Project Area A

A1

Siegfried Roth

The evolution of gene regulatory networks for dorsoventral patterning in insects

Abstract

Patterning the *Drosophila* dorsal-ventral (DV) axis relies on Toll/NF-kB signalling, and represents one of the best understood gene regulatory networks. Toll signalling provides highly accurate positional information by establishing a nuclear concentration gradient of the NF-kB transcription factor Dorsal which controls the spatial expression of about 50 target genes in a concentration-dependent manner. Insects with more basal phylogenetic position, like the red flour beetle *Tribolium*, the jewel wasp *Nasonia* and the milkweed bug *Oncopeltus* show a stepwise replacement of the Toll signalling morphogen gradient by a BMP signalling gradient. We plan to compare the DV gene regulatory network of such insects to that of *Drosophila* and to analyse how the relative roles of the two signalling pathways have changed during insect evolution. This work will unravel how Toll/NF-kB signalling which has an ancestral function in innate immunity was co-opted for DV patterning in insects and how the NF-kB transcription factor Dorsal gained its dominant role as morpho-regulatory gradient in the evolutionary lineage leading to *Drosophila*.

A5

Wolfgang Werr

The evolution of seed plant stem cell niches

Abstract

Robust phylogenetic reconstructions of the *WUSCHEL-related homeobox* (*WOX*) gene family define three discrete subclades: i) the ancestral WOX13 lineage, which is conserved from green algae throughout extant land plant radiations; ii) the intermediate WOX9 branch, which evolved with vascular plants, and iii) the most recent or modern (> 350 mya) stem cell-promoting clade that appeared with leptosporangiate ferns and according to symplesimorphies was subject to amplifications prior the gymnosperm/angiosperm split. Characteristic for each clade or sub-branch therein are amino acid signatures in the DNA-binding homeodomain (HD) conserved over long independent evolutionary trajectories and despite the interchangeability of some proteins. Mutual information implies coevolution of some HD positions and guides functional analyses in *Arabidopsis thaliana*. The *Arabidiopsis* WOX13 and WUS HDs are exemplary crystallised for X-ray diffraction and will serve to identify adaptations in DNA sequence specificity. This unique combination of data not only will outline the functional relevance of specific amino acid side chains but also allow the interpretation evolutionary data and mutual information with respect to HD function.

Keywords
Homeodomain evolution, mutual information, adaptations in protein structure, DNA sequence specificity

A9

Martin Hülskamp

Microevolution of a regulatory network in Brassicaceae

Abstract

In this proposal we consider five traits, root hair, trichome, seed coat mucilage, anthocyanidin and pro-anthocyanidin production that are all involved in the plant`s responses to environmental changes and show great variation in natural populations. To understand the molecular mechanism of how plants adapt to new environmental situations we propose to study by genome wide association analysis how the variation of these traits is genetically linked. This should reveal which changes in some traits come with costs or benefits for others and how this is molecularly realised. In a complementary approach we plan to study the evolution of the regulatory network responsible for root hair and trichome patterning by a comparison between *Arabidopsis thaliana* and *Arabis alpina*. Based on *A.thaliana* network models we plan to use an *in silico* evolutionary approach to explain the observed differences in root hair and trichome pattern in the two species. The theoretical results will be used to focus the molecular and genetic experiments.

Keywords
in silico evolution, regulatory network evolution, developmental genetics

A10

George Coupland

Evolution of transcriptional regulation during the divergence of annual and perennial plant life histories

Abstract

Flowering plants exhibit a variety of different life histories. Annuals live for less than a year and reproduce only once, whereas perennials live for many years and reproduce multiple times. The *Arabis* genus of the Brassicaceae is a model system for studying the divergence of these life history traits. I propose to analyse this process using the progeny of interspecies crosses between *A. alpina* and its annual sister species, *A. montbretiana*. The transcription factor PERPETUAL FLOWERING 1 (PEP1) is a repressor of flowering that plays major roles in the perennial cycle of *A. alpina.* Three specific directions are proposed: (1) Analysis of the contribution of *cis* and *trans* effects to the distinct patterns of expression of PEP1 observed in *A. montbretiana* and *A. alpina* (2) Genetic analysis of *A. montbretiana* genes that modify the phenotypic effect of *A. alpina PEP1* on the perennial life cycle (3) Analysis of direct target genes of PEP1 in *A. alpina* and to which extent these are conserved in *A. montbretiana*.

Keywords
Evolution of transcriptional regulation; diversity in plant reproduction; inter-species variation

A

Martin Hasselmann

Gene duplications as a driving force in the evolution of social insects

Abstract

Novel gene functions are often associated with an increase of organismal complexity, speciation and adaptation. One possible source for novel gene functions represent duplicated genes. We will test the hypothesis that the rich repertoire of physiological and behavior aspects characteristic for social insects (e.g. the hymenopteran groups honey bees, bumble bees and stingless bees) is associated with lineage specific gene duplications.
Remarkable traits that differentiate castes of female bees (worker and queens) are body size, behavior and life-span. For honey bees it has been shown, that these traits are linked to two interconnected fundamental regulatory pathways: Insulin/Insulin-like signaling (IIS) and target of rapamycin (TOR). Interestingly, at least three key genes (*ILP, InR, S6K*) of these pathways are duplicated within the hymenopteran lineage.
We will i.) study the molecular evolution of these genes over broad range of social and non-social insects and ii.) experimentally test single paralogous copies of these genes to elucidated their function in the pathways affecting caste determination.

Keywords
molecular evolution, gene expression, RNAi, pleiotropic effects

A

Kristen Panfilio

Upstream and downstream of macroevolutionary changes in the insect *zen* genes' roles in extraembryonic development

Abstract

The extraembryonic membranes (EEMs) of insects represent an adaptive synapomorphy of this arthropod class. Once specified, their morphogenetic program ensures correct tissue configurations to protect the early embryo. Concomitant with the origin and evolutionary diversification of the EEMs are several macroevolutionary changes in the *zen* gene locus. This locus encodes a Class 3 Hox homeodomain transcription factor and has undergone multiple instances of (tandem) duplication and neo- or subfunctionalization. To investigate *zen* orthologue and paralogue evolution, we will take both bioinformatics and *in vivo* functional approaches in the beetle *Tribolium castaneum* and the bug *Oncopeltus fasciatus*, which provide informative taxonomic sampling and have initial *zen* gene characterizations. We will thereby elucidate at which levels of regulation – transcription or translation of the *zen* genes themselves or transcription of their downstream targets – *zen* genes have acquired roles in either specification or morphogenesis, and relate these to the evolution of the insect EEMs.

Keywords

evolution of development; neofunctionalization; functional divergence of paralogues; insect extraembryonic membranes; RNA-seq; RNAi; new genome annotation; microRNA regulation?; transcriptional insulators?; enhancer bashing?

A

Miltos Tsiantis

An interdisciplinary approach to understanding development and evolution of leaves

Abstract

Two key challenges in biology are to understand how biological forms are generated, and to elucidate the basis for their diversity. We will address these problems by studying the development and diversity of leaf forms. To this end, we will integrate developmental biology and computational modelling to compare, contrast, and put in an evolutionary context the mechanisms that shape the simple leaf of the model plant Arabidopsis thaliana and the dissected leaf of its relative *Cardamine hirsuta.* These models will integrate genetic interactions the pattern of growth of individual cells, mechanical interactions between cells, and vein pattern formation. We will investigate to what degree our models can capture the considerable variation in leaf shape seen in seed plants. We will also exploit our modelling work in a phylogenetic context to help conceptualise the mechanistic underpinnings of evolutionary transitions between divergent leaf shapes.

Keywords
evolution of development, comparative genetics, computational modelling

Project Area B

B7

Eric von Elert

Evolutionary adaptation of *Daphnia* to protease inhibitors in cyanobacteria

Abstract

Cyanobacteria are known to produce a variety of dietary chymotrypsin inhibitors (CIs), which specifically inhibit the chymotrypsins in the gut of *Daphnia*. Quantitative traits of clonal cultures of *Daphnia magna* from populations existing in the presence or absence of cyanobacteria point at local adaptation of *Daphnia* to CIs. Among populations chymotrypsin alleles differ with respect to non-synonymous mutations. The meaning of these mutations for tolerance to CIs will be investigated by enzyme-kinetics of the respective proteins obtained from heterologous expression and by modelling. In addition the role of plasticity for local adaptation to CIs will be investigated by comparing changes in chymotrypsin expression in response to CIs using RNASeq. Furthermore, the role of copy number variation of the three chymotrypsin genes for local adaptation to CIs will be examined.

Keywords
Population genetics, gene expression, RNASeq, copy number variation

B10

Jane Parker & Maarten Koornneef

Temperature modulation of plant immune responsiveness and fitness

As sensors of pathogen interference, *NB-LRR* immune receptor genes represent the most variable plant gene family. Polymorphic *TIR-NB-LRR* genes underlie a number of epistatic allelic interactions leading to immune-related hybrid incompatibility (necrosis), potentially also driving genetic diversification. Our analysis of *Arabidopsis* incompatible hybrids and 'auto-activated' immune backgrounds reveals an intimate relationship between temperature and TIR-NB-LRR triggered immune pathway activation, affecting plant development and fitness. Evidence is emerging for actions of TIR-NB-LRR receptors at the nuclear chromatin to reprogram cells for defence. We will continue to explore molecular and evolutionary mechanisms underlying a TIR-NB-LRR (RPP1) driven hybrid incompatibility. We will further measure the impact of temperature on *Arabidopsis* transcription dynamics by (i) sampling RNA populations and (ii) comparing epigenetic signatures of selected immune-activated and immune-defective backgrounds exposed to different temperatures. This study should reveal how even moderate changes in temperature might alter plant stress homeostasis and thereby environmental adaptation.

B11

Berenike Maier

Cost and benefit of bacterial transformation

Abstract
Horizontal gene transfer can speed up adaptation in varying environments. In particular, many bacterial species are naturally competent for transformation, i.e. for import of DNA from their environment and its heritable integration. We have developed an assay for directly visualizing transformation at the single cell level using the naturally competent species *Neisseria gonorrhoeae.* Moreover, we found that within hours individual *N. gonorrhoeae* lost their competence for transformation through phase variation, i.e. through a reversible mutation at a mutational hot-spot affecting competence. We are currently determining the fixation rates of both transformants and variants in well-mixed environment and in range expansion experiments. The goal of our future project is to quantify the cost of being competent for transformation and to find conditions in which the benefit of transformation outweighs its cost. Furthermore, we will investigate the interplay between phase variation and transformation.

Keywords
gene transfer, experimental evolution, microbial genetics

B

Andreas Beyer

Non-neutral effects of silent mutations

Abstract

When studying molecular evolution it is usually assumed that synonymous (silent) mutations have neutral effects. Although in general the phenotypic impact of 'silent' mutations may be smaller than that of non-synonymous mutations, they may also play a significant role during adaptation to new environments. Silent mutations may for example affect transcript structure or the efficiency of translation. This project aims at improving our knowledge about the relevance of silent mutations for molecular traits. Using high-throughput transcript- and protein-level data from crosses of different yeast species the project will identify local mutations impacting on protein levels without changing the corresponding mRNA levels. The yeasts are completely sequenced, thus subsequently it will be possible to identify candidate polymorphisms inside coding regions and outside (i.e. in untranslated regions, UTRs). Computational analysis will investigate the extent of synonymous mutations affecting protein levels and their potential molecular mechanisms by investigating their effect on the codon adaptation index, splicing, or other structural aspects of the transcript (e.g. hairpin formation).

Thereby this project will contribute to our understanding to what extent silent or synonymous mutations may in fact be non-neutral.

Keywords
silent mutations, population genetics, regulation of translation

Michael Nothnagel

Project number B

Runs of homozygosity in the human genome: disentangling the causes

Abstract
Runs of homozygosity (ROHs) are genomic stretches extending over at least 500 kb where a substantial number of single-nucleotide polymorphisms (SNPs) that are polymorphic in the population are homozygous in a particular individual. Suggested causes include, besides inbreeding and chance, selective sweeps as an evolutionary signature. This projects aims at identifying sequence features that allow classifying ROHs with regard to the underlying cause. This includes polymorphism density, linkage disequilibrium structure, marker allele frequencies and modelling of regional mutational patterns, among others. Extensive data simulations and analysis of publicly available genome-wide data, including the HapMap and 1000 Genomes projects, and of those produced at the CCG (Prof. Nürnberg) will be used to develop and validate discrimination algorithms. Mutational pattern modelling will benefit from collaborations with theoretical physicists within the SFB. Disentangling the causes for ROH will help annotating genetic variation for genetic-epidemiological studies.

Keywords
population genetics, genetic epidemiology

Project Area C

C1

Johannes Berg, Michael Lässig, Andreas Beyer (?)

Comparative analysis of gene expression

Abstract
Previously, we have compared gene expression across species at the level of mRNA. This focus was determined by the available data: microarray and RNAseq technology made mRNA data abundant. However, for nearly all genes the final product is protein, and for those genes it is protein levels that are under selection. Advances in mass-spectroscopy proteomics now allow cross-species comparison of protein levels. We will construct statistical models for the joint evolution of mRNA and protein levels, aiming to quantify the impact of post-transcriptional regulation. Initially, this will be done at the level of single genes, while our long term goal is to quantify selection on entire biological pathways.

Keywords
evolution of transcriptional regulation, post-transcriptional regulation

C2

Michael Lässig

Evolution of influenza

Abstract
The human influenza A virus is an ideal system to study fast adaptive evolution in asexual populations. In previous work, we have shown that the influenza surface protein haemagglutinin evolves under clonal interference, and we have developed theoretical models for this mode of asexual evolution. Based on these results, we will build a comprehensive, predictive model of influenza evolution. Its core will be fitness model for individual strains based on genomic and phenotypic data, which establishes a link between molecular evolution and epidemiology of influenza. This model will inform a season-to-season prediction of influenza strain frequencies. On the theoretical side, we will develop selection inference methods for non-equilibrium evolutionary processes under (partial) genetic linkage.

Keywords
asexual evolution, interference selection, protein structure

C3

Joachim Krug & Arjan de Visser

Epistasis, recombination and predictability in adaptive evolution

Abstract

The project will extend the collaboration between a theoretical and an empirical group that was started in the last funding period in several directions. First, we will explore the origins of the patterns of epistatic interactions that were identified in our previous work with the antibiotic-resistance enzyme TEM-1 β-lactamase, using explicit models of the genotype-phenotype and phenotype-fitness maps -- adapted to specific biological situations. Second, we intend to study how recombination affects the dynamics and predictability of adaptation on fitness landscapes with varying topographies, using tools from spectral landscape theory developed during the current period. Predicted effects from recombination will be tested in experiments with TEM-1 β-lactamase, where the intragenic crossover position and frequency of recombination is varied by the choice of restriction enzymes. Third, we will extend our analyses of determinants of evolutionary predictability from a single gene (TEM-1 β-lactamase) to the entire genome of *Escherichia coli*.

Keywords
Epistasis, fitness landscape, recombination, TEM-1 β-lactamase, evolutionary predictability, antibiotic resistance

C4

Thomas Wiehe

Evolutionary innovations - a case study on chromatin insulators

Abstract

Chromatin insulators have a central role in spatial and temporal organization of eukaryotic gene regulation. However, there is substantial variation of these proteins between and within clades. For instance, while a single insulator is known in vertebrates, Drosophila utilises seven such proteins. Six of them have been succesively acquired in the protostome lineage and two after the split of flies and mosquitos. We hypothesize that the expansion of the insulator family is an ongoing process and possibly not limited to arthropods. Combining macro- and microevolutionary perspectives, we seek to reconstruct in detail the evolutionary events which have led to the present distribution. In particular, we want to assess the roles of de novo gene birth, domain duplication and neo-functionalization. Further, we want to understand the adaptive component in the expansion of insulator proteins. We will examine genetic diversity in insulator genes and their potential cis- and trans-interaction partners in Drosophila.

Keywords
chromatin insulators; evolutionary genomics and proteomics; lineage specific genes; population

Martin Lercher

C7

Replaying the evolution of E. coli and Salmonella metabolic networks in silico

Abstract

We will reconstruct the metabolic genome of the most recent common ancestor of *E. coli* K12 and *Salmonella typhimurium*, based on gene gains and losses inferred along a carefully reconstructed phylogenetic tree. The metabolic networks at all inner branching points will be used to define sets of environments that were relevant for the selection under which these lineages evolved. We approximate fitness by the yield of biomass production. We set up a genomic mutational model that allows changes in specificity as well as gains and losses of transporters and enzymes. Under a weak mutation / strong selection model, we can then repeatedly simulate the stochastic evolution of bacterial populations under these conditions. As a result, we (i) quantify the role of chance and necessity in metabolic evolution; (ii) examine the importance of pre-adaptation; and (iii) test to what extent bacterial evolution is predictable.

Keywords
metabolic networks; evolutionary systems biology; ancestral reconstructions

Kay Hofmann

C

Unusual processes in the evolution of multi-domain proteins

Abstract

Most signaling proteins of pro- and eukaryotes do not have a monolithic architecture but rather consist of multiple structural and functional domains that fold independently of each other. The evolutionary processes of ‚domain shuffling', consisting of a combination of gene fission, fusion and (partial) duplication events, are well appreciated as the source of modular protein diversity. During our bioinformatical work on modular proteins of the ubiquitin-modification system or the innate immunity pathways, we noticed two widespread but underappreciated trends of domain- and motif-evolution. The first of these processes has been called 'non-orthologous domain displacement' and is characterized by the evolutionary replacement of one functional domain by an unrelated but functionally equivalent domain. The second process, which we call 'pathway fidelity', has not been formally described so far: Over long evolutionary distances, certain classes of protein domains undergo substantial functional changes, but remain within the same biological pathway. Since the two processes often affect the same domain types, they are probably linked. By tracing and analyzing the ancestral states of proteins involved in ubiquitination and innate immunity, we intend to shed light on two mysterious evolutionary processes that shape modern signal transduction pathways.

Keywords

functional domains, domain shuffling, bioinformatics

C

Achim Tresch

The evolution of repetitive DNA and its methylation

Abstract

Deep sequencing of bisulfite converted DNA measures the DNA methylation rate at cytosine positions. Typically, reads mapping to repetitive regions are discarded, because they cannot be mapped unambiguously to the genome. We turn this into an advantage by mapping these reads to prototypes of repetitive DNA sequences. The huge coverage ($> 10^5$) obtained this way allows us to quantify the effect of mutations of repetitive sequences on their methylation pattern at unprecedented precision. Conveniently, we can recur on species-specific methylation data available in public databases.

Keywords

Bisulfite sequencing, methylation of repetitive DNA