COLOGNE SPRING MEETING 2012 | FEBRUARY 22–24

MOLECULAR ECOLOGY AND EVOLUTION

AN INTERNATIONAL SYMPOSIUM OF SFB 680

SATELLITE MEETING | FEBRUARY 24–25
VIRAL EVOLUTION: FROM GENETICS TO EPIDEMICS

University of Cologne
Institute for Genetics
Institute for Zoology
Institute for Theoretical Physics

Max-Planck-Institute for Plant Breeding Research

SFB 680
MOLECULAR BASIS OF EVOLUTIONARY INNOVATIONS

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This year's Cologne Spring meeting brings top international speakers together to discuss links between ecology and evolution at the molecular level. Specific topics include microbial and viral systems, host-pathogen interactions, plant communities and food webs.

ORGANIZERS

Eric von Elert (Zoological Institute, University of Cologne)
Jonathan Howard (Institute for Genetics, University of Cologne)
Maarten Koornneef (Max-Planck-Institute for Plant Breeding Research, Cologne)
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Thomas Wiehe (Institute for Genetics, University of Cologne)

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SPEAKERS

Ian Thomas Baldwin | MPI Jena
Nitin Baliga | SB Seattle
Andrew Beckerman | University of Sheffield
Michael Boots | University of Sheffield
John Colbourne | Indiana University
David Conway | LSHTM London
Santiago Elena | IBMCP Valencia
Duncan Greig | MPI Plön
Bryan Grenfell | Princeton University
Eddie Holmes | Pennsylvania State University
Peter Keightley | University of Edinburgh
Britt Koskella | University of Oxford
Juliette de Meaux | University of Münster
Thomas Mitchell-Olds | Duke University
Hélène Morlon | Ecole Polytechnique Paris
Wayne Potts | University of Utah
Michael Purugganan | New York University
Andrew Rambaut | University of Edinburgh
Walter Salzburger | University of Basel
Johanna Schmitt | Brown University
Ralf Sommer | MPI Tübingen
Miltos Tsiantis | University of Oxford
Diethardt Tautz | MPI Plön
Daniel Weinreich | Brown University

COLOGNE SPRING MEETING 2012 – MOLECULAR ECOLOGY AND EVOLUTION

WEDNESDAY | FEBRUARY 22

14:00–14:10	Welcome: Michael Lässig
Session 1	**Chair: Maarten Koornneef**
14:10–14:50	**Thomas Mitchell-Olds**, Evolution of complex traits in complex environments
14:50–15:30	**Johanna Schmitt**, Mapping local adaptation in Arabidopsis thaliana
15:30–16:10	**Juliette de Meaux**, Evolution of microRNA824 in Arabidopsis thaliana
16:10–16:40	**Coffee Break**
16:40–17:20	**Ralf Sommer**, The nematode Pristionchus pacificus as a model system for integrative studies in evolutionary biology and ecology
17:20–18:00	**Michael Purugganan**, Adaptation and regulation: Insights from single gene to genome-wide perspectives
18:00–18:40	**Britt Koskella**, Evolution of microbes and viruses in plant communities
19:00	**Welcome Reception** at the Institute for Genetics

THURSDAY | FEBRUARY 23

Session 2	**Chair: Eric von Elert**
08:30 – 09:10	**Miltos Tsiantis**, Towards understanding development and evolution of leaf shape
09:10 – 09:50	**Diethardt Tautz**, Tracing the genetic basis of adaptations in the house mouse
09:50–10:30	**Peter Keightley**, Inferring adaptive evolution in the mouse genome
10:30–11:00	**Coffee Break**
11:00–11:40	**John Colbourne**, Duplicating genes allow Daphnia populations to thrive in toxic environments
11:40–12:20	**Andrew Beckerman**, The molecular ecology of species interactions
12:20–14:00	**Lunch Break**
Session 3	**Chair: Thomas Wiehe**
14:00–14:40	**Walter Salzburger**, Evolution in Darwin's dreamponds - The adaptive radiations of cichlid fishes in East Africa
14:40–15:20	**Hélène Morlon**, Explosive radiation of a bacterial species group
15:20–15:50	**Coffee Break**
15:50–16:30	**Daniel Weinreich**, Adaptation in changing environments: Influence of changing patterns of epistasis in the TEM-1 antibiotic-resistance gene
16:30–17:10	**Nitin Baliga**, Environmentally structured evolution of complex biological networks
17:10–17:50	**Duncan Greig**, Reproductive isolation in Saccharomyces

PUBLIC LECTURE

	Chair: Jonathan Howard
18:00–19:00	**Ian Thomas Baldwin**, Insect resistance for the long haul: Lessons from a native plant

FRIDAY | FEBRUARY 24

Session 4	**Chair: Jonathan Howard**
08:30 – 09:10	**David Conway**, Parasite population genomic studies towards develop ment of a blood stage malaria vaccine
09:10 – 09:50	**Michael Boots**, Ecology and epidemiology as drivers of host-parasite coevolutionary dynamics
09:50–10:30	**Wayne Potts**, Experimental evolution of viral fitness and virulence: satisfying the red queen
10:30–11:00	**Coffee Break**
11:00–11:40	**Santiago Elena**, Uncertainties in estimating viral fitness
11:40–12:20	**Eddie Holmes**, Cross-species transmission and emergence of viruses
12:20–13:30	**Lunch Break**
13:30 – 14:10	**Bryan Grenfell**, Vaccination and the dynamics of immune escape
14:10–14:50	**Andrew Rambaut**, Linking spatial epidemiology with antigenic and genetic evolution of human influenza virus
15:00	**Satellite Meeting starts**

Ian Thomas Baldwin

Insect resistance for the long haul: lessons from a native plant

A large fraction of our agricultural crops are protected from attack by insect pests by a single protein: the BT toxin. Whether this is a stable pest control strategy remains to be seen, but studies of how native plants have evolved resistance strategies for their eon-long battles with insect herbivores, suggest otherwise. Native plants produce a plethora of secondary metabolites, long thought to be metabolic waste products, but are now recognized to play a variety of different ecological roles in the life of a plant that include protection against abiotic and biotic stresses and communication with other plants and heterotrophs in the surrounding community. The best way to uncover the roles that these metabolites play is to query the ecosystem in which the plants evolved; however, the research is this field has a history of anthropomorphic metaphors which may prevent researchers from placing their experiments in an appropriate ecological context. This talk will describe an approach which attempts to "phytopomorphize" the researcher by using field experiments with wild-type and transformed lines of the native tobacco, *Nicotiana attenuata,* which are silenced in their ability to produce or respond to particular secondary metabolites. Examples of the roles that secondary metabolites play in herbivore defence will illustrate the approach and describe the lengths that plants must go to evolve enduring resistance against co-evolved herbivores.

References:

Weinhold A, Baldwin IT. Trichome-derived O-acyl sugars are a first meal for caterpillars that tags them for predation. 2011 PNAS 108 (19) 7855 – 7859 www.pnas.org/cgi/doi/10.1073/pnas.1101306108

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Heiling, S., Schuman M.C., Schoettner, M., Mukerjee, P., Berger, B., Schneider, B., Jassbi, A.R., Baldwin, I.T. 2010 Jasmonate and ppHsystemin regulate key malonylation steps of 16-hydroxygeranyllinalool diterpene glycosides, the most abundant and effective direct defense against herbivores in *Nicotiana attenuata*. **The Plant Cell** 22: 273-292.

Nitin Baliga

Environmentally structured evolution of complex biological networks

Microbes exist almost everywhere including environments that were previously considered uninhabitable. Understanding the molecular and systems level basis for this remarkable colonization ability of microbes holds the key to solving some of the most challenging environmental and health problems. I will discuss how evolutionary constraints imposed by a structured environment makes it possible to infer and mathematically model the architecture of biological networks associated with niche adaptation of any organism. These models link systems scale behavior to molecular mechanism and predict cellular responses to new and previously unstudied environmental perturbations. By enabling rational systems re-engineering with parts from any organism, these multiscale models will unlock the biotechnology potential of the vast biodiversity on our planet.

Andrew Beckerman

The Molecular Ecology of Species Interactions

Organisms are naturally exposed to a range of biological enemies, the most important of which are predators and parasites. Few would argue that a diverse set of strategies have evolved in animals to defend against predators and parasites, including structural barriers, changes in growth and reproduction, and the immune system. Despite decades of research into these defences, very little is known about how animal defence against predators and parasites might interact or whether they share a similar physiological basis. Here I suggest and document that the ability to deal with stress is largely based on shared developmental mechanisms that govern the timing of life cycles and the spatial patterning of development. I suggest that molecular ecology is poised to reveal a conserved set of molecular and physiological mechanisms that underpin adaptation in the face of stressful interactions.

Michael Boots

Ecology and epidemiology as drivers of host-parasite coevolutionary dynamics

Understanding the processes that generate and maintain diversity in hosts and parasites is critical to understanding the epidemiology, evolution and control of infectious disease. Considerable variation in the infectivity of parasites and the harm that they cause on the one hand and the resistance and tolerance of hosts on the other is a characteristic of a wide range of infectious disease interactions. This diversity determines individual disease risk, influences disease outbreak and spread, and has the potential to affect control interventions and treatment. It is also clearly fundamental to the evolution of hosts and parasites. However, we lack an understanding of how processes such as infection specificity, demography and epidemiology determine the evolution and maintenance of host-parasite diversity. Here I present a theory on the role of epidemiological feedbacks in the evolution and coevolution of host and parasites. Firstly I describe how ecological processes may lead to the evolution of diversity in host resistance. In particular I show how genetic variation in both transmission and susceptibility generated by processes such as greater interactions between related individuals in social groups, can select for polymorphism. In this case epidemiology creates a selection pressure that promotes the evolution of different resistance levels to relatives. Dimorphisms, often between highly resistant and highly susceptible types, evolve when infection is determined only by an individual's relative susceptibility or when transmissibility and susceptibility are simply correlated. Overall I show when and how ecological feedbacks generate diversity in host defence, and demonstrate when polymorphism can evolve without co-evolution with the parasite. Next I consider coevolutionary dynamics and describe how they may lead to evolution of diversity through a process of multiple branching from monomorphic populations only when there is specificity such that some combinations of host types and parasite strains lead to no infection (qualitative resistance). I describe a range of infectivity relationships that capture the characteristics of common realistic frameworks including universal infectivity, and gene-for-gene and matching allele infection genetics. Without qualitative resistance and specificity, dimorphic populations of hosts and parasites is the maximum level of diversity that can evolve. This diversity is static "red king" rather than the classic temporally cycling "red queen" and therefore provides a mechanism for the generation of stable diversity in host parasite interactions. The population level epidemiological feedbacks, life-history trade-offs in hosts and parasites and in particular specificity of infection are critical to the generation and maintenance of diversity in infectious disease.

John Kenneth Colbourne

Duplicating Genes Allow *Daphnia* Populations to Thrive in Toxic Environments

Daphnia, or the water flea, is a sentinel species of freshwater ecosystems. Their populations are defined by the boundaries of ponds and lakes, are sensitive to modern toxicants in the environment, and thus are used to assess the ecological impact of environmental change. Their short generation time, large brood sizes, and ease of laboratory and field manipulation have assured *Daphnia*'s importance for setting regulatory standards by environmental protection agencies, for testing chemical safety, for monitoring water quality, and as a model for environmental genomics research. A hallmark of the genome sequence is a large number of duplicated genes that are most responsive to ecological challenges and are specific to the *Daphnia* lineage. In this study, we take advantage of maturing genomics tools to understand the molecular basis for evolved tolerance to toxic levels of certain metals. We also test the adaptive significance of *Daphnia*'s genome structure. Natural populations that have faced severe chemical challenges for over a century of industrial iron/ore smelting demonstrate evolved tolerance to cadmium. Other reference populations that have no history of chemical stress are clearly harmed by metal exposure, showing slower growth rates, lower fecundity and higher mortality. By measuring the distribution of copy number variants and interrogating differential expression of 31,000 annotated genes from sampled populations across chemical conditions, this study provides new insights into the functional interactions between genome structure and environment. This research benefits from and contributes to the *Daphnia* Genomics Consortium, http://daphnia.cgb.indiana.edu.

David Conway

Parasite population genomic studies towards development of a blood stage malaria vaccine

The memory component of acquired immune responses causes frequency-dependent selection on pathogens, leading to distinctive patterns of polymorphism in genes encoding important target antigens. These are detectable by evaluating statistical signatures of balancing selection, using either allele frequency-based or polymorphism-versus-divergence indices. This is illustrated in analyses of genes encoding particular malaria parasite antigens that are candidate targets of naturally acquired immunity. Screening for such signatures to prospectively identify targets of immunity among panels of relatively poorly known or hypothetical parasite proteins has been effective. For a

comprehensive screen to discover targets of immunity in *Plasmodium falciparum*, endemic populations in West Africa have been sampled and genome sequence data obtained by paired-end Illumina shotgun reads, yielding high coverage sequence data on almost all protein coding genes (excluding those for which unambiguous alignment of orthologous sequence is difficult such as large sub-telomeric gene families). Analyses of the polymorphic site frequency spectrum show particular utility for identification of new antigen genes. Genes previously studied by capillary re-sequencing in independent population samples had highly concordant indices in the genome-wide analysis, including several replicated outlier loci, and this has identified other genes with stronger evidence of balancing selection, now prioritised for functional study and vaccine candidacy.

Santiago F. Elena and Jasna Lalić

Uncertainties in estimating viral fitness

Virus emergence is a complex, multilevel problem that results from a combination of ecological and genetic factors. To forecast when and how new viruses may emerge we must first identify the factors determining the distribution of genetic variants within the reservoir host as well as across all potential new ones, since this will ultimately condition the chance that different viral genotypes may have to persist in the reservoir and to successfully replicate in the new host. To this end, the following information is crucial: (*i*) what is the distribution of mutational fitness effects (DMFE) on the reservoir host and (*ii*) how it changes on different hosts (i.e., genotype-by-host interactions - *G´E*), (*iii*) the way in which multiple mutations hitting the same genome interact in determining fitness (i.e., genetic-by-genetic interactions aka epistasis - *G´G*) in the reservoir host, and finally, (*iv*) how different hosts may affect the form of epistasis (i.e., epistasis-by-host interactions - *G´G´E*).

In this contribution, we will review some recent work with tobacco etch potyvirus (TEV) addressing the above questions. We characterized the DMFE for genotypes carrying single nucleotide substitutions across a set of eight hosts of decreasing genetic relatedness with the primary one. We found a significant *G´E* interaction, which was sustained by differences in genetic variance for fitness and the pleiotropic effect of mutations among hosts. We also found that the DMFEs were markedly different between natural and non-natural hosts and, notably, the fraction of possible beneficial mutations was larger in the latter.

Next, we generated random pairs of mutations whose separated effects were known and the combined deleterious fitness effects were determined. We found that many pairs had significant epistasis for fitness (*G´G*), including both positive and negative deviations from the null hypothesis of additive effects. Furthermore, we explored the contribution of sign epistasis to these non-additive effects and found that a large fraction consisted of cases of reciprocal sign epistasis, where the sign of the effect of mutations at two loci are dependent on each other.

Finally, we characterized the distribution of epistasis among pairs of random mutations across four hosts that differ in their taxonomic proximity. We provide first evidence that the distribution of epistatic interactions significantly varied among hosts (*G´G´E*), and that average epistasis was stronger in the primary host but more independent as host's genetic relatedness decreased.

The existence of significant *G´E*, *G´G* and *G´G´E* imply that no precise predictions on the fitness effect of an individual mutation can be made since it will depend on the genetic background in which it appears and in the host wherein the virus replicates.

Duncan Greig

Reproductive isolation in *Saccharomyces*

Speciation is one of the most interesting processes in evolution, but underlying causes of reproductive isolation are not well understood. I will discuss the results of many experiments on reproductive isolation between yeast species of the Saccharomyces sensu stricto group. Hybrids between these species are easy to make in the laboratory, but, if given a choice of species to mate with, some are able to avoid hybridization. F1 hybrids are viable but sexually sterile: their gametes are inviable. The major cause of this hybrid sterility is antirecombination – the inability of diverged chromosomes to form crossovers during F1 hybrid meiosis. Chromosomal rearrangements can contribute to hybrid sterility, but they are not a general mechanism for yeast reproductive isolation. Although incompatibility between genes expressed from different species' genomes seems to evolve readily in the laboratory, it is not a major cause of F1 hybrid sterility, although it may contribute to reproductive isolation at other stages of the life cycle.

Bryan Grenfell

Vaccination and the dynamics of immune escape

We begin by reviewing current understanding of the dynamics of immune escape. We then describe two case studies; first, we discuss the potential impact of rotavirus

vaccine on strain structure of the virus and the possibility of strain escape from prevailing immunity. We then explore the impact of novel broad spectrum vaccines on the epidemiology and ecology of influenza. We conclude with a discussion of open questions in multi-strain pathogen dynamics.

Edward C. Holmes

Cross-Species Transmission and Emergence of Viruses

Most viral infections of humans have their origins in other animal species. However, despite the importance of cross-species virus transmission for public health, its evolutionary basis is often uncertain. In addition, little is known about how virulence will evolve following a species jump: mathematical models suggest that when viruses like HIV or avian influenza jump into human populations, evolution in the subsequent epidemic(s) can make the disease more harmful or less harmful depending on the biological particulars. Herein, I will use three model systems to explore the cross-species transmission and emergence of viruses. Using carnivore parvoviruses I will explore the evolutionary genetic basis of host adaptation and the role played by parallel hosts in cross-species transmission. With mxyoma virus of rabbits I will explore aspects of the evolution of virulence following a host jump, focusing on the parallel epidemics in Australia and Europe. Finally, using avian influenza viruses from wild birds I will explore the patterns and dynamics of virus spread at a continental scale, and how this can be used to model the spread of novel influenza viruses.

Peter Keightley

Inferring adaptive evolution in the mouse genome

We have employed Illumina shotgun sequencing to obtain genome sequences of 10 individual house mice of the subspecies Mus musculus castaneus at a high depth of coverage. From these sequences we are able to accurately determine the distribution of polymorphic nucleotide sites in the genome. I will describe the results of ongoing analysis of the polymorphism data, along with divergence to two related species (Brown rat and Mus famulus), that aims to infer the distribution of selective effects of new mutations and strength and rate of adaptation in coding and noncoding DNA.

Britt Koskella

Evolution of microbes and viruses in plant communities

Understanding coevolutionary dynamics, in particular between hosts and parasites, is critical to understanding both biodiversity and ecosystem functioning. Recently, major strides forward have been made due to a burgeoning empirical and theoretical literature that consider how environmental heterogeneity influences the outcome of species interactions. Microbial systems provide an exciting opportunity to examine these complex dynamics with tractable methods both in the field and the laboratory. In addition, microbial communities are of key importance to the health of human, agricultural, and natural populations. A key challenge is to understand how these communities are influenced by interactions with both their eukaryotic hosts and their viral parasites (bacteriophages). In this talk, I first present data on the scale at which bacteriophages adapt to infect their host bacteria within natural populations living in and on their plant host, the horse chestnut tree. I then examine the specificity of these natural phages and the potential consequences of phage-mediated selection on bacterial adaptation to plant hosts, and finally explore the importance of historical contingency in shaping fitness trade-offs. I discuss these findings both in light of phage therapy for regulating bacterial populations and, more generally, to highlight the importance of understanding the spatial scale and biotic complexity of species interactions in successfully predicting the outcome of coevolution.

Jinyong Hu, Filippos Klironomos, Johannes Berg and **Juliette de Meaux**

Evolution of microRNA824 in Arabidopsis thaliana

Mutations affecting gene regulation are thought to contribute prominently to phenotypic change and adaptive evolution. Non-coding RNAs are post-transcriptional regulators of gene expression enjoying unique evolutionary attributes because of their structural properties. Yet, evidence for their involvement in adaptive evolution is lacking. We analyzed the effect of variation at ath-miR824 on the expression level of its target, AGL16, a MADS-box transcription factor controlling stomata patterning in Arabidopsis thaliana. We find that a structural polymorphism in the miRNA precursor molecule affects the synthesis of mature miRNA. We demonstrate that miR824 polymorphism affects stomata patterning, and we investigate the fluctuations of selection pressure on the miR824 alleles in a field trial over three consecutive generations. Our work showcases the unique adaptive properties of non-coding RNAs for the adjustment of gene expression at the post-transcriptional level and illustrates how these properties come into play in a natural population.

K. Prasad, B-H Song, C. Olson-Manning, J. Anderson, C-R Lee, M.E. Schranz, A. Windsor, and **Thomas Mitchell-Olds**

Evolution of complex traits in complex environments

Although many studies provide examples of evolutionary processes such as balancing selection or deleterious polymorphism, the relative importance of these processes for phenotypic variation within and among populations is unclear. In order to understand the evolutionary forces that influence complex trait variation, one approach is to clone ecologically important QTLs in natural populations, and then measure the fitness of alleles in the populations where they evolved. This is our approach in Boechera stricta, a perennial wild relative of Arabidopsis. By positional cloning we identified a QTL controlling insect resistance and plant defense in wild populations in the Rocky Mountains. In the original populations where these alleles evolved, we quantified insect resistance and the survival and reproduction of contrasting alleles. Ecological measurements of selection indicate that this polymorphism is influenced by spatially heterogeneous natural selection.

Hélène Morlon

Explosive radiation of a bacterial species group

Hélène Morlon, Center for Applied Mathematics, Ecole Polytechnique, Paris

The current diversity of life on earth is the product of macroevolutionary processes that have shaped the dynamics of diversification. While the tempo of diversification has been studied extensively in macroorganisms, much less is known about the rates of diversification in the exceedingly diverse and species-rich microbiota. Decreases in diversification rates over time, a signature of explosive radiations, are commonly observed in plant and animal lineages. However, the few existing analyses of microbial lineages suggest that the tempo of diversification in prokaryotes may be fundamentally different. I will present a study using multi-locus and genomic sequence data, along with recently developed phylogenetic inference methods that can account for missing taxa, to test hypotheses about the rate of diversification in a well-studied pathogenic bacterial lineage, *Borrelia burgdorferi* sensu lato (sl). This study supports the hypothesis that an explosive radiation of lineages occurred near the origin of the clade, followed by a sharp decay in diversification rates. These results suggest that explosive radiations may be a general feature of evolutionary history across the tree of life.

Jason Kubinak and **Wayne Potts**

Experimental evolution of viral fitness and virulence: satisfying the red queen

Extreme levels of allelic diversity observed at vertebrate major histocompatibility complex (MHC) loci are associated with susceptibility to most infectious and autoimmune diseases. The evolutionary forces maintaining this important genetic diversity has been controversial. Here, we test the antagonistic coevolution (or red queen) model of MHC evolution and demonstrate rapid adaptation and virulence evolution of a pathogen to its mammalian host (*Mus*) across multiple MHC genotypes. Critically, a portion of this adaptive response is host MHC-specific as the fitness of pathogens is significantly higher when pathogens infect hosts carrying a familiar versus unfamiliar MHC genotype. adaptive response also results in significantly enhanced disease virulence in familiar MHC genotypes. These data are the first to experimentally confirm the requisite conditions of the antagonistic coevolution model of MHC evolution and provide quantification of fitness effects for pathogen and host. These data help explain the unprecedented diversity of MHC genes, including how disease-causing MHC alleles are maintained.

We have also applied this approach to hosts that differ across the whole genome with similar results - pathogen fitness and virulence increases rapidly and is host genotype specific. These combined data sets allow us to estimate the relative contribution of MHC and non-MHC genes as targets of pathogen adaptation. The MHC represents only 0.1% of the genome, but is responsible for about 72% of the fitness tradeoffs observed between infections of post-passage virus in familiar vs unfamiliar host genotypes. Finally, to test the prediction that genetic diversity in host populations creates an impediment to pathogen adaptation we compared patterns of adaptation between pathogens passaged through a series of genetically identical hosts or through alternating host genotypes. Rapid pathogen adaptation and virulence evolution was only observed in pathogens serially exposed to genetically identical hosts. Thus, host genetic diversity is an impediment to pathogen adaptation and also reduces the severity of infectious disease. Combined, these findings provide the first experimental confirmation of all the requisite conditions for red queen coevolutionary dynamics for a pathogen with a vertebrate host. They have important implications for the evolution of sex, management of endangered species and the emerging global-antibiotic-resistance problem caused by prophylactic antibiotic use in domestic animals.

Michael Purugganan

Adaptation and regulation: Insights from single gene to genome-wide perspectives

Understanding the genetic basis for adaptation is widely considered as a central challenge of modern evolutionary genetics, and the identification of these genes offers an unprecedented opportunity to finally investigate the molecular basis of adaptive evolution. The paucity of isolated genes that have been analyzed at the molecular level, however, has made it difficult to address issues surrounding the origins and spread of selected mutations, the molecular mechanisms that lead to evolutionary change and the relative role of protein structural mutations vs. cis-regulatory changes in adaptive evolution. Using the model wild plant *Arabidopsis thaliana*, we have been used both single-gene and genome-wide systems biology approaches to study both life history evolution as well as plant responses to complex, dynamic environments. We discuss these two areas in this talk. One is the nature of *cis*-regulatory variation in the *CONSTANS* gene, which encodes a MADS-box transcription factor and controls daylength sensitivity of flowering time. Our results have identified a promoter polymorphism that appears to have been under recent directional selection and is associated with flowering time variation. Second, we also report on genome-wide gene expression patterns in the wild in two natural accessions of *A. thaliana* and examined the nature of transcriptional variation throughout its life cycle in the field. We identified between ~110 and 190 time-varying gene expression clusters in the field, many of which were significantly overrepresented by genes that are regulated by abiotic and biotic environmental stresses. The two main principal components of vegetative shoot gene expression were shown to correlate to temperature and precipitation occurrence in the field.

Andrew Rambaut

Linking spatial epidemiology with antigenic and genetic evolution of Human influenza virus

"I will discuss our recent advances in integrating models of antigenic evolution into the molecular phylogenetic framework, BEAST. These approaches are motivated by the fact the antigenic properties we are most interested in for vaccine selection are a function of the viruses protein sequence. We examine two ways of modelling the evolution of these properties on the estimated phylogenetic tree: The first is to assign each virus a location in a 2-dimensional ‚antigenic space' as proposed by the Antigenic Cartography approach. This location is considered to be a property of the virus and its evolution is modelled as a bivariate diffusion in the space conditioned on the estimated phylogenetic tree that relates the viruses to their inferred common ancestors. The second approach is to posit the existence of a number of discrete antigenic clusters and then assign viruses to these clusters using measured antigenic distances with the cluster count and occupancy drawn from a Dirichlet process. This approach is appealing because it assumes discrete antigenic variation which is what we would predict if this property arises from the amino acid sequence. The occupancy of the clusters is then modelled as a trait of the virus rather than the location in antigenic space. This allows for the ready examination of potential correlates of antigenic change using the existing machinary of BEAST, including genetic change, spatial structure and epidemiology."

Walter Salzburger

Evolution in Darwin's Dreamponds: The adaptive radiations of cichlid fishes in East Africa

More than 152 years after the publication of Charles R. Darwin's *The Origin of Species*, the identification of the processes governing the emergence of novel species remains a fundamental question to biology. Why is it that some groups have diversified in a seemingly explosive manner, while other lineages have remained unvaried over millions of years? And what are the external factors and environmental conditions that promote diversification? A key to these and related questions is the comparative study of exceptionally diverse yet relatively young species assemblages that have radiated in geographically well-defined areas, such as the Darwin's finches on the Galapagos archipelago, the Caribbean *Anoles* lizards or the cichlid fishes in the Great Lakes of East Africa. Lakes Tanganyika, Malawi, and Victoria are each teeming with a unique set of hundreds of endemic cichlid species, which are likely to have evolved in the last few millions to several thousands of years only. East Africa's cichlid species differ greatly in ecologically relevant, hence naturally selected, characters such as mouth morphology and body shape, but also in sexually selected traits such as coloration. One of the most fascinating aspects of cichlid evolution is the frequent occurrence of evolutionary parallelisms, which has led to the question whether selection alone is sufficient to produce these parallel morphologies, or whether a developmental or genetic bias has influenced the direction of diversification.

Johanna Schmitt

Mapping local adaptation in *Arabidopsis thaliana*

Adaptation to local environments has been observed experimentally in many organisms, and will be critical for species persistence in the face of rapid environmental change. However, the genetic mechanisms underlying local adaptation are still largely unexplored. We tested for local adaptation to climate by growing a set of *Arabidopsis thaliana* ecotypes across the species native climate range in common garden field experiments in Finland, England, Germany, and Spain. Genotypes originating in climates similar to the site of planting had high relative fitness in each site, providing direct evidence for adaptation to climate in this model species. However, genotypes originating in climates historically warmer than the site of planting had higher relative fitness than native genotypes in every site, evidence of lagging adaptation in response to changing climate. A genome-wide association study of survival and lifetime reproduction revealed that the genetic basis of local adaptation differs among regions. Effect sizes of the SNPs associated with fitness were weakly correlated across field sites, and different SNPs were most strongly associated with fitness traits in each site. Thus distinct environment-specific loci contributed to fitness variation in each geographic region. Moreover, the molecular functions under- or overrepresented among genes linked to associated SNPs were also different across traits and sites. Alleles conferring higher fitness within each site were distributed significantly closer to that site than genomic controls in all sites except Finland, providing a geographic signature of local selection. Alleles of strongly fitness-associated SNPs in each site also exhibited higher levels of climate specialization than genomic controls.

Ralf Sommer

The nematode *Pristionchus pacificus* as a model system for integrative studies in evolutionary biology and ecology

The nematode *Pristionchus pacificus* has been established as a model system in evolutionary biology with genetic, genomic and transgenic tools. Detailed investigations of vulva formation and other developmental processes revealed that developmental mechanisms differ strongly between *C. elegans* and *P. pacificus.* While evo-devo can provide fundamental insight into morphological evolution, the limitations of its gene-centered and development-centered view necessitate the synthesis of evo-devo with other areas of evolutionary biology. Synthesis with "population genetics" can reveal how phenotypic evolution is initiated at the micro-evolutionary level and synthesis with "evolutionary ecology" can add an ecological perspective to these evolutionary processes (Sommer, Nat Rev Genet., 2009).

The well-defined association of *P. pacificus* with scarab beetles, the apparent plasticity of this beetle association, and the ability of this widespread species to thrive in a variety of geographic ranges and ecological conditions, make *P. pacificus* an ideal model organism for the merger of evo-devo, population genetics and evolutionary ecology. We have started to analyze the ecological interactions of *P. pacificus* in the beetle ecosystem and more than 400 strains of *P. pacificus* have been isolated from around the world. In the last few years, our biogeographic work focused on island biology and we discovered that La Réunion, a young volcanic island in the Indian Ocean harbours the complete worldwide genetic diversity of *P. pacificus* due to independent invasions of this nematode with different carrier beetles. Thus, La Réunion represents a microscosm for studies of population genetic and ecology.

After a conceptual introduction, I will report from our most recent work focusing on 1) the evolution of novel morphological structures (*P. pacificus* forms dimorphic teeth involved in predatory feeding), 2) the integration of this novel predatory behaviour into an existing nervous system, and 3) intraspecific nematode competition in the decaying beetle ecosystem by the means of small molecules. These case studies will highlight the importance of integrative and interdisciplinary approaches in modern biology from development and evolution to ecology and organic chemistry.

Diethardt Tautz

Tracing the genetic basis of adaptations in the house mouse

Although the house mouse is a well-established model system for biomedical research, it has received much less attention by evolutionary biologists so far. However, there are many aspects that make it particularly suitable for studying evolutionary questions. It has a well-defined history of population expansions and colonization of new habitats, ranging from desert climates to sub-Antarctic islands. Several colonizations have occurred during historic times, which allows studying the earliest phases of evolutionary adaptations. The mouse has also a broad behavioral repertoire making it suitable for analyzing social communication and mate choice. In the past years, we have build up a large collection of samples and animals from natural populations. I will report on the experimental approaches that we are using to better understand the genetics of adaptation and population differentiation. We can make full use of the genomic resources that were developed for the laboratory mouse, allowing us to do genome scans for adaptive trait genes and to map the genetic basis for complex traits,

such as the unusual growth of mice on some islands. We find evidence that adaptive introgression of alleles from other populations plays a larger role in shaping the genomes than previously considered.

Miltos Tsiantis

Towards understanding development and evolution of leaf shape

A key challenge in biology is to understand how diversity in organismal form is generated. Genetic analyses in model systems have identified key regulators that sculpt the body plans of metazoa and seed plants. However, less is known about how the action of such regulators produces particular organ shapes, or how the balance of conservation versus divergence of such form regulating pathways generated the tremendous morphological diversity of multicellular eukaryotes. One impediment to answering these questions is the paucity of experimental platforms where genetic tools can be utilized to unambiguously study morphogenesis and its evolution in a genome-wide, unbiased fashion. To circumvent this problem we developed the Arabidopsis thaliana relative Cardamine hirsuta into a versatile system for studying morphological evolution. We aim to understand the molecular mechanisms through which leaf morphology evolved in these species, resulting in simple, undivided leaves in A. thaliana and dissected leaves with distinct leaflets in C. hirsuta. This presentation will discuss our progress towards understanding the genetic pathways that specify dissected versus entire leaf shapes and that regulate the number, position and timing of leaflet production.

Daniel Weinreich

Adaptation in Changing Environments: Influence of Changing Patterns of Epistasis in the the TEM-1 Antibiotic-Resistance Gene

Modern methods of molecular biology are allowing us to characterize the consequences of individual mutations in an organism. This work has in turn highlighted the empirical importance of epistasis, namely interactions among mutations in determining traits of an organism. Here we describe recent work describing the influence of changing environment on epistatic interactions among mutations, and hence on opportunities for adaptation. TEM-1 beta-lactamase is an enzyme that confers bacterial resistance to beta-lactam antibiotics such as penicillin. We previously constructed all combinations of five point mutations in this gene that jointly increase bacterial resistance 100,000-fold. We now report drug resistance conferred by each construct at each of six temperatures. Analysis of these data reveals important changes in the underlying epistatic structure among mutations as well as implications for models of protein evolution in changing environments.

SATELLITE MEETING | FEBRUARY 24–25

VIRAL EVOLUTION: FROM GENETICS TO EPIDEMICS

The satellite meeting aims at bringing together experts on genome evolution, population genetics, and epidemiology of viral systems to discuss emerging links between these fields. Key topics include evolution and epidemiology of the influenza and HIV viruses.

ORGANIZERS

Michael Lässig (Institute for Theoretical Physics, University of Cologne)

Thomas Lengauer (MPI Saarbrücken)

SPEAKERS

Trevor Bedford | University of Edinburgh

Nico Beerenwinkel | ETH Zürich

Julia Gog | University of Cambridge

Alice McHardy | University of Düsseldorf

Sergei Kryazhimskiy | Harvard University

Michael Lässig | University of Cologne

Ville Mustonen | Welcome Trust Sanger Institute

Richard Neher | MPI Tübingen

Nico Pfeifer | MPI Saarbrücken

Oliver Pybus | University of Oxford

Annemie Vandamme | University of Leuven

SATELLITE MEETING
VIRAL EVOLUTION: LINKING GENETICS TO EPIDEMICS

FRIDAY | FEBRUARY 24

15:00–18:45 **Chair: Thomas Lengauer**

Sergey Kryazhimskiy, Directionality and epistasis: How natural selection shapes the influenza A genome

Coffee Break (30 min)

Michael Lässig, Influenza A: Genomics of a red queen race

Ville Mustonen, Distinguishing driver and passenger mutations in an evolutionary history categorized by interference

Break (20 min)

Trevor Bedford, Canalization of the evolutionary trajectory of the human influenza virus

Julia Gog, Age-structured model of influenza evolution

SATURDAY | FEBRUARY 25

09:00–13:00 **Chair: Michael Lässig**

Oliver Pybus, Unifying the phylogeography and mathematical epidemiology of emerging epidemics

Alice McHardy, Phylodynamic techniques for the study of evolutionary dynamics of influenza A viruses

Annemie Vandamme, Current controversies on the origin and adaptation of epidemic HIVs

Coffee Break (30 min)

Richard Neher, The recombination rate of HIV and the role of recombination in chronic infection

Nico Pfeifer, Modeling HIV evolution in treatment-naïve patients

Nico Beerenwinkel, Estimating the structure of viral quasispecies from deep sequencing data

Organizers: **Michael Lässig, University of Cologne**
Thomas Lengauer, MPI Saarbrücken

Trevor Bedford

Canalization of the evolutionary trajectory of the human influenza virus

Since its emergence in 1968, influenza A (H3N2) has evolved extensively in genotype and antigenic phenotype. However, despite strong pressure to evolve away from human immunity and to diversify in antigenic phenotype, H3N2 influenza shows paradoxically limited genetic and antigenic diversity present at any one time. Here, we propose a simple model of influenza that displays rapid evolution but low standing diversity and simultaneously accounts for the epidemiological, genetic, antigenic and geographical patterns displayed by the virus. In this model, antigenic phenotype is represented by a N-dimensional vector and virus mutations perturb phenotype within this continuous Euclidean space. We implement this model in a large-scale individual-based simulation, and in doing so, find a remarkable correspondence between model behavior and observed influenza dynamics. We find that evolution away from existing human immunity results in rapid population turnover in the influenza virus and that this population turnover occurs primarily along a single antigenic axis. Thus, selective dynamics induce a canalized evolutionary trajectory, in which the evolutionary fate of the influenza population is surprisingly repeatable and hence, in theory, predictable.

Nico Beerenwinkel

Estimating the structure of viral quasispecies from deep sequencing data

Next-generation sequencing allows for cost-effective probing of virus populations at an unprecedented level of detail. The massively parallel sequencing approach can detect low-frequency alleles and it provides a snapshot of the structure of the entire virus population. However, analyzing ultra-deep sequencing data obtained from mixed virus populations is challenging, because (i) data sets are very large, (ii) reads contain amplification and sequencing errors, and (iii) the read length is typically shorter than the genomic region of interest. Thus, ultra-deep sequencing experiments provide only indirect evidence of the underlying viral population structure. We will discuss computational and statistical methods for separating technical artifacts from true biological variation in these data. Specifically, we will address read error correction, haplotype reconstruction, and haplotype frequency estimation in HIV. We present several bioinformatics approaches, analyze their performance on simulated data and on data obtained from control experiments and clinical HIV samples.

Julia Gog

Age-structured model of influenza evolution

Population models that include multiple strains of an infectious disease are typically intractable unless strategic assumptions are taken. Additional complications, such as age-structure, are usually therefore avoided in models. However, we show that explicit inclusion of age-structure actually greatly simplifies strain models: a sort of two for the price of one. Using this, we can explore the consequences of „original antigenic sin" in influenza A, including generation of models that can be confronted by age-structured serological data. This model structure might open up some interesting future areas, such as which age groups are most important for driving the observed evolution of influenza.

Alice McHardy

Phylodynamic techniques for the study of evolutionary dynamics of influenza A viruses'

Phylodynamic techniques combine epidemiological and genetic information to analyze the evolutionary and spatiotemporal dynamics of rapidly evolving pathogens, such as influenza A or human immunodeficiency viruses. We introduce 'allele dynamics plots' (AD plots) as a method for visualizing the evolutionary dynamics of a gene in a population. Using AD plots, we propose how to identify the alleles that are likely to be subject to directional selection. Furthermore, we have developed ‚phenotype trees', a method to distinguish mutations that determine an organism's phenotype from (near-) neutral ‚hitchhikers' based on pairwise phenotype distances. We analyze the merits of both methods with detailed studies of the evolutionary dynamics of seasonal influenza A viruses. AD plots for the major surface protein of seasonal influenza A (H3N2) and the 2009 swine-origin influenza A (H1N1) viruses show the succession of substitutions that became fixed in the evolution of the two viral populations. They also allow the early identification of those viral strains that later rise to predominance, which is important for the problem of vaccine strain selection. ‚Phenotype trees' predicted antigenic distances with comparable accuracy to antigenic cartography. Additionally, it identified both known and novel sites, and amino acid changes with antigenic impact in the evolution of influenza A (H3N2) viruses from 1968 to 2003. Both methods can be applied for the study of other rapidly evolving species or viruses.

Sergei Kryazhimskiy

Directionality and epistasis: How natural selection shapes the influenza A genome

Influenza A is an RNA virus that infects millions of people every year. What allows influenza to evade our immune response and persist in our population is the incredibly high rate at which mutations are incorporated into its genome. For instance, about 20% of sites in its surface protein hemagglutinin have changed over the course of 20 years. We do not know the precise mechanisms that promote such rapid evolution, but we do know that much of this evolution is adaptive. In this talk, I will discuss our computational work that uncovers different modes by which natural selection has shaped the influenza A genome over the past 30 years. Large number of available genetic sequences allows us to understand the molecular evolution of influenza in great detail. We can pinpoint specific amino-acid residues that were selected for and against in the course of evolution and determine some pairs of sites that exhibit genetic interactions (epistasis). This genomic information will eventually help us understand the evolutionary success of influenza.

Michael Lässig

Influenza A: Genomics of a red queen race

The seasonal influenza A virus undergoes rapid evolution to escape human immune response. Adaptive changes occur primarily in antigenic epitopes, the antibody-binding domains of the viral haemagglutinin. We show that influenza A (H3N2) evolves by strong clonal interference. This mode of evolution is a red queen race between viral strains with different beneficial mutations. Clonal interference explains and quantifies several features of influenza genome evolution. We infer that this process is driven by at least one strongly beneficial amino acid substitution per year. Our results imply that mode and speed of influenza evolution are governed not only by positive selection within, but also by background selection outside antigenic epitopes: immune adaptation and conservation of other viral functions interfere with each other. We discuss the implications for predictability of influenza evolution.

Christopher Illingworth and **Ville Mustonen**

Distinguishing Driver and Passenger Mutations in an Evolutionary History Categorized by Interference

In many biological scenarios, quantifying the selection acting on observed mutations is a central question. One difficulty in answering this question is the complexity of the background upon which mutations can arise, with multiple potential interactions between genetic loci. We here present a method for discerning selection from a population history that accounts for interference between mutations. Given sequences sampled from multiple time points in the history of a population, we infer selection at each locus by maximizing a likelihood function derived from a multi-locus evolution model. We apply the method to the question of distinguishing between loci where new mutations are under positive selection (drivers) and loci that emit neutral mutations (passengers) in a Wright–Fisher model of evolution. Relative to an otherwise equivalent method in which the genetic background of mutations was ignored, our method inferred selection coefficients more accurately for both driver mutations evolving under clonal interference and passenger mutations reaching fixation in the population through genetic drift or hitchhiking. We further describe the application of our method to influenza sequence data, and present preliminary results.

Richard Neher

The recombination rate of HIV and the role of recombination in chronic infection.

The HIV populations within a patient is an effectively sexual population since genetic material can be exchanged between different viral lineages via template switching of the reverse transcriptase. Such effectively sexual reproduction requires coinfection of host cells with several genetically distinct virus particles. We estimated the effective HIV recombination rate and the coinfection probability, which turns out to be much lower than previously thought. This low recombination rate has implications for our understanding of HIV evolution, since different parts of the genome no longer evolve independently. I will present theoretical results on the evolution of viral populations with infrequent recombination.

Nico Pfeifer

Modeling HIV Evolution in Treatment-Naïve Patients

Abstract: Even after more than 25 years of HIV research no vaccine for HIV has been approved yet. This is mainly due to the high variability of the virus that creates the need for the immune system to constantly adapt to the new variants. Nevertheless, there exists a small group of people called elite controllers that are able to control HIV such that their viral load is low even without treatment. Therefore, studying viral evolution directed by the pressure of the immune system could eventually help to identify important parts of the HIV proteome that could be included in a peptide-based vaccine for HIV.

When modeling HIV evolution directed by host HLAs one has to account for the phylogenetic relationships between different HIV strains as well as for co-variation among sequence positions. We were able to build a graphical model that represents the complex interplay between these different types of variables and showed that the model can be used to answer interesting questions on data from treatment-naïve patients in Africa.

Oliver Pybus

Unifying the phylogeography and mathematical epidemiology of emerging epidemics

The spread of a single pathogenic lineage of West Nile Virus across North America represents the most significant recent example of wave-like invasion by an emerging infectious disease. Here, I introduce a new method to estimate the epidemic's spatial behaviour, including its diffusion coefficient, directly from sampled virus genomes. I find that WNV's epidemic diffusion is greater and far more variable than previously thought. As a result many current models of WNV in North America are unrealistic and have over-estimated the virus' basic reproductive number. This new approach provides a formal link between phylogenetics and mathematical spatial epidemiology, and enables the inference from genetic data of spatial ecological dynamics that are otherwise difficult to quantify.

Annemie Vandamme

Current controversies on the origin and adaptation of epidemic HIVs

Both for HTLV and HIV a combination of phylogenetic, molecular clock and population dynamics analyses can be used as a powerful framework for constructing and testing hypotheses about viral epidemics. The time-frame for the zoönotic transmissions at the origin of both groups of viruses has been set by several research teams including ours. This simian to human transmission occurred several times over a period of thousands of years for HTLV and several times over a period of less than hundred years for HIV. This time paradox between both viruses has been the topic of hot debate and epidemiological research. Two major hypotheses remain, unsafe needle injections were crucial at the origin of HIV, and, genital ulcer diseases were to blame. We argue in favor of a critical role for enhanced heterosexual transmission at the epicenters of epidemic HIV groups. We have demonstrated that the conditions for heterosexual spread were particularly favorable around the time and locations when and where epidemic HIV strains first emerged. The main factor was an unprecedented peak of bacterial genital infections, such as syphilis and chancroid (which greatly increase the efficiency of HIV transmission) in nascent colonial cities (Sousa et al. (2010) PLoS ONE 5(4): e9936: http://www.plosone.org/article/info:doi/10.1371/journal.pone.0009936). We do not deny that unsafe injections might have played a role in the initial „passaging" of the simian-adapted viruses in humans to allow for adaptation to the new host. However, beyond this „qualitative" contribution, injection campaigns need not have played an important „quantitative" role in the initial spreading of HIV epidemics. The origin of the epidemic HIV strains can be parsimoniously explained by the same factors that even today promote their spread by the heterosexual route in Africa: sexual promiscuity and genital ulcer diseases, particularly among prostitutes and their clients. Our arguments include a plausible and coherent explanation for the timing, geographical origin, and scarcity of epidemic HIV strains.

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