURES
(SPEAKERS IN ALPHABETICAL ORDER)

The holobiont revolution: why bacteria will change the way you think about evolution and development

For a long time, the main purpose of host-associated microbiology was to study pathogenic bacteria and infectious disease; the potential benefit of good bacteria remained unrecognised. However, individuals from Hydra to man are not solitary, homogenous entities but consist of complex communities of many species that likely evolved during a billion years of coexistence. The hologenome theory of evolution considers the holobiont with its hologenome as a unit of selection in evolution. Defining the individual microbe-host conversations in these consortia, therefore, is a challenging but necessary step on the path to understanding the function of the associations as a whole. Here I introduce Hydra as model for examining the entire innate immune system with its inputs, outputs and the interconnection. I will present observations which may have profound impact on understanding a strictly microbe/symbiont-dependent life style and its evolutionary consequences.

PUBLIC LECTURE

Thomas C. G. Bosch
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The good, the bad and the ugly: extraintestinal pathogenic or commensal Escherichia coli - what makes the difference?

PUBLIC LECTURE

Ulrich Dobrindt
Institute of Hygiene
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The species *Escherichia coli* is physiologically and metabolically versatile and includes non-pathogenic variants which belong to the normal gut flora of humans. In contrast, so-called extraintestinal pathogenic *E. coli* (ExPEC) can cause urinary tract infection, newborn meningitis or sepsis in humans, but also extraintestinal infection in animals. Acute ExPEC infection can sometimes develop into an asymptomatic state, where ExPEC persistently colonize the organ without causing overt symptoms of infection. This asymptomatic long-term colonization resembles commensalism. To understand which traits may distinguish ExPEC from *E. coli* commensals, and which bacterial traits are required to cause symptomatic infection, we are interested in virulence mechanisms, adaptation and evolution of ExPEC. Our current research activities include global functional and comparative studies of the *E. coli* genome content, gene regulation and physiology in order to better understand and eventually control ExPEC infection. Upon infection, ExPEC face not only the host and its immunological defense systems but they also have to compete for resources and thus the strategic plan of the persisting bacteria must include overcoming or evading the host, but also adaptation to the growth conditions in the new niche.

Our results indicate that ExPEC variants with the ability to cause persistent infection employ colonization strategies to withstand or avoid the induction of host immune responses as well as to efficiently utilize nutrients provided in this niche. In case of asymptomatic urinary bladder colonization, prolonged growth in the host is accompanied with genomic alterations that result in bacterial attenuation, thus contributing to bacterial adaptation to their host niche and a reduced activation of host immune responses. Our comparative and functional genome analyses of different pathogenic and non-pathogenic *E. coli* variants also indicate that differential expression of conserved genes can also contribute to a strain's ability to efficiently colonize certain niches and cause infection. These results further demonstrate the importance of *E. coli* genome plasticity as a prerequisite for adaptation and diversification of this species.

Function and dysfunction: the evolution of normative differences

Organs have functions within an organism, as do enzymes and other cellular and extracellular components. They may fulfill their function better or worse, adequately or impaired. Mal- and dysfunction may lead to conditions of disease. Functionality, thus, refers to a normal or even beneficial state. Being normative, however, the concept of function is in danger of conceptualizing nature as being goal-directed; it seems to import teleological thinking into natural science. In order to save the scientific status of biological function ascriptions, a naturalist account of functionality is required.

The first, conceptual part of the talk will discuss the basic options of how to deal with a normative concept of function within a naturalist framework, i.e., how to identify norms in nature without referring to anything like goals, aims, and intentions as possible sources of normativity.

The second, applied part will address the question how normative differences could evolve from a non-normative, prebiotic, physico-chemical world at first hand, and how new functions can come into being during evolutionary processes thereafter. I will show that the answers to those questions depend partly on the theory of function which is accepted. The final part of the talk demonstrates that asking the question for the evolution of functionality and of disease affects theory and model building in biology.

PUBLIC LECTURE

Ulrich Krohs
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From protein interactions to gene expresion distributions

PUBLIC LECTURE

Sarah Teichmann
EMBL-EBI and
Wellcome Trust Sanger Institute
Cambridge

The work in my group is focused on the evolution and dynamics of protein interactions and transcriptional regulatory interactions. Over the past decade, we have taken a global and genomic view in analysing large datasets using computational and mathematical methods in these two areas.

In the first part of my presentation, I will talk about our work on the evolution and assembly of protein complexes. We used three-dimensional structures of protein complexes to identify interface size as a determining principle of both evolutionary and assembly pathways (Levy et al., Nature, 2008; Marsh et al., Cell, in press), and to quantify the role of conformational change in assembly (Marsh & Teichmann, Structure, 2011). We also use crystal structures in recent work showing that the magnitude of the evolutionary constraint on a protein's surface is directly correlated to a protein's abundance in the cell (Levy et al., PNAS, 2012). These results have implications for structure prediction and engineering of protein complexes.

In the second part of my presentation, I will talk about recent work in which we have dissected the distribution of gene expression levels in mammalian cell populations, revealing two distinct mRNA abundance classes (Hebenstreit et al., 2011a). We provide evidence, including correlation of the two mRNA abundance classes with epigenetic modifications (Hebenstreit et al., 2011b), supporting the notion that these two classes correspond to functional versus non-functional proteins in cells. These findings have important implications, since the absolute abundances of both protein and mRNA levels are important not only for understanding evolutionary constraints, as mentioned above, but also many functional aspects of RNAs and proteins (stoichiometry, regulation etc).

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Talks
(speakers in alphabetical order)

Biogeochemistry nurturing metagenomics: nitrogen flows from ecosystems to genes

The elucidation of the genetic basis of ecosystem processes is one of the open challenges in the metagenomics era. Nitrogen, a fundamental building block of nucleotides and amino acids, often limiting in natural environments, plays a key role in shaping the interdependence between ecological and evolutionary dynamics. Recent advances have shown a direct impact of nitrogen availability from the environment on the evolution of genes and proteins of species, suggesting that the material costs of evolutionary change play a pivotal role in constraining the evolution of species in response to nutrient limitations in natural ecosystem. However, these results have primarily relied on few well established laboratory model organisms, leaving the question of the relevance of adaptation to nutrient availability in natural environments only partially addressed.

Recent advances in metagenomics allow to extend our understanding of the impact of the evolutionary history of nutrient limitation on molecular evolution providing a major arena to directly quantify the allocation of nutrients from the abiotic habitat to genes and proteins in environmental samples. Here we present an interdisciplinary approach merging biogechemistry and metatranscriptomics in an evolutionary framework. Central to our common research is a set of seawater samples collected at different depths of the water column during a cruise with RV Meteor during February 2012 in the Tyrrhenian Sea, where we have complemented biogeochemical analyses (dissolved nitrate concentration, nitrate isotopic composition, C/N ratio of particulate organic matter) with environmental genomics. Our focus is on the biogeochemical cycling of nitrogen in sea waters, and we investigate the evolutionary consequences of ecosystem nitrogen demand on the material costs of genetic change across trophic levels.

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Bacterial virulence gene regulation: just new pearls on a string or a new loop for the string?

Michael Berger
Microbial Genome Plasticity
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University of Münster

Escherichia coli (E.coli) is a gram negative, rod shaped commensal bacterium in the gut of animals and humans. By taking up foreign DNA, symbiotic *E.coli* can acquire genes that make the bacteria harmful and eventually result in disease or even death of the host organism. But in order to cause these negative effects, horizontally acquired virulence genes have to be expressed “properly”, i.e. to the “right” extent and at the “right” time. The mechanisms of gene regulation are extensively studied in the model organism *E.coli* and the available data makes it possible to create detailed maps of transcriptional networks that are based on the classic operon model of bacterial gene regulation by Jacob and Monod. This talk addresses the question, how horizontally acquired virulence gene clusters are integrated in pre-existing regulatory networks during genome evolution - and if the regulation of these strings of genes is best explained by the operon model.

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Nitrogen is a crucially limiting nutrient in oceans, and its availability varies substantially across latitude and longitude on surface waters (e.g., coastal and estuary regions are typically richer in nutrients than open ocean). Thus, oceans provide an ideal set of related ecosystems to study the effect of environmental nitrogen limitation on the evolution of protein composition in natural communities.

With a bioinformatics approach based on Hidden-Markov-Models we have extracted sets of homologous sequences of proteins involved in the metabolic response to different levels of nitrogen availability from over 40 million sequences coming from 30 different samples (Global Ocean Survey). A parsimonious strategy of nitrogen allocation in proteins predicts protein nitrogen content to be lower in open ocean than in coastal and estuary communities. Indeed, our analysis of the evolutionary dynamics of sets of homologous proteins shows a signature of nitrogen thrift in variable, fast-evolving sequences in communities adapted to open oceans.

These results directly link the environmental availability of nitrogen to different adaptive strategies of genome evolution, and reinforce the relevance of environmental nutrient availability in shaping evolutionary change.

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How are flowering time and immune defense related in Arabidopsis thaliana?

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In order to survive, plants rely heavily on having an effective immune system to combat pathogens, especially because they are not able to move and run away from danger. The plant immune system consists of two main pillars: an unspecific first-line defense mostly against bacteria on leaf surfaces and the R- (Resistance) gene mediated defense against specific pathogens. R-genes are highly specific against coevolving virulence factors of specific pathogens. Just like animals and humans, a plant can have a stronger (effective) or weaker defense machinery. They are resistant/tolerant or susceptible to a given class of pathogens, depending on which alleles they carry on immune gene loci.

Another trait of major importance for plants is flowering time. When looking at *Arabidopsis thaliana*, the longer the time until flowering, the bigger it grows and the more seeds are being produced. Generally, we can say that a plant with a late flowering time has a higher fitness. But if the plant waits a long time until producing seeds, the risk of an infection and dying before reproducing becomes higher. Thus, we would expect late flowering plants to benefit from investing more resources into strong immune defense. In nature we find large inter- and intra-specific variation regarding flowering time and indeed, what has often been observed by plant breeders, gardeners and scientist is that early flowering plants tend to be more susceptible to diseases than late flowering plants.

However, the genetic and evolutionary basis of the correlation between defense and development is not understood. Two mechanisms could be responsible: Genetic pleiotropy where the same gene(s) underlie both traits and variation is directly linked to both phenotypes; or co-evolution, where selection of one trait is linked to that of the other.

We have grown a collection of Recombinant Inbred Lines (RILs) from two parental accessions showing strong differences in flowering time (Bur-o and Col-o). With these plants, a QTL (Quantitative Trait Loci) analysis of flowering time will be associated with gene expression profiles of early and late flowering lines. If both traits have common underlying genes, we expect to find a correlation of the expression patterns of defense related genes, compared to non-defense related genes with flowering time and flowering time QTLs.

Bacteria in Paramecium - what kind of symbionts?

All eukaryotes are subject of parasitic attacks by various prokaryotes. More accurately, eukaryotes offer plenty of habitats for prokaryotes, and with the occurrence of eukaryotes and their great diversity new niches developed for the prokaryotes. Even at the present, eukaryotes - unicellular as well as multicellular - are still infected by bacteria or archaea. New infections are observed, that may turn out to be mutualistic or parasitic. New symbionts may originate from free living bacteria, but many are transferred from other hosts. Often, lateral transfers of certain genetic tools may have been a prerequisite for the successful adaptation of a (new) symbiont.

We know a lot about the biology of some free-living protists and their symbionts, e. g. Paramecium and some of its bacterial symbionts, but we are far from understanding the population biology/epidemiology, although such populations are found even in the *Schlossgräfte* in front of the *Institute for Evolution and Biodiversity* just across the street. As will be explained, even the valuation as beneficial symbiosis or pathogenic infection (disease) often is impossible at least on population level.

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Variations in marine nitrate concentration, Tyrrhenian Sea

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Nitrate is an important nutrient for primary producers in aquatic environments and its concentration varies widely within the marine water column. In the photic zone nitrate is frequently a limiting factor due to high consumption during primary production. This effect occurs particularly in water bodies with insufficient mixing of the water column and during times of high photosynthetic activity. Nitrate is released again to the water column during (aerobic) respiration and concentrations somewhat homogenize due to deep(er) water circulation.

Nitrate concentrations in the water column were determined for depth profiles at seven sites in the Tyrrhenian Sea, Mediterranean Sea. Samples were collected during the RV Meteor cruise M86/4 in February 2012. Immediately after recovery, samples were frozen at -80°C and kept frozen until the measurements.

High concentrations of chloride in seawater inhibit measurements of low nitrate concentrations using standard ion chromatography. Instead, nitrate concentrations were measured photometrically using a method well established in marine chemistry (Grashoff, 1983). Thereby, nitrate is reduced to nitrite by copperized cadmium fillings in a reduction column and fixed in a complex by sulphanilamide, which shows a color reaction with N-(1-naphtylethylenediamine dihydrochloride).

Nitrate concentrations in Tyrrhenian Sea depth profiles range from 0.02 µmol/L to 8.92 µmol/L and display a nutrient-like distribution in the water column with low concentrations in the upper column and high concentrations near the sea floor. Below the photic zone, nitrate concentrations were high and stable, except for a clear decrease in nitrate concentration (on average around 30%) at all sites in water depth between 1000 and 1500 m.

Grashoff, K. (1983) (ed) Methods of Seawater Analysis. Weinheim, Verlag Chemie.

Even though the use of data from natural communities offers a realistic picture of the dynamics affecting natural environments, those data tend to be difficult to interpret ecosystem evolution since there are many other factors that can have an influence. Therefore, parallel to the analysis of environmental samples, we are studying the response to nitrogen starvation experimentally. Here, we use controlled laboratory conditions, in which nitrogen is the only stress factor, which could have an impact on genome evolution of the model organism *Escherichia coli*.

In this context, we want to investigate whether over macro-evolutionary time scales the composition of transcripts and proteins highly expressed in response to nitrogen starvation has been shaped by selection, favoring monomer usage biases that conserve nitrogen. To test this hypothesis, we first have designed a starvation experiment integrating transcriptomics and metabolomics to follow the flow of nitrogen from the environment into the building blocks of the bacterial genome.

Second, we are currently using experimental evolution of *E. coli* by culturing this fast evolving species in continuous culture in which nitrogen limitation is directly controlled for about 1000 generations. In this setting, we expect to see evolution in action, reducing nitrogen allocation (especially in genes that are highly expressed) by promoting the use of bases and amino acids that contain fewer nitrogen atoms. Proteomics, transcriptomics and metabolomics data will be produced at regular intervals of time, enabling a direct comparison of the evolutionary dynamics of genes and proteins in response to different levels of nitrogen availability.

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The effects of genotype and social experience during adolescence on anxiety-like behaviour later in life: pathology, constraint or adaptation?

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Across mammalian species, behavioural traits like anxiety are means to optimally cope with environmental challenges. However, in their exaggerated form they pose serious psychiatric problems to human societies and are regarded as pathologies from a biomedical viewpoint. Yet, from an evolutionary perspective these traits might represent adaptations to the present environment. Levels of anxiety can be shaped by genotype and experiences during development, in particular the time of adolescence. To elucidate (1) how levels of anxiety are shaped by genotype and experience during adolescence and (2) whether the resulting traits adjust the individuals to the current environmental conditions, experiments were conducted with serotonin-transporter (5-HTT) knockout mice, a well-established model for the study of anxiety.

During adolescence, males of all three genotypes (wildtype, heterozygous and homozygous 5-HTT knockout mice) either experienced an excellent social environment or found themselves in an adverse situation. In accordance with our hypothesis, social adversity resulted in increased levels of faecal corticosterone metabolites, especially in homozygous 5-HTT knock-out mice, and a reduced body weight gain.

Surprisingly, however, unfavourable conditions during adolescence led to a decrease in anxiety-like behaviour and an increase in exploratory locomotion. Whereas adverse social conditions seem to adapt the animal towards a more exploratory and risk-prone behaviour, beneficial social conditions may lead to an adaptation towards a resource defence strategy.

Coevolution between the red flour beetle Tribolium castaneum and the bacterium Bacillus thuringiensis

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Parasites and pathogens are everywhere, and they often strongly reduce the fitness of their hosts. Hosts, on the other hand, employ a diversity of defence mechanisms that may help them to cope with their parasites. Because of the high selection pressures involved, co-evolution between hosts and parasites is supposed to be characterised by fast evolutionary change. Host-parasite interactions are therefore ideal systems for studying co-evolutionary processes of adaptation and counter-adaptation (Red Queen Dynamics) in the laboratory.

We performed evolution experiments using the red flour beetle as a host and *Bacillus thuringiensis* bacteria as a microparasite. Both antagonists were allowed to co-adapt, while in control treatments only one side was allowed to adapt. We will report the first results on parasite virulence and host resistance after seven host generations of selection.

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The generation and distribution of tailless retropseudogenes: News from an old acquaintance

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In 2004, Schmitz et al. detected a new kind of mammalian-specific retropseudogenes, prominently deduced from highly expressed RNAs. They are flanked by specific target site duplications indicating that they were generated by a LINE1-dependent retrotransposition event within the genomic DNA. However, the also expected poly(A)-tail (and sometimes further bases) at the 3'-end of the retropseudogenes is missing why these forms are called "tailless retropseudogenes". Although they represent a new aspect of LINE1-derived retroposition, this novel class of pseudogenes is mainly unexplored and still unacknowledged.

Now, newly conducted analyses give us additional insights into the distribution of these elements throughout the domain of eukaryotes. We could figure out that the appearance and quantity of tailless retropseudogenes fits very well the LINE1-activity within the huge amount of different species analyzed during this work. Furthermore, we could detect some previously unknown and intriguing features of the LINE1-dependent mechanism of retroposition. Among other things, the LINE1-element shows activity not only within the cytoplasm but also within the nucleoplasm providing a new approach for future analyses growing on the activity of this transposable element. In addition, it seems that this element provides the possibility to recognize RNAs without any poly(A)-tail. Thereby, mainly producing tailless retropseudogenes via reverse transcription and re-integration of the established complementary DNA back into the genome.

This presentation should help to understand and to acknowledge this fascinating type of LINE1-derived retroposition events while illustrating the possible mechanism of amplification and the distribution of tailless retropseudogenes within the clades of eukaryotes.

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Detection of rare genetic variants involved in congenital heart disease in the Quebec founder population

Congenital heart disease (CHD) is the most common birth defect, the most common cause of infant morbidity and the second most common cause of infant mortality in the western world and Asia. It is defined as a structural malformation of the heart and/or great vessels that is present at birth and is of functional significance. The global burden of CHD exceeds that of other major diseases with underlying complex genetic basis such as diabetes, hypertensive heart disease, asthma, or rheumatoid arthritis.

We have recently sequenced 96 individuals which have been recruited in Quebec with congenital heart disease, consisting of septal defects, aortic valve lesions, co-arctation, and supraventricular arrhythmias. Several individuals are members of multiple affected families for which genetic linkage to the disease has been established. However, since genetic and phenotypic heterogenity is the norm even for rare mendelian disease such as CHD, it remains a challenging tasks to make accurate and clinically useful predictions regarding the disease phenotype.

Here, we present our recent results from a whole-exome sequencing approach highlighting that genomic, phenotypic and population context matters. We identified a number of rare disease variants co-segregating in different families which are enriched for disease related molecular pathways (KEGG) and overlap with a number of biological plausible candidate genes reported in the literature. In order to test our hypothesis whether the excess of rare, population specific variants contributed to disease risk we will validate the identified variants in an independent control cohort from Quebec.

Evolutionary analysis will serve as a powerful tool to determine the origin and frequencies of the derived mutations. Our long-range goal is to understand why mutations with a strong deleterious effect remained in the French-Canadian population, if they are dependent on a specific genetic background and if both common and rare variation in modifier genes account for the large differences between individual disease risk.

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B3GAT2 - A NOVEL SUSCEPTIBILITY LOCUS FOR PEDIATRIC THROMBOSIS SUBJECT TO RECENT SELECTION

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Recently, we implicated genomic regions exhibiting traces of recent selective sweeps and a clustering of association signals in the genomic architecture underlying common, complex diseases. Here, a genome-wide association study (GWAS) in 212 nuclear families with pediatric venous thromboembolism (VTE) is presented as an example of a disease that supports our initial hypothesis. In this study, 3 SNPs exceeded the threshold for genome-wide significance as determined by 100.000 bootstrap permutations (p<10-5). Among these, 2 SNPs reside in a region on chromosome 6q13 comprising the gene for beta-1,3-glucoronyltransferase 2 (B3GAT2), a member of the human natural killer 1 (HNK1) carbohydrate pathway, and are associated with pediatric VTE (rs1304029; p=7.76x10-7 and rs2748331; p=1.5x10-6). SNP rs2748331 was replicated (p=0.00719) in an independent study sample coming from our GWAS on pediatric thromboembolic stroke (TS) (combined p=2.152x10-7). B3GAT2 has been associated with fetal hemoglobin levels and an increased risk for sickle cell anemia in an African population, a trait that has been shown to be subject to natural selection. Estimation of iHS and FST values in YRI and CEU populations revealed signatures of recent selection at the B3GAT2 locus in both populations. Our study suggests that genetic hitchhiking may have led to the accumulation of deleterious alleles conferring risk for VTE, and further underlines the importance of a chronically activated coagulation system in the pathogenesis of VTE.

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Comparative metatranscriptomic analyses along a depth gradient in the marine water column

Metatranscriptomics, the direct sequencing of RNA molecules from the environment, is one of the emerging technologies to investigate environmental adaptations in natural communities. The tremendous potential of this approach lies primarily on the possibility to identify pools of functional genes involved in the key biogeochemical reactions directly in natural ecosystems and to distinguish between active and inactive members of bacterial communities.

Here we use metatranscriptomics to study adaptation to different levels of nitrogen availability in marine habitats. The availability of nutrients varies substantially across the water column, with sea surface environments poor in nitrogen and exposed to high-light intensity and deep sea environments at lower light intensity, limiting photosynthesis. Thus, the marine water column provides an ideal set of related ecosystems to study the effect of nitrogen limitation on the evolution of protein and transcript composition. Under conditions of severe N limitation, growth and reproduction of individuals with lower allocation of nitrogen in their genes should be favored by natural selection. Thus, species exposed to chronic nitrogen limitation are expected to exhibit a progressive evolutionary shift in the frequencies of nucleotides towards an enrichment of nucleotides that contain lower relative concentrations of nitrogen (compared to carbon).

In order to test this hypothesis, we are using samples collected during the RV Meteor cruise M86/4 in February 2012, complementing the biogeochemical analyses by Halama et al. with biological data on community structure and transcriptional activity. RNA was extracted using mechanical cell disruption and a combination of phenol- and column-based purification methods which exclude small RNA types such as tRNAs and 5S rRNA. After fragmentation and conversion to cDNA, the total metatranscriptome is sequenced using a paired-end approach on an Illumina GAIIx System. Ribosomal RNA gene sequences will be filtered from the dataset and the remaining reads will be assembled into larger mRNA transcripts. These data will allow to study the evolutionary dynamics of nitrogen allocation in sets of homologous in the different microbial communities adapted to different ocean depths along the nitrogen gradient.

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Origin of the 1918 pandemic H1N1 influenza A virus as studied by codon usage patterns and phylogenetic analysis

The pandemic of 1918 was caused by an *H1N1 influenza A* virus, which is a negative strand RNA virus; however, little is known about the nature of its direct ancestral strains. Here we applied a broad genetic and phylogenetic analysis of a wide range of influenza virus genes, in particular the PB1 gene, to gain information about the phylogenetic relatedness of the 1918 *H1N1* virus. We compared the RNA genome of the 1918 strain to many other influenza strains of different origin by several means, including relative synonymous codon usage (RSCU), effective number of codons (ENC), and phylogenetic relationship. We found that the PB1 gene of the 1918 pandemic virus had ENC values similar to the *H1N1* classical swine and human viruses, but different ENC values from avian as well as *H2N2* and *H3N2* human viruses. Also, according to the RSCU of the PB1 gene, the 1918 virus grouped with all human isolates and "classical" swine *H1N1* viruses. The phylogenetic studies of all eight RNA gene segments of influenza A viruses may indicate that the 1918 pandemic strain originated from a *H1N1* swine virus, which itself might be derived from a *H1N1* avian precursor, which was separated from the bulk of other avian viruses in toto a long time ago. The high stability of the RSCU pattern of the PB1 gene indicated that the integrity of RNA structure is more important for influenza virus evolution than previously thought.

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The barA/uvrY two-component system determinants: mutational hotspots and its role for urovirulence in uropathogenic Escherichia coli

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Urinary tract infections (UTI) are among the most common infectious diseases in humans and thus constitute a major health problem. Escherichia coli is responsible for more than 80 % of all UTI cases which may be symptomatic or asymptomatic. Asymptomatic bacteriuria (ABU) is the most common form of UTI and represents long term bacterial colonization of the urinary tract without characteristic symptoms of an infection. The reason why ABU-associated *E. coli* isolates fail to induce a host inflammatory response is so far not completely understood yet. Many ABU E. coli isolates seem to lack virulence phenotypes, even though they possess significant numbers of known virulence genes of uropathogenic *E. coli* (UPEC). It has been shown that such isolates do not express functional UPEC virulence factors. This seems to be the consequence of an evolutionary pressure that results in attenuation of virulence genes and genome reduction and finally in a commensal lifestyle. Besides the presence of UPEC virulence factors, the regulation of virulence gene expression contributes to the virulence potential. Two-component regulatory systems (TCS) play an important role for rapid adaptation to changes in environmental conditions.

One TCS that was shown to be involved in regulation of urovirulence is the BarA/UvrY two-component system. It controls a global regulatory network that affects a multitude of cellular functions, e.g. regulation of carbon metabolism, biofilm formation or motility and adhesion. As genes coding for components of the BarA/UvrY TCS were shown to be frequently mutated upon prolonged growth in the bladder, we hypothesized that this could constitute an adaption strategy to achieve long-term persistence in the bladder.

In order to learn more about the role of BarA/UvrY in prolonged growth in the urinary tract, we chose two complementary approaches. (i) We sequenced the barA and uvrY genes in 192 *E. coli* strains representing ABU, UTI and fecal isolates, in order to see, if the these genes represent a specific mutational hotspot for bacterial adaption during long-term growth in the urinary tract. (ii) based on the occurrence of SNPs, barA and uvrY variants were selected to be tested in functional assays in order to test the impact of these SNPs on protein function in vitro. The results will be discussed in the light of bacterial adaption strategies to changing environmental conditions.

What is the evolutionary significance of DNA splicing?

DNA splicing can drive extensive rearrangements in the eukaryotic genome. Perhaps best known for shaping immune genes in vertebrates, this process crucially mediates the elimination of germline DNA sequences when germline cells differentiate into somatic cells.

DNA splicing plays a central role in the life of ciliated protozoa. In ciliates, DNA splicing mediates the excision of regions of DNA from the entire zygotic genome at every event of sexual reproduction. More specifically, ciliates are characterized by nuclear dimorphism. These single-celled organisms contain 1) a diploid "germline" micronucleus (MIC), silent during the vegetative life of the cell, which undergoes meiosis and transmits the genetic information to the next sexual generation; and 2) a polyploid "somatic" macronucleus (MAC), which is a streamlined version of the germline genome and determines the phenotype. The assembly of a functional MAC is influenced by the configuration of the maternal MAC (which is lost soon after the sexual event) and requires the precise excision or splicing of thousands of interstitial DNA segments known as the Internal Eliminated Sequences (IES).

Although much work has been done to understand the mechanics of DNA splicing, the evolutionary significance of this process remains essentially unexplored. In the ciliate Paramecium, IES excision has been found to be typically precise, however events of incomplete excision occur and generate distinct DNA isoforms, which can influence the assembly of the MAC at the subsequent sexual generation. Thus, DNA splicing "errors" in one generation might ultimately become integrant part of the MAC and acquire biological significance in subsequent sexual generations.

In my PhD work, I am planning to apply comparative genomics approaches to examine the evolution and the turn over rate of IESs as well as their differential excision/retention between several closely-related species of Paramecium. Moreover, I am planning to test the biological function and the physiological effects of already reported and newly uncovered events of differential DNA splicing between species, some of which might be associated with recent events of speciation.

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Analysis of retrogene repertoire in two green algae Volvox carteri and Chlamydomonas reinhardtii

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Retroposition, one of the processes of copying the genetic material, is an important RNA-based mechanism leading to the evolution of new genes. Most of the retroposed genes encounter strong purifying selection right after their insertion into their new genomic location, however, some endure and contribute to the formation of lineage-specific traits. Despite numerous studies of retrogenes in animals, like human or fruit fly, or plants, like arabidopsis or maize, very little is known about retrogene landscape in green algae. The current availability of algal genomes, both uni- and multicellular, provides a perfect opportunity to investigate this phenomenon. Here we present the results of a comparative analysis of *Chlamydomonas reinhardtii* and *Volvox carteri* genomes to identify retrogene candidates. As a result of our survey we report identification of 295 retrogene candidates in both genomes, with the vast majority being found in the genome of multicellular Volvox. Many of the identified retrocopies were found to be lineage-specific and some of them seem to have lost their parental genes as they were only found in the outgroup species. The study was enriched with phylogenetic and functional analyses to conclude whether retrogenes could have contributed to the evolution of multicellularity in the green algae.

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Eukaryotic genes contain exons and (spliceosomal) introns. Exons are sequences included in mature messenger RNAs and are used for protein manufacturing, whereas introns are intervening sequences which are eliminated before the synthesis of amino acid chains, in a crucial step known as splicing. The splicing process is not error-free: intronic/exonic sequences can be included or excluded from mRNAs, leading to multiple alternative transcripts of the same gene. The origin and the evolution of the exon-intron structure remain mysterious.

Intronization is the conversion of exonic sequences into intronic sequences. Catania and Lynch have proposed a model based on intronization (PLoS Biology, 2008; BioEssays 2013) in which the physical establishment and preferential distribution of introns along a transcript results from the interactions between cellular processes such as mRNA splicing, mRNA surveillance, mRNA capping, and mRNA cleavage/polyadenylation. This model introduces the concept of mRNA territories which affect splicing efficiency, puts forward predictions, and suggests ramifications that require thorough examinations. We have begun this exploratory work and we present here some of our preliminary results.

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Horizontally acquired adaptation and pathogenicity of Mycobacterium tuberculosis

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The *Mycobacterium tuberculosis* complex is the causative agent of tuberculosis, which still infects one people out of three worldwide. Emergence of highly virulent and drug-resistant strains raised therapeutic failures. Models of evolution of the Mycobacterium tuberculosis complex genomes proposed that harmless soil mycobacteria gained genes in a step-by-step manner through horizontal transfers that brought pathogenic lifestyle, host adaptation and virulence variability [1]. To detect those acquired elements, we adapted two parametric methods based on the tetranucleotide frequencies and codon usage [2]. We selected 29 genomes gathering nonpathogenic soil mycobacteria, pathogenic species of variable animals host range including Human and species of the *Mycobacterium tuberculosis* complex which are responsible for tuberculosis in mammals. Horizontally acquired orthologous genes were clustered with the Reciprocal Best Hit criterion and then used to built a phylogenetic tree under a presence/absence parsimony model, thus giving the most probable node of acquisition of each transfer.

In the 29 genomes, an average of 11,7% of genomic DNA is horizontally acquired, including 4271 groups of orthologous genes acquired all along the evolution history of the species of this study. We selected genes that were acquired around the nodes that segregate phenotypes of pathogenicity, adaptation to the Human and the formation of the *Mycobacterium tuberculosis* complex. Those three major evolutionary nodes present several acquisitions in which we found characterised virulence factors and essential products. Analysis of the origin of these transferred regions showed an evolution of the donor species spectrum, with an increase in proteobacteria and viruses compared to the transferred regions found in the soil species. Functional classes of the transferred genes significantly differs from the rest of the genome with an enrichment in virulence and adaptation factors, phages and insertion sequences and PE/PPE proteins family members. The results match and support the proposed model of evolution of the *Mycobacterium tuberculosis* complex, in which the horizontal gene transfers provided features that changed life style and host range.

[1] Jichan Jang, Jennifer Becq, Brigitte Gicquel, Patrick Deschavanne, and Olivier Neyrolles. Horizontally acquired genomic islands in the tubercle bacilli. Trends Microbiol, 16(7):303–8, Jul 2008.
[2] Jennifer Becq, Cécile Churlaud, and Patrick Deschavanne. A benchmark of parametric methods for horizontal transfers detection. PLoS One, 5(4):e9989, 2010.

Long-term adaptation of Staphylococcus aureus in the airways of a cystic fibrosis patient: RNA-sequencing studying transcriptional changes

The lung of Cystic fibrosis (CF) patients is often colonized or infected by *Staphylococcus aureus* for many years, mostly by one individual clone. To survive in this hostile environment (abundance of neutrophils, antibiotic therapy, co-infections with other pathogens), *S. aureus* needs to adapt. Here, we study the adaptation of two isogenic *S. aureus* isolates, "early" and "late", which were recovered from a CF patient 13 years apart.

To detect transcriptional changes in the late isolate, we cultivated both isolates under *in vitro* CF lung conditions and sequenced rRNA-depleted total RNA using Illumina RNA-sequencing (RNA-Seq). Bioinformatic analyses were carried out using the DNASTAR-QSeq software (Lasergene) selecting *S. aureus* strain MSSA476 as reference sequence. Comparative transcriptomic analysis via scatter plot view revealed significant differences between the early and late isolate under aerobic conditions. Overall, of the 2,339 genes expressed in both isolates, 513 (21.9%) genes were up-regulated in the late isolate with ≥ 2-fold change (f), 96 genes ≥ 4f, and 17 genes ≥ 8f, respectively. In contrast, only 143 (6.1%) genes were down-regulated ≥ 2f, 41 genes ≥ 4f, and 14 genes ≥ 8f.

The ≥ 8f up-regulated genes comprised, for example, the virulence associated gene *spa*, encoding for an IgG-binding protein that is essential for immune evasion. Of the Ess secretion pathway, which comprises a cluster of at least 9 genes, five genes, namely *esxA, esaA, essA, essB* and *essC* were also up-regulated in the late isolate. Among the ≥ 8f down-regulated genes, all genes of the *kdpFABC* operon, except *kdpF*, were detected. Quantitative real-time-PCR confirmed the changes determined by RNA-Seq.

In summary, RNA-Seq enables us to characterize the whole transcriptome. Our findings will elucidate crucial steps in the host-pathogen interaction responsible for long-term adaptation of *S. aureus* in the CF lung.

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Does personality correlate with immune competence?
An experimental design for the stickleback-schistocephalus system

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The three spined stickleback (*Gasterosteus aculeatus*) has long been a model organism in behavioural and immunological studies. Recently, there has been a growing interest in personality or behavioural syndromes in sticklebacks. Both terms refer to consistent individual differences in the expression of correlated behaviours across time and/or across contexts. Such a link was reported for aggressive and risk-taking behaviours in several populations of sticklebacks around the world. If a population exhibits correlated behaviours, the individuals will differ in their behavioural types: for example, one individual tends to be bolder and more aggressive relative to the others. These various behavioural types might be exposed to infection pressure by parasites in a different manner. Bold, more explorative individuals might encounter higher infection risk, than shy, less explorative individuals. Consequently, a bold individual should have a higher immune activity than a shy individual.

In my PhD project, I will explore whether personality is linked to immunity and resistance. Therefore, I will assess if laboratory bread sticklebacks from a German population near Münster exhibit different behavioural types and if these behavioural types are consistent across contexts and across time. A number of behavioural challenges will characterize their aggressive and risk-taking behaviours at different stages of the life history of the individuals (juvenile, subadult and adult). After the juvenile phase but before the animals reach the subadult stage, a fraction of the fish will be exposed to the tapeworm *Schistocephalus solidus*. At the end of the observation period, leucocytes from the head kidneys of the sticklebacks will be isolated and functional immune parameters, such as the respiratory burst activity and the granulocyte to lymphocyte ratio will be determined. Behavioural and immunological data will be explored for potential correlations.

Evidence of nitrogen limitation in the transcriptional response to nutrient stress in Saccharomyces cerevisiae

MÜNSTER GRADUATE SCHOOL OF EVOLUTION

Merging the perspective of biological stoichiometry and molecular evolution, recent evidence has revealed that the atomic composition of proteins and genes have adaptive significance. It was shown that this phenomenon plays a key role in many diverse fundamental molecular processes such as gene duplication, and evolution of cellular components and metabolic functions (reviewed in [1]). Organisms constantly perceive and integrate information from their habitats, and have evolved mechanisms to respond to limiting availability of key nutrients with specific transcriptional responses. In this context, the possibility exists that over macro-evolutionary time scales the composition of proteins over-expressed in response to nitrogen starvation has been shaped by selection, favoring amino acid usage biases that conserve nitrogen.

To test this hypothesis, we use *S. cerevisiae* as a model organism to assess the role of nutrient stress on the evolution of the transcriptional response. With a genome-wide approach, we quantify the level of nitrogen allocation in proteins highly expressed in response to nitrogen starvation (data from 3]). Comparing over 5000 proteins, our results show a significant decrease of over 5% in nitrogen content of proteins highly expressed under nitrogen starvation compared to the rest of the proteome. We show that eco-physiological selection for nitrogen conservation specifically targets proteins highly expressed in response to nitrogen limitation. To understand the nitrogen conservation mechanism more precisely we go beyond the nitrogen content marker and observed lower composition of N rich amino acids, specifically in highly expressed proteins in response to nitrogen starvation. To rectify the effect of functional constraints on compositional biases of N rich amino acids in highly expressed proteins in response to nitrogen starvation, we dissect the functional constraints from the material cost by estimating the evolutionary rate for each residue and taking it as a proxy for functional constraints. Our results shows selection for elemental allocation in sites, which are under lower functional constraints.

These findings suggest that the evolutionary history of nitrogen availability has directly constrained the molecular architecture of the biotic processes that enable cells to respond to this ecologically relevant environmental cue.

1.TREE , 2010, 26:38-44; 2.Mol Syst Biol, 2006, 1038/msb4100069; 3.Plos Comp. Biol.,2009, 5(1): e1000270

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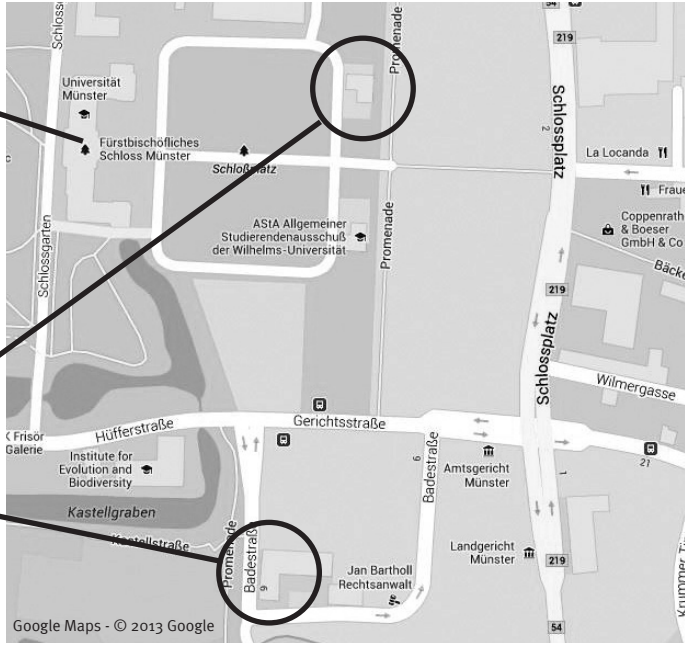

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Impressum

Abstract book

3rd annual Münster Graduate School of Evolution Symposium

12 - 13 June 2013
University of Münster

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Manuel Talarico

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