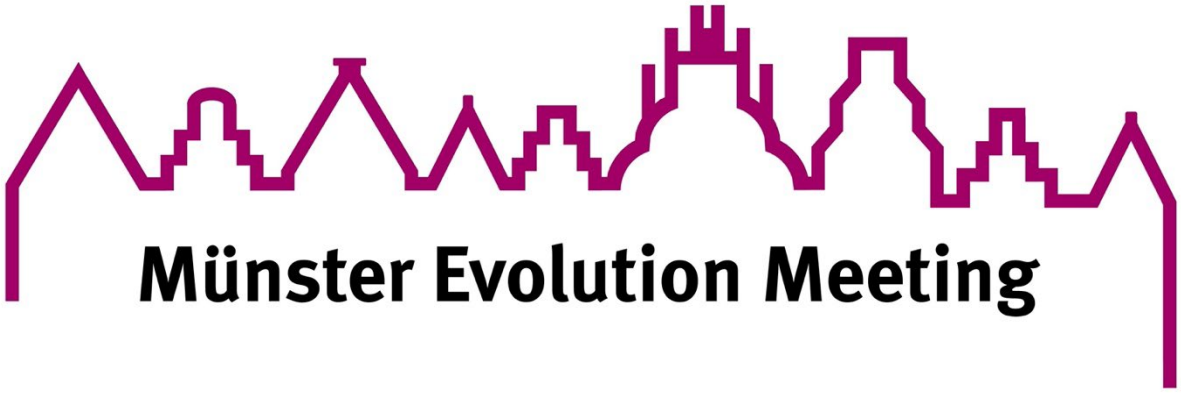

2023

ABSTRACT BOOK POSTERS

Münster Evolution Meeting

LANGE, Andreas

Poster number: 01

A guide to facilitate putative de novo proteins expression

Over the past decade, evidence has accumulated that new protein coding genes can emerge de novo from previously non-coding DNA. These proteins are called *de novo* proteins. Their properties are still poorly understood, and their experimental analysis faces major obstacles. This might be due to difficulties in handling de novo proteins in vitro, as most are predicted to be short and disordered. Here we propose a guideline for the effective expression of eukaryotic de novo proteins in *Escherichia coli* (*E.coli*) with the help of chaperones. We used 11 sequences from *Drosophila melanogaster* and 10 from *Homo sapiens*, that are predicted *de novo* proteins from former studies, for heterologous expression. The candidate de novo proteins have varying secondary structure and disorder content. Using multiple combinations of purification tags, *E. coli* expression strains and chaperone systems, we were able to increase the number of solubly expressed putative de novo proteins from 30% to 62 %. Our findings indicate that the best combination for expressing putative de novo proteins in *E. coli* is a GST-tag with T7 Express cells and co-expressed chaperones. We found that, overall, proteins with higher predicted disorder were easier to express. Since the expression of de novo proteins is quite difficult and time consuming, we also want to present another method to determine whether a protein is properly folded or not, the so-called Twin-arginine Translocation assay (TaT-assay). The assay selects for protein folding in living bacterial cells by using the intrinsic protein transport pathway. This pathway depends on the correct folding of the protein. In this assay, the target protein is expressed between an N-terminal transport signal and a C-terminal β-lactamase reporter protein, conferring ampicillin resistance. When growing the bacterial cells on a selective medium containing ampicillin, only the cells containing folded protein can survive.

Münster Evolution Meeting

MÜLLER, Annika

Poster number: 02

Investigating the effect of environmental sex determination on gene regulation

Most eukaryotes reproduce sexually – a mechanism that allows for recombination and therefore faster evolution. Many multicellular organisms have developed sexually specialised phenotypes which can be determined via two different systems: genetically (GSD), for example, by sex chromosomes, or environmentally (ESD). These sex determination systems are not necessarily mutually exclusive and rather represent a continuum within which the systems can transition into each other. Sexual dimorphism is mostly due to differential gene expression which plays an even more important role in species with ESD where the entire genome is identical in the sexes. In my project, I am investigating how genome evolution and gene regulation differ between organisms with ESD and GSD. I am comparing genomic and transcriptomic data from carefully chosen species to identify the role of sex-biased expression and splicing. I am aiming to identify overarching evolutionary mechanisms rather than lineage specific patterns. Therefore, I will compare organisms of these two modes of sex determination among highly divergent taxa, including crustacean, reptile, and fish species. Based on initial data from Daphnia, I hypothesise that gene duplication and sex-biased or sex-specific expression of the copies is more frequent in species with ESD than in those with sex chromosomes. The results of my project could shed light on fundamental genetic and regulatory mechanisms involved in the establishment of the two sexual phenotypes and how the mode of sex determination contributes to the evolution of both gene repertoire and gene regulation. Additionally to the evolutionary approach and the identification of general patterns, I would like to investigate transitions between different sex determination systems on a molecular level. A system that I find especially intriguing is the evolution of sex determination in wild and domesticated forms of zebrafish. Interestingly, zebrafish quickly lose their sex chromosomes when held in captivity, leading to the establishment of polygenic sex determination in the domesticated strains. I would like to better understand the mechanisms and consequences of this rapid loss of sex chromosomes on gene regulation.

Münster Evolution Meeting

PROENCA, Audrey M.

Poster number: 03

The deterministic side of bacterial heterogeneity (that you cannot see in a flask)

Bacterial populations, even when grown from a single starting cell, exhibit wide variation in physiological states and stress responses. Although this broad variability can determine which individuals survive environmental pressures, phenotypic heterogeneity is often disregarded as non- relevant variation. Nonetheless, advances in single-cell microscopy have shown that much of the physiological variance in bacteria is due to deterministic factors. As cells grow and divide, even the morphologically symmetric *Escherichia coli* gives rise to lineages with distinct growth physiology. We have explored the mechanisms of this phenotypic asymmetry through time-lapse microscopy and microfluidics techniques, following bacterial populations over several generations. We show that asymmetric patterns emerge with every division and are maintained over time, resulting in stable fitness differences between mother and daughter cells. Mother cells maintain lower elongation rates over time, while producing faster-growing daughters. Whereas this physiological asymmetry is constant over generations, we also demonstrate that certain aspects of cell size determination change over time due to cellular aging. Finally, using fluorescent reporters, we demonstrate that these patterns also refer to the expression of genetic components. We have followed the expression of transcription factors related to stress responses, observing that the source of asymmetry among mother and daughter was already produced within the mother itself prior to division. Together, these results demonstrate that bacterial populations can create complex patterns of phenotypic heterogeneity, with the coexistence of distinct growth states and responses to intracellular damage that derive from deterministic components. This deterministic aspect is masked in batch cultures, but evident once the life history of bacterial lineages is taken into consideration — and it can be essential in assessing the response of a population to environmental pressures.

Münster Evolution Meeting

KEMENA, Carsten

Poster number: 04

DomainWorld - Facilitating protein domain based analyses

Protein domains are reusable building blocks of proteins that allow to easily recombine functional units and therefore to change the function of proteins during evolution. Despite their known importance, the development of domain-based analysis tools is still at the beginning. Therefore, we are developing DomainWorld, a set of tools aiming to facilitate domain based analysis, beginning with their annotation and extending to different tools to analyze the evolution and recombination of protein domains. We provide support for different annotation tools, so users can choose their own way of annotating their proteomes. To increase maintainability, we have developed a C++ core library that is reused in the different programs. Here, we will specifically present RADIANT, a tool to annotate even large proteomes very rapidly. Instead of using Hidden Markov Models, it uses a database of words that can be searched quickly and is used to assign domains to protein sequences. RADIANT has been shown to have an accuracy almost as good as PfamScan while having a much shorter runtime.

Münster Evolution Meeting

LAUENROTH, Dana

Poster number: 05

Persistence and adaptation in perennial weeds with complex life cycles

Perennial weed species like *Sorghum halepense* are a bane of agricultural produce and lead to significant economic losses. Such weeds can spread quickly and are particularly hard to control due to their ability to reproduce sexually via seeds and asexually through rhizomes. Control strategies are frequently investigated through field experiments in the short run. However, mathematical modelling has proved to be a valuable approach to studying the long-term population dynamics of weeds. Still, few models have dealt with perennial weed species because of their complex life cycles. I present a population-based model of seed and rhizome propagated perennial weeds, specifically *Sorghum halepense*, incorporating the complete complex life cycle and control measures of herbicide application and tillage. I highlight the pivotal role of the sexual phase of the life cycle, including self-pollination and seed bank dynamics, in the persistence and rapid adaptation of *Sorghum halepense*. In particular, I illustrate how the seed bank helps preserve genetic diversity under recurrent selective pressure imposed by herbicides. Moreover, I indicate the potential of the seed bank to ensure population survival under control by delaying extinction and increasing the probability that resistant mutants establish. I show that the speed of resistance evolution increases under self-pollination in such seed and rhizome-propagated weeds.

Münster Evolution Meeting

AKSU, Ekin Deniz

Poster number: 06

Primate enhancers: identification, annotation, evolution

Enhancers are genomic regulatory regions that interact with promoters to increase transcription of their gene targets. They are distal to transcription start sites and contain binding sites for transcription factors (TFs) that are thought to be mainly responsible for their activity. Locations of enhancers in the genome are predicted chiefly by two means: from experimental data (such as ChIP- seq or DNase-seq) or from evolutionary conservation between multiple species. When different vertebrates are compared, there is the drawback of missing recently evolved enhancers. In order to improve on this problem and characterize enhancers in primates, we revisit previously developed methods (such as phylogenetic shadowing). First, we identify primate-shared enhancers by analyzing multiple sequence alignments of primate reference genomes. Second, we annotate identified regions with their genomic and tissue context as well as their predicted targets. Furthermore, we compare results of comparative-genomics-based enhancer identification to biochemically-defined enhancers. Third, we try to understand how enhancer sequences evolve in primates by focusing on TF binding site and single nucleotide polymorphism evolution.

Münster Evolution Meeting

CELEMIN, Enrique

Poster number: 07

Evolutionary history and seascape genomics of Harbour porpoises (*Phocoena phocoena*) across environmental gradients in the North Atlantic and adjacent waters

The Harbour porpoise (*Phocoena phocoena*) is a highly mobile cetacean species which primarily occurs in coastal and shelf waters across the Northern hemisphere. It inhabits heterogeneous seascapes that vary broadly in salinity and temperature. Here we produced 74 whole genomes at intermediate coverage to study Harbour porpoise's evolutionary history and investigate the role of local adaptation in the diversification into subspecies and populations. We identified ~6 million high quality SNPs sampled at 8 localities across the North Atlantic and adjacent waters, which we used for population structure, demographic, and genotype-environment association analyses. Our results support a genetic differentiation between three subspecies, and three distinct populations within the subspecies P.p. phocoena: Atlantic, Belt Sea and Proper Baltic Sea. Effective population size and Tajima's D levels suggest a population contraction in both Black Sea and Iberian porpoises while a population expansion in the P.p. phocoena populations. Phylogenetic trees indicate a post-glacial colonization of Harbour porpoises from a southern refugium. Genotype- environment association analysis identified salinity as a major driver in genomic variation and we identified candidate genes putatively underlying adaptation to different salinity levels. Our study highlights the value of whole genome resequencing to unravel subtle population structure in highly mobile species and shows how strong environmental gradients and local adaptation may lead to population differentiation. The results have great conservation implications as we found major levels of inbreeding and low genetic diversity in the endangered Black Sea subspecies and identified the critically endangered Proper Baltic Sea porpoises as a separate population.

Münster Evolution Meeting

ZEBARDAST, Fatemeh

Poster number: 08

Genes with positive selection in humans might be involved in Alzheimer pathology

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by extracellular amyloid plaques, intracellular neurofibrillary tangles and selective neuronal loss. Since AD seems to be a human specific disease, it is likely that some genomic changes related to the evolution of the human brain also play a role in the pathology of AD. To test this possibility, we analyzed which co-expression changes are common to AD patients and subsequently investigated the evolutionary history of AD specifically co-expressed genes. We used four genome-wide transcriptomics datasets of different cortex regions from 717 AD patients and 332 controls to construct a gene co-expression network using weighted topological over- lap (wTO) method. From "AD pathway" as defined in the KEGG database and the literature we selected thirteen well-known AD-related genes and extracted their graph neighbourhood in order to enrich the set of genes potentially involved in AD pathology. A comparison of the AD and control networks revealed that 2538 genes are specifically co-expressed with these thirteen AD key genes in the AD network, while only 66 genes are specifically co-expressed with these genes in the control network and 159 genes are co-expressed with them in both networks. We tested all these co-expressed genes for positive selection on the human branch using the branch-site model of PAML v4.9 including about 60000 orthologs from 23 non-human primate species. Our results showed that 25 genes of the AD specifically co-expressed genes and one of the genes co-expressed specifically in the control network have positively selected codon/s in the human lineage, while none of the genes with co-expression in both networks showed signs of positive selection in humans. Interestingly, two AD specifically co-expressed genes are evolutionary young genes; ZNF439 a positively selected gene that arose within the primate clade, and SRGAP2C which is human specific. Both genes are the first neighbours of ADAM10 in the AD wTO network, which codes for the main α- secretase that cleaves the amyloid precursor protein in the non-amyloidogenic pathway inhibiting the formation of the β-amyloid peptide. SRGAP2C has already been associated with brain development, providing further support for an interplay between evolutionary and pathological changes.

Münster Evolution Meeting

DEWAN, Ian

Poster number: 09

Evolution of multiple drug resistance in bacterial populations through plasmid-mediated heterozygosity

Bacterial plasmids and other extra-chromosomal DNA elements frequently carry genes that have important effects on the fitness of their hosts. Because plasmids often exist in the bacterial cell in multiple copies, different plasmid copies can carry distinct alleles, allowing for heterozygosity not possible for loci on haploid chromosomes. Plasmid-mediated heterozygosity may increase the fitness of bacterial cells in circumstances where there is an advantage to having multiple distinct alleles (heterozypgote advantage); for example, plasmid-mediated heterozygosity of antibiotic resistance alleles can produce multiple drug resistance, in which a single bacterial strain is resistant to multiple antibiotics, a serious problem in the clinical context. However, plasmid-mediated heterozygosity is also subject to constant loss due to random segregation of plasmids on cell division: each division has some probability of producing a homozygous daughter cell. We present a mathematical model of the evolution of plasmid-borne bacterial traits in a heterozygote advantage scenario, focusing on the establishment of a novel resistance allele on a plasmid in a bacterial population already adapted to one antibiotic but undergoing demographic decline due to simultaneous treatment with multiple antibiotics (an evolutionary rescue scenario). We derive the minimum threshold on the selective advantage of heterozygotes required to overcome segregative loss and make population persistence (rescue) possible; this threshold decreases with increasing copy number of the plasmid. We further show that the formation of cointegrates from the fusion of plasmids increases the probability of rescue, as multiple alleles on cointegrated plasmids are less subject to stochastic loss. These results contribute to understanding both the contribution of the evolution of plasmid-level traits, such as copy number, to bacterial evolution and the evolution of antibiotic resistance in complex selective environments.

Münster Evolution Meeting

KRAMER, Jos

Poster number: 10

KillerProfiles – Unraveling the effects of deeply divergent predators on the evolution of microbial communities

Predation shapes biological evolution at multiple scales, from genomes and organisms to entire ecosystems. Albeit traditionally studied in larger organisms, predation also pervades the microbial world: nematodes and protists ingest prey whole via phagocytosis; many Bdellovibrio-and-like organisms (BALOs) invade their prey and reproduce in a virus-like fashion; and group-hunting myxobacteria deploy a whole arsenal of diffusible and contact-dependent mechanisms to kill and lyse their prey. Despite their expected great impact, we know little about how such deeply divergent predators affect the composition and dynamics of microbial communities over extended evolutionary times. Moreover, it remains unclear how predators adapt to their prey, and whether predator-prey coevolution leads to Red Queen dynamics at the community level. We will perform a large-scale evolution experiment to shed light on these issues. Specifically, we will use three species each of five divergent classes of predators – myxobacteria, BALOs, dictyostelids, cercozoa, and nematodes – and coevolve them with a defined community of 20 soil bacteria. By comparing the effects of each predator species and class on prey community evolution, we will gain systematic insights into how distinct major predators of microbes shape the evolution of species-rich communities. Metagenomic sequencing will moreover allow us to test how different predators and their prey (co-)evolve. Overall, our comparative approach will foster a more comprehensive understanding of the evolutionary ecology of microbial predation.

Münster Evolution Meeting

MEYER ZU RIEMSLOH, Katharina Natascha

Poster number: 11

Novel task for a novel caste – Controlling microbial growth

The gut microbiome of ants has been shown to contribute to their metabolism and immunity. Controlling the composition of the gut microbiome is essential for the host to avoid overgrowth of harmful microorganisms and their spread through the whole colony. *Myrmecocystus mendax* belongs to an ant genus which evolved a special worker caste, called repletes or honeypots. Healthy honeypots have enlarged crops and gasters filled with a nutrient-rich liquid, which can be dispensed to the colony if required. Honeypots specialize early after metamorphosis and once fully developed, they cannot revert. Besides the capacity to enlarge their abdomen, honeypots need mechanisms to conserve their gut content and prevent unwanted microbial growth. We repeatedly found that honeypots in isolated worker groups accumulate gas in their crop and often die. We hypothesized, that the variation between healthy and gas-filled honeypots is due to a dysbiosis of their crop microbiome. This was supported by microscopic analyses of the crop liquid, which showed that yeast-like cells were abundant in gas-filled honeypots but almost absent in healthy honeypots. To further verify this finding, we used a cultivation-dependent approach to identify dominant microbes in extracted crop liquid of healthy and gas-filled honeypots by using different growth media. Strikingly, several colonies of the yeast *Zygosaccharomyces bailii* were isolated from the gas-filled honeypots, which did not appear in healthy repletes, while bacteria from the genus *Fructilactobacillus fructivorans* were isolated from both honeypot variants. This suggests, that growth of the yeast *Zygosaccharomyces bailii* can lead to a dysbiosis of the crop microbiome of *Myrmecocystus mendax*, with adverse effects on the survival of the repletes. Next, we will analyze the composition and diversity of the crop microbiome of both honeypot variants by 16S amplicon sequencing and compare the microbiomes with samples of other worker castes (nurses and foragers) of *Myrmecocystus mendax* and with the microbiome of its sister genus *Lasius niger*. In combination with RNAseq analyses we want to understand the underlying mechanisms that help to preserve the crop content of honeypots and hence the necessary physiological and evolutionary changes associated with the evolution of honeypots as a new worker caste in the ant genus *Myrmecocystus*.

Münster Evolution Meeting

WILHELM, Léonore

Poster number: 12

Cellular regulation of a neurotoxin produced by the Colorado potato beetle

The Colorado potato beetle (CPB), a major potato crop pest, produces a neurotoxin that circulates in its hemolymph. The toxin, leptinotarsin, evolved from a duplication of the juvenile hormone esterase gene. It is present in every developmental stage of the beetle, including the egg. Preliminary data suggest that leptinotarsin is produced by a specific type of hemocyte. I aim to study the cellular regulation of leptinotarsin and its evolution in the genus Leptinotarsa. My first goal is to identify the cells producing leptinotarsin. For that purpose, I am performing a Gene Expression Atlas of the Colorado potato beetle tissues and single-cell sequencing of its hemocytes.

Münster Evolution Meeting

MACIT, Maide Nesibe

Poster number: 13

Genome-wide detection of selection and local adaptation in *Temnothorax longispinosus* to its dulotic parasite *T. americanus*

Parasitism as a highly specialized and evolutionary successful mode of life requires a reshaping of morphology, gene expression and genome of the exploiter to adjust to its host. In social parasitism, an exploitation based on altruistic behaviour, the focus of adjustment of behaviour of the parasite to its host is of special importance. In the case of the obligate dulotic ant *Temnothorax americanus* and its host *Temnothorax longispinosus*, their common ancestry and shared environment posed a viable opportunity for social parasitism to evolve, with reciprocal adaptations of the host leading to a coevolutionary arms race. To investigate the genomic basis of this coevolution and its mosaic- like structure created by local adaptations, we collected 100 host colonies and varying numbers of parasitic colonies from ten independent populations in the NE of the USA during the summer of 2021. We pooled DNA of these host populations to generate data to conduct a Pool-GWAS that is yet to be analysed. We plan to investigate population structures of the host by associating the locally adapted genomes to i) genes linked to the local parasite prevalence, ii) antennal transcriptional data investigating communication, iii) levels of aggression in behavioural assays, iv) diversity in chemical profiles and lastly, v) map these findings to climate data to investigate associations to the local environment. With this, we hope to shed light on the effect that the local occurrence of the parasite had/has on the genome structure and subsequent adjustments in the phenotype of the host.

Münster Evolution Meeting

LAMMERS, Mark

Poster number: 14

Transcriptomic basis of rapid host adaptation and multigenerational phenotypic plasticity.

Phenotypic plasticity allows individuals to exploit a larger variety of environments. When a novel environment (e.g. new host species) emerges, more plastic individuals may succeed to use its resources. Subsequent generations may rapidly adapt to the novel environment, e.g. through genetic accommodation if the population is genetically diverse. Population dynamics of the aphid-parasitoid system are characterized by boom-and-bust dynamics in nature. Collapse of an aphid population forces parasitoid wasps to disperse and attack other hosts. Consequently, the wasps' life-history alternates between local inbreeding followed by dispersal, a lifestyle hypothesized to select for phenotypic plasticity but reduce genetic diversity. We inbred *Aphidius ervi*, purging >98% of heterozygosity, and then subjected to experimental evolution, rearing them either on a novel smaller host, or on the original host. Only few inbred lines successfully reproduced on the novel host showing signs of adaptation. However, long-term survival of inbred lines was only observed on the original host. After adaptation to the novel host, we tested performance of surviving lines in a common garden experiment and sequenced tissue-specific transcriptomes to ask whether rapid host adaptation is linked to behavioral changes (RNAseq of brains), maternal imprinting (ovaries) or host manipulation (venom glands) and whether losses and gains of phenotypic plasticity are reflected in transcriptomic changes.

Münster Evolution Meeting

BOLDER, Maximilian

Poster number: 15

Investigation into the reversibility of queen specialization in ants

Major evolutionary transitions gave rise to biological systems that function based on the cooperation of specialized components that occur at multiple phenotypic scales. Examples of such systems include multicellular organisms and insect societies. One fundamental question in biology is to understand the ontogeny of biological systems, i.e., the process that produces the self-assembly and specialization of multiple components from a single unit. While extensive research efforts have focused on the ontogeny of multicellular organisms, leading to the formation of the entire field of developmental biology, there are few experimental investigations into the ontogeny of insect societies. In most social insect species, queens found their colonies independently, and during this stage express a variety of behaviors, including brood care. Only when the first workers have been produced do the queens become specialized in egg production. Recent research has revealed that established queens of two ant species revert to expressing brood care upon experimental removal of their workers. This reversibility highlighted the importance of worker presence in initiating and maintaining the queen's specialization in egg production. Preliminary experiments suggest that this reversibility may be found in multiple species of ants, but queens of some species appear to perform very little brood care irrespective of the social environment. We plan to investigate the reasons for this variability in queen behavioral specialization inside the ant phylogeny. So far, we have analyzed the behavior of some species in the most species-rich and evolutionary distinct subfamilies Formicinae, Myrmicinae and Dolichoderinae, and are actively seeking to increase the number of subfamilies and species to achieve a wide phylogenetic sweep. Collecting data across species with distinct specificities, such as queen morph, colony foundation strategy and others, would allow us to trace the evolutionary history of queen behavioral specialization and would help us better understand the division of labor in insect societies.

Münster Evolution Meeting

KULIKOV, Nikita

Poster number: 16

Machine learning can be as good as maximum likelihood when inferring phylogenetic tree topologies and determining the best evolutionary model on four taxon alignments

Phylogenetic tree reconstruction with molecular data is one of the biggest challenges for evolutionary biologists. To address this issue, numerous methods have been developed in the past. The classical algorithms fall into two main categories: distance-based, i.e, UPGMA, NeighbourJoining and character-based, i.e. maximum likelihood, Bayesian methods. Among the classical approaches, the Maximum Likelihood method is considered as the gold standard when it comes to accuracy. Additionally to the aforementioned methods several machine learning based algorithms, including neural networks, have been presented in recent years. However, the majority of the machine learning algorithms presented so far have not been able to achieve the accuracy of the maximum likelihood method. We have shown that for quartet trees, neural networks trained on the site pattern frequency distribution can be as good as the Maximum Likelihood method to infer the best tree topology and the best model of sequence evolution for nucleotide as well as amino acid sequences. For this purpose we simulated data sets for a wide range of branch lengths, evolutionary models and model parameters and compared the topologies and inferred models obtained with Machine learning with those obtained with the Maximum Likelihood and the Neighbour Joining method. Our results have shown that neural networks are a promising avenue for determining relatedness between taxa, which is likely to accelerate the construction of phylogenetic trees in the future, while maintaining a high accuracy.

Münster Evolution Meeting

GUSEV, Nikolai

Poster number: 17

A locus specific analysis of all MHC class II genes of the Harbor porpoise (*Phocoena phocoena*)

The evolutionary arms race between hosts and their pathogens revolves around the ability of the hosts to recognize their pathogens – and the pathogens to avoid recognition by the hosts. In vertebrates, a set of proteins of the Major Histocompatibility Complex (MHC) is central to pathogen recognition. These proteins present the cellular and intracellular peptidome to the T-Cells which, in turn, initiate the immunological cascade. Being capable of presenting the most frequent pathogenic variant is favourable for the host. Vice versa, pathogens undiscovered by most frequent MHC alleles have increased fitness. This relationship can cause negative-frequency depending selection on both hosts and pathogens, leading over time to balancing selection, hence maintaining adaptive genetic variability. In the present project, we focus on the genetics of MHC class II proteins from Harbor porpoises (*Phocoena phocoena*). Our recently produced reference genome and a wide sampling across the Atlantic and adjacent waters enabled us to annotate all MHC class II loci and subsequently amplify them separately. With this locus-specific data from all MHC class II genes, we are able to better understand the nature and evolution of cetacean MHC. We can infer prevalent selection for each locus, thereby inferring whether they are functional or have become pseudogenes. We further see, in how far allelic diversity is correlated across MHC loci, both on the individual and population level. Comparison to neutral markers allows to control for population structure and thereby to infer possible local adaptations to differential pathogenic regimes. In summary, our study highlights the potential of locus-specific MHC typing and its feasibility in non-model organisms.

Münster Evolution Meeting

TRETTER, Sandra

Poster number: 18

The genomic and neural constituents of ant olfaction in the light of social complexity

Olfactory perception is an essential trait for most animals, as it is involved in many vital behaviors such as mating and foraging. Odorant receptors (ORs) are one of the main types of olfactory receptors and constitute a rapidly evolving and highly diverse gene family. Within insects, the number of ORs varies considerably and is especially increased in ants, where chemical communication shapes the cohesion of social insect societies. The expansion of the OR gene family in ants is thus suspected to be associated with the evolution of sociality and social complexity e.g., nestmate recognition and division of labor in the colony. Neuroanatomical studies further suggest that the OR family expansion is responsible for an increase of the olfactory processing system in the ant brain, as the number of glomeruli in the antennal lobes is correlated with the number of functional ORs expressed in the antennae. To study the interplay of antennal lobe complexity and OR diversification in ants, we combine microCT scans with comparative genomics across 52 ant species from seven different subfamilies. We will correlate the expansion of the OR gene family and the emerging changes in the olfactory lobes of ants, with regard to antennal lobe volume, glomeruli count, and glomeruli volume. This will allow us to test the prediction that changes in social complexity (e.g., larger colonies, larger worker polymorphism, etc.) are accompanied with changes in the olfactory system at both the genomic and neuroanatomical level in ants.

Münster Evolution Meeting

ÇOBAN, Abdulbaki

Poster number: 19

A domain-based approach to simulate protein evolution

Protein domains are building blocks of proteins. The rearrangements of protein domains create new proteins with novel functions, therefore expand the protein repertoire. Protein domains are rearranged by at least six possible events: fusion, fission, terminal domain loss, terminal domain gain, single domain loss and single domain gain. Even though there are studies on the domain rearrangements of a given set of proteins, the tools to benchmark protein evolution tools, specifically in terms of domain rearrangements, are scarce. While previously reported tools (e.g. DomArchov) simulate domain arrangements with low resolution considering only insertion and deletion events, here we calculated the frequencies of six rearrangement events defined above and used the frequencies to build a simulation tool to benchmark domain based programs. Currently, we built the simulation tool for the vertebrates, but we plan to expand it to include other clades. Our simulation framework models protein evolution through a chain of domain rearrangement events with their estimated frequencies. The simulated data were tested with DomRates, a tool to infer domain rearrangement events, and compared to the naturally found domain arrangements sets. Preliminary results show that our simulation tool models protein evolution at the domain level comparable to what we observe in data sets with real proteins and, therefore, it allows researchers to test and benchmark current tools in the field of protein evolution.

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COCULLA, Angelica

Poster number: 20

Temporal niche choice in *Drosophila melanogaster*

In a world characterized by regular fluctuations, biological systems have developed mechanisms to anticipate and respond to environmental changes to increase their fitness and survival. Circadian clocks consist of highly preserved molecular feedback loops allowing organisms to adapt their behavior to the 24 hours environmental light and temperature changes. Circadian clocks enable biological systems to restrict their activity to specific periods of the day, which is defined as temporal niche. Correct temporal niche choice is important for the fitness and survival of an organism, and it is regulated by an intricate interplay between the animal's circadian clock and environmental fluctuations. As temporal organization is genetically determined, mutations in circadian clock genes can allow organisms to explore and to occupy different temporal niches, increasing the adaptation of a population to different environmental conditions. However, the molecular and cellular mechanisms that drive niche choice remain to be fully understood. Under non-stressful conditions, individuals show low inter-specific phenotypic variability, despite being genetically different. However, they may accumulate genetic mutations that are not phenotypically visible, called cryptic genetic variations. When a sudden environmental stressor occurs, cryptic genetic variations introduce new phenotypes that are then bottlenecked by natural selection to favor fitness and survival of the population. Heat shock protein 90 (HSP90) has been identified as a possible candidate to unveil the genetic potentials of biological systems. Previous studies (Hung et al., 2009) indicate that loss of HSP90 leads to increased behavioral variation in *Drosophila*, including multiple transitions from rhythmic to arrhythmic behavior. Here, we investigate whether individuals can actively explore and choose temporal niches that support their own fitness, and the possible role of HSP90 in the molecular pathways leading to variable intraspecific temporal niche choices.

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FAJARDO-CASTRO, Johana

Poster number: 21

Exploring the role of taxonomically restricted genes in neuronal diversity of hydra

There are many unanswered questions regarding the origin and evolution of different cell types within a cell lineage. One of them is the origin of diverse neuronal populations in metazoa, and how they are related to each other. Although the genes generally expressed by neurons were already present in the ancestor of metazoa, or even earlier, specific neuronal populations might express a battery of lineage specific genes. A related question is whether neuronal populations in different organisms share a common origin, or whether they have diversified independently within each taxon. To address these questions, we used a combination of phylostratigraphy, single-cell RNA sequencing and functional bioinformatic predictions in order to identify taxonomically restricted genes—i.e. specific to the phylum, genus or species—in brown hydra, responsible for the diversification of neuronal populations. Recent genome and transcriptome data from a variety of cnidarian species, allowed us to look at gene age more accurately than before. We found that, although the genes necessary for the formation of neurons were indeed present in the ancestor of metazoa, evolutionary younger genes are enriched in differentiated neuronal types in hydra. Our results showed that neuronal populations have diversified in Hydra through adoption of lineage-specific, taxonomically restricted genes. This finding supports the idea that terminally differentiated neuronal populations have lineage specific identities.

Münster Evolution Meeting

STEIDLE, Johannes

Poster number: 22

The emergence of ecotypes in a parasitoid wasp: a case of incipient sympatric speciation in Hymenoptera?

To understand which reproductive barriers initiate speciation is a major question in evolutionary research. Despite their high species numbers and specific biology, there are only few studies on speciation in Hymenoptera. This study aims to identify very early reproductive barriers in a local, sympatric population of *Nasonia vitripennis* (Walker 1836), a hymenopterous parasitoid of fly pupae. We studied ecological barriers, sexual barriers, and the reduction in F1-female offspring as a postmating barrier, as well as the population structure using microsatellites. We found considerable inbreeding within female strains and a population structure with either three or five subpopulation clusters defined by microsatellites. In addition, there are two ecotypes, one parasitizing fly pupae in bird nests and the other on carrion. The nest ecotype is mainly formed from one of the microsatellite clusters, the two or four remaining microsatellite clusters form the carrion ecotype. There was slight sexual isolation and a reduction in F1-female offspring between inbreeding strains from the same microsatellite clusters and the same ecotypes. Strains from different microsatellite clusters are separated by a reduction in F1-female offspring. Ecotypes are separated only by ecological barriers. This is the first demonstration of very early reproductive barriers within a sympatric population of Hymenoptera. It demonstrates that sexual and premating barriers can precede ecological separation. This indicates the complexity of ecotype formation and highlights the general need for more studies within homogenous populations for the identification of the earliest barriers in the speciation process.

Münster Evolution Meeting

PETIT, Jules

Poster number: 23

Phenotypic plasticity under urbanisation: A meta-analysis

Human-induced rapid environmental change (HIREC) is a major concern of the last decades due to the many perturbations it generates in ecosystems. Urbanisation is a widespread and common HIREC which involves disturbances such as fragmentation of habitat, noise pollution or pollutants. A key area of current research is studying how animals cope with these new challenges. Yet, much research performed till now, investigates patterns and changes of individual average responses to face urbanisation. These studies showed that individuals seem to have different ecotypes between rural and urban habitats, but it often remains unclear whether this pattern is due to an adaptive process (e.g., local adaptation) or results from phenotypic plasticity, such as reversible individual flexibility. The aim of this study is to test the hypothesis that individuals of urban populations are more flexible than those of rural populations. Using a meta-analysis approach, we aim to (i) summarise studies that have measured individual variation at the within- and between-individual level under urbanisation for labile traits (behaviour, physiology, life-history), (ii) quantify the proportion of within- and between-individual variation and (iii) test whether the degree of urbanization explains differences for each partition of individual variation. We expect that urban individuals express higher levels of phenotypic plasticity across all types of labile traits although the clearer differences should be present in behavioural traits since they are the most flexible. Specifically, we test three predictions (i) within individual variation (i.e. reversible flexibility) is higher in urban compared to rural populations. (ii) between-individual variation (i.e. indicating individual specialisation) is lower in urban compared to rural populations and, therefore, (iii) urban populations have lower traits expression consistency (i.e. lower repeatability) than rural populations. We hope that our study will help to clarify the mechanisms driving adjustment to urbanization and particularly by revealing how individual variation in the form of reversible plasticity or increased generalisation play a role to mitigate the effects of urbanisation.

Münster Evolution Meeting

EICHOLT, Lars

Poster number: 24

Structure Predictions of de novo emerged Proteins in the Age of Machine Learning

During the last two decades, it became clear that new genes and hence proteins, can emerge from formerly non-coding DNA; they have emerged de novo. Contemporary protein families can be seen as island in the vast ocean of possible sequences. Only a few have surfaced during the course of evolution while most remained submerged or plunged. De novo proteins are novel island dis- tant from the rest in this ocean and could further provide unique structures and folds. Completely unique structures would further confirm de novo status since structure is more conserved than sequence and an entirely new structure would be highly unlikely arising from an ancestral structure. Experimental structural determination of de novo proteins can yet only be performed via NMR due to their small size and high disorder. We have recently established a protocol to express many de novo proteins solubly and allow for the first time further characterisation of multiple de novo proteins. AlphaFold2 seems to reliably predict the structure of de novo proteins while the question remains how if does that, since they lack sequence homology, and how AlphaFold2 performs on de novo proteins in comparison to natural language based predictors. Our goal is to use combinations of experimental and computational tools to elucidate the novel structures we hope to find in de novo arisen proteins and open up new lanes for protein engineering and our understanding of early protein evolution.

Münster Evolution Meeting

DORONINA, Liliya

Poster number: 25

Intron loss in mammals and other vertebrates

The loss of spliceosomal introns and the merging of the two neighboring exons is a well-known phenomenon at the genomic level. However, its mechanisms and evolutionary dynamics remain elusive. Here, we used the recently developed n-way tool to perform comparative genome analyses of intron loss events in vertebrates. We conducted pairwise whole-genome alignments on intron annotations of human, mouse, cow, dog, opossum, and zebra finch genomes (about a million introns). The screening and subsequent manual orthology verification revealed 153 cases of intron losses in 11 groups of mammals, 11 intron losses in birds, 1 case in reptiles, 5 in amphibians, and 46 in bony fishes. Although the loss of an intron represents a rare event, some mammalian groups were particularly exposed to this phenomenon, namely rodents, shrews, and tenrecs, suggesting that similar population dynamic/generation times lead to similar accelerated genome dynamics. Among vertebrates, the largest observed burst of intron losses in bony fishes may be connected with high transposable element diversity and activity and agrees with previous studies. Moreover, we detected 38 introns lost repeatedly in vertebrate evolution, including cartilaginous fishes and lampreys. We propose that some introns present a hotspot that can easily be lost, resulting in a phylogenetic mosaic of losses today.

The most frequently proposed mechanism for intron loss is ectopic recombination of intron-containing genes with intron-less retropseudogenes derived from reverse transcribed and genomic reintegrated spliced cDNAs. Surprisingly, we also observed intron loss in the bird lineage leading to Galliformes, where only a small number of retropseudogenes are known. However, in many analyzed loci, we detected identical sequence stretches of up to 15 nt around the 5'- and 3'-splice sites, suggesting that microhomology-mediated intron loss, described, for example, in Drosophila, may represent an alternative mechanism for losing introns in vertebrate genomes. We further detected two cases of intron gains that were not described before: (1) in the ancestor of Gnathostomata and (2) in Theria. Thus, our data reject the previously proposed hypothesis that no novel introns occurred, e.g., during mammalian evolution.

Münster Evolution Meeting

SCHRADER, Lukas

Poster number: 26

The Global Ant Genomics Alliance (GAGA): towards a phylogenomic understanding of ant social evolution

The ants have evolved a stunning global diversity with more than 15,000 extant species belonging to over 330 genera. Their ecological success is rooted in division of labour comparable to what somatic cells in a metazoan body achieve. GAGA was launched in 2017 to generate and study high-quality genomic, transcriptomic, microbial and ecological data for >200 ant species. During the first phase of the project, samples of more than 250 species have been obtained, involving 64 collectors in 30 countries. We have now reached our first milestone of >140 high-quality and contiguous genome assemblies, covering twelve of the world's 16 ant subfamilies. Combined with extensive life history and phenotypic data sets, these genomes allow us to explore the molecular mechanisms underpinning ecological adaptations and complex social systems. Our analyses offer evidence for convergent evolution associated with parallel increases in social complexity, and exhaustive annotation of odorant receptor genes suggest that the expansion of this gene family had already happened in the common ancestor of all extant ants. We will provide an overview of GAGA's progress so far, and of the major questions that ongoing analyses are addressing to better understand the astonishing adaptive radiation of the ants.

Münster Evolution Meeting

POHL, Marius

Poster number: 27

The impact of the river Comoé and its tropical surrounding gallery forest on the praying mantis composition of the Comoé National Park, Ivory Coast.

The Comoé National Park (CNP), located in the north-western part of the Ivory Coast, is strongly influenced by the river Comoé, which is eponymous for the park. The river is located in the eastern part of the country and stretches from the southern coast of Ivory Coast to the northern border with Burkina Faso. The river is accompanied by an evergreen, tropical gallery forests in the CNP, which is otherwise characterized by forest islands, open grasslands, bush and tree savannas of various density. All those different habitats harbor a huge variety of invertebrates. Praying mantids (Insecta: Mantodea) are predatory, polyneopteran insects and well morphologically adapted to their specific habitat. They are sensitive bioindicators for ecosystems and show a high diversity south of the Sahara. Typically, one would expect to find mostly savanna praying mantis species in the CNP, but the tropical gallery forest allows forest species to migrate further into the northern part of the country. Specimens were collected in 2000, and between 2017 and 2019, resulting in 35 species in 13 families. Two species, *Ischnomantis werneri* (Giglio-Tos, 1916) and *Solygia sulcatifrons* (Audinet-Serville, 1838) were recorded the first time for the Ivory Coast. Six forest species (most notably from the genera Congoharpax and Chrysomantis) and 24 typical savanna taxa were recorded. Four species are generalists, found in both habitats (*Phyllocrania paradoxa*, *Heterochaeta strachani, Polyspilota aeruginosa, Sphodromantis lineola*). Here we compare the morphology and distribution patterns of savanna and forest taxa of the CNP. Furthermore, we present a preliminary checklist of praying mantis species, including 47 species, which are expected to occur in the CNP.

Münster Evolution Meeting

RODRIGUEZ, Matias

Poster number: 28

Mobilome of Apicomplexa parasites

Transposable elements (TEs) are interspersed repetitive sequences that can be found in almost all eukaryotic genomes and constitute a major part of them. TEs are so ubiquitous that most genomes have been colonized by different types of transposable elements and they're so diverse that new elements continue to be discovered. Apicomplexa is a phylum that comprises a large number of protozoan organisms which are obligate intracellular parasites of a wide range of vertebrate and invertebrate hosts. Apicomplexa protozoans are responsible for multiple pathologies of medical and veterinary importance, including malaria, toxoplasmosis and cryptosporidiosis in humans, eimeriosis in poultry, and theileriosis in cattle. Up to date, only a handful of TEs were identified in Apicomplexa species. For instance in RepBase, one of the most commonly used databases of repetitive sequences from eukaryotic organisms, there are only three well characterized TEs in Apicomplexa, each of them from different Eimeria species, seven unknown repetitive elements from *Plasmodium falciparum*, two from *Toxoplasma gondii* and one from *Theileria parva*. For a comprehensive study of the TE repertoire in Apicomplexa and to get an overview of the distribution of these elements, we analyzed 64 genomes available in public databases. We used a 'de novo' approach combining different strategies for the identification of TEs. We classified TEs into families and performed functional analysis of unclassified elements and predicted domains associated with TE proteins. After an exhaustive manual curation of the results, we annotated the elements identified in their respective genomes. This study allows us to get a clear picture of the distribution of TEs in Apicomplexa, which type of elements are more prevalent in different genus, their abundance and how different elements are related. Many species included in this study have no TEs described before, so we have the opportunity to offer an insight into the abundance and type of TEs that occupy these genomes.

Münster Evolution Meeting

SARFERT, Melanie

Poster number: 29

Deeper insights in transcriptional regulation of homothorax

Homothorax (Hth) is a homeodomain transcription factor, which plays a key role in the development of the dorsal rim area (DRA) of the *Drosophila melanogaster* eye. Although the gene *hth* is known to exist across species, it is currently unclear whether it is also involved in the development of DRA in other species and how conserved the underlying molecular networks are. As a first step to answer this question, we performed a sequence analysis of *hth* and its DRA enhancer across 149 drosophilidae species. Within the enhancer sequence we screened for conserved transcription factor binding sites by testing enriched sequences against different background sets with three different programs (Xstreme, Ciiider, Homer). As a result, we obtained 10 different conserved binding motifs corresponding to 10 different transcription factors. Currently those putative transcription factor binding sites were experimentally validated, using different fly lines with point mutations in these sites.

Münster Evolution Meeting

ŞAHIN, Özge

Poster number: 30

A Bulked Segregation Analysis for an HSP90-Released Reduced Eye Phenotype in *Tribolium castaneum*

The molecular chaperone heat shock protein 90 (HSP90) has been considered a potential capacitor for evolution because this protein is supposed to mediate the storage and release of cryptic genetic variation (CGV). Such variation is not expressed under normal conditions, but it can lead to phenotypes when the organism is subjected to stressful conditions. However, it is still debated how exactly such evolutionary capacitance works and which specific traits are affected by it. A 'reduced eye' phenotype was recently reported in *Tribolium castaneum* (Coleoptera: Tenebrionidae, red flour beetle) after experimental inhibition of HSP90 (Aboelsoud & Kurtz, 2019), and was subsequently assimilated, such that we have lines of beetles that stably express this phenotype. In the present study, we characterized in detail the inheritance, penetrance, and morphology of this trait across the life stages of the beetle. However, the genetic basis of this phenotype is still unclear. We thus pursued a bulked segregation analysis (BSA) combined with whole genome sequencing (WGS) to identify single nucleotide polymorphisms (SNPs) associated with the phenotype. Two large areas on linkage groups 3 and 10 were found strongly associated with the phenotype. Functional gene analyses using RNAi are currently ongoing and focus on candidate genes within these regions. Some of these genes are even directly affected by SNPs differentiating between normal and reduced eye phenotypes. In summary, the obtained results will enable us to determine the genetic basis of an HSP90-regulated trait and thereby improve our understanding of how evolutionary capacitance works in detail.

Münster Evolution Meeting

STEFFEN, Raphael

Poster number: 31

NewickTreeModifier and paPAML – Computational tools to simplify selection analysis of protein-coding sequences

Selection analysis relies on phylogenetic species trees simplified in the text-based Newick format, grouping related species with parentheses. However, this usually requires modifying or pruning existing phylogenies to select relevant species. Doing so manually is time-intensive, and format errors are likely to occur. Therefore, we developed the NewickTreeModifier (NTM), a flexible web-based tool that uses the trimming function of the Python package ete3 to prune and modify tree files in Newick format automatically. Additionally, we implemented multiple reference trees, e.g., a large vertebrate "master" tree from a species list resembling the NCBI ortholog gene database, as a standard reference for our tool. Users can create Newick trees using predetermined or customized reference trees. Furthermore, branching orders can be manually modified using the connected Java-based graphic tree editor TreeGraph 2 developed by Ben Stöver and Kai Müller at the IEB Münster. Comparative analyses of protein-coding sequences may tell us retrospectively about episodic relaxed, preserving, or adaptive evolution. The ratio of genetic sites with nonsynonymous (amino acid altering) versus synonymous (non-amino acid altering) mutations indirectly measures selection pressure. This kind of analysis can be performed with the Phylogenetic Analysis by Maximum Likelihood (PAML) software. To facilitate and accelerate the large-scale analysis of selection, e.g., acting upon our previously detected 1151 exonized protein-coding sequences, we developed parallel PAML (paPAML). Our tool speeds up calculation times by parallelizing codeml runs, simplifies the resulting output, and includes detecting negatively selected sites. paPAML compiles results of multiple tests for both episodic and preserving selection pressures, including site, branch-site, and branch model comparisons, and outputs them in a codon list with labeled significance values and a fasta file which eases mapping the results to the original sequences. Our tool accelerates the computing speeds of PAML analysis up to the number of allocated computer threads and makes selection analyses easier for beginners and experienced users. NTM is accessible via a web-based interface, and both NTM and paPAML are available as downloadable command-line versions, allowing for integration in larger selection analysis pipelines.

Münster Evolution Meeting

SAAGER, Rebecca S.

Poster number: 32

Differential Gene Expression in Eusocial and Solitary Mole Rats: An Analysis of Sociality and Vocalization

Sociality and vocalization are closely connected in many animal species, whereby social animals rely heavily on communication to coordinate their behaviour and establish social bonds. In this study, in order to further examine the relationship between these two behavioural phenomena at the functional genomic level, we investigate the variations in the activity of genes that are associated with these traits in the brains in three mole rat species. Due to their highly social behaviour and eusocial organization of colonies, *Heterocephalus glaber* (HG) and *Fukomys damarensis* (FD) depend a lot on vocalization as part of their communication system. In contrast, *Bathyergus suillus* (BS) is a solitary species with no need for communication. The RNA-seq data from the brainstem, cortex and hypothalamus for two to six individuals of each species was examined. Since no reference transcriptome exists for BS and the existing reference transcriptome for FD and two reference transcriptomes for HG did not produce sufficiently good mapping results, we created a reference transcriptome for each species yielding improved mapping results and used these for differential gene expression analysis. The phylogenetically closer pair BS/FD, as well as eusocial species FD and HG, shared more common differentially expressed genes between the brain regions, 20.8% and 20.1% respectively, than the BS/HG pair (only 2.1%). Furthermore, among the eusocial species genes associated with behaviour were enriched across the differentially expressed ones. In a second step, we focused on a manually curated list of genes associated with sociality or vocalization. From this list, there were more differentially expressed genes between the brain regions of eusocial species than in the solitary species. No differentially expressed genes were found as common in BS and HG, whereas the eusocial species share 15.1% and the two phylogenetically close species 17,4%. Taken together, these results suggest that there are genetic differences in the activity of certain genes between eusocial and solitary mole rat species that may be related to social behaviour and vocalization, while there is also an effect of phylogenetic proximity between species exhibiting different social behaviour.

Münster Evolution Meeting

PRÜSER, Tobias

Poster number: 33

Effects of a reduced eye phenotype mediated by HSP90 reduction on the light-dependent behavior of *Tribolium castaneum*

Niche choice means that individuals choose the environment, with which they are interacting to improve their phenotype-environment match. Genetic variation can enable individuals to access novel niches and thereby enhance their fitness. Evolutionary capacitance is an important process by which populations 'store' more individual variation in the form of cryptic genetic variation (CGV), which can be released under stressful environmental conditions. Such release of CGV is supposed to be mediated by Heat Shock Protein 90 (HSP90), which is a molecular chaperon acting as an evolutionary capacitor. In our previous work with the red flour beetle *Tribolium castaneum*, the reduction of HSP90 led to the release of a new 'reduced eye' phenotype and its subsequent assimilation. These beetles have much smaller eyes and less defined ommatidia. Potential fitness benefits of this phenotype were reported under continuous light stress. We also have indications from locomotor activity and circadian rhythm assays that individuals with the reduced eye phenotype show a lower startling response to the morning light. Along with these results we will present first results from ongoing experiments to further investigate if this reduced eye phenotype leads to a different niche choice in the beetles. We hypothesize that the reduced eye will lower the light avoidance of the individuals, and that it enables the beetles to maintain a strong rhythmic behavior even under more intense light conditions. In addition to the Drosophila Activity Monitor (DAM) system that we have established for the work on *Tribolium*, we will use for this new experiment infrared cameras to record the precise position of the beetles in a light-choosing set up. Overall, our work aims to contribute to understand HSP90's evolutionary role in diversifying the niche choice of individuals in a population, and how it can enhance the fitness in a stressful environment.

Münster Evolution Meeting

LURIA, Victor

Poster number: 34

Novel genes enable protein structural innovation and function in the brain

How genuinely new protein-coding genes originate is a central question in biology. Long thought impossible to arise from non-coding sequence, novel genes arising de novo from intergenic genomic DNA long considered to be “junk”, or from long non-coding RNAs, were recently found in eukaryotic genomes. Novel genes are taxon-restricted and may encode structurally novel proteins with new protein domains. To understand how novel genes arise, we built a mathematical model based on gene and genome parameters and dynamic factors such as mutation. We combined phylostratigraphy and proteogenomics to identify novel genes in 25 eukaryotic genomes ranging from human to paramecium and evaluated their predicted biophysical properties. Compared to ancient proteins, novel proteins are shorter, more fragile, disordered and promiscuous, yet less prone to aggregate or to form toxic prions. We performed biophysical experiments comparing novel and ancient proteins and showed that novel genes function in vivo in zebrafish brains. We examined gene regulation in humans and found that the number of regulatory elements is smaller in novel genes than in ancient genes but higher than that of control intergenic open reading frames (ORFs). Furthermore, GTEx human RNA expression analysis shows that novel genes are expressed at levels lower than those of ancient genes but higher than control intergenic ORFs. Using single-cell RNA-Seq and proteomics, we found that novel genes are expressed in human brains at multiple ages. Thus, genomic sequence turnover generates many novel genes encoding short proteins, of which some are maintained and encode proteins that have distinct structural features and are expressed in the brain. Variation in large eukaryotic genomes, that have large intergenic “dark matter” regions, continuously generates new protein structures and new functions.

Münster Evolution Meeting

YILMAZ, Yeliz

Poster number: 35

Identification of bacterial traits involved in interference with horizontal gene transfer in *Escherichia coli*

Since the discovery of penicillin by Alexander Fleming (1928), the consumption of antibiotics in human and veterinary medicine has increased continuously. This has resulted in strong selection pressure on bacteria and the spread of multi-resistant microbes. Horizontal gene transfer (HGT) is an important process triggering bacterial evolution. Conjugation is the most important form of HGT, resulting in the rapid spread of plasmids among bacteria. Conjugative plasmids carry genes for self-transfer and play thereby a major role in the transmission of antibiotic resistance genes. We hypothesize that the increased use of antibiotics since the discovery of penicillin is a selective force that affects HGT rates in bacteria. The transfer frequency of a defined plasmid is known to be affected by many biotic and abiotic factors, such as growth phase, donor:recipient ratio, environmental conditions (temperature, pH, mating time, etc.), and other plasmids that are already present in the bacterium. However, factors affecting the capability of the recipient bacterial cell to take up foreign DNA are less well understood. Since the inhibition of conjugation could be a useful strategy to reduce resistance gene spread among bacteria, a better understanding of the molecular mechanisms that control plasmid spread is essential. Here, we tested the *E. coli* isolates collected in the early twentieth century, which means before the introduction of antibiotics in human medicine, and recent clinical isolates from patients with urinary tract infections. The conjugation frequency, transformation efficiency as well as plasmid content of selected historical strains were analyzed. We examined the recipient and donor properties of each historical and clinical *E. coli* isolate in matings using two different conjugative plasmids, the highly transmittable plasmid RP4 and the ESBL-plasmid pO104_90. We observed that the recent isolates often showed better recipient properties than the historical isolates, whereas the latter often showed better donor properties than the recent isolates. These results should serve as a basis for us to learn more about the mechanisms and possible selection pressures involved in bacterial adaptation to increasing exposure to antibiotics and the emergence of multidrug-resistant strains.

Münster Evolution Meeting

TAWFIK, Youssef

Poster number: 36

Age at death estimation from ancient human methylomes

Paleodemography is the study of the population characteristics and dynamics of past human populations using archeological, biological, and historical data. This subfield of anthropology has virtu- ally ceased to exist for the past 30 years due to the lack of precision in determining age at death in skeletons. It has been shown that DNA methylation is correlated with age and can thus be used to estimate the age of individuals. This led to the development of computational tools that estimate the chronological age of individuals called epigenetic clocks. During the past decade, advances have been made in the field of paleo-epigenetics, with the publication of the first genome-wide ancient methylome and the generation of computational tools able to infer methylation in ancient genomes. In our study, we try to make use of all these advancements, and we aim to set up, optimize, and validate a computational pipeline to predict age at death from ancient epigenomes. We tested five different epigenetic clocks using publicly available Whole Genome Bisulfite Sequencing (WGBS) data and methylation array data, with known ages of individuals and tissues used. We quantified and compared their prediction accuracies by calculating the root mean square error (RMSE) between true and inferred ages. As ancient data is generated from bones or teeth, we also compiled array datasets of bone tissue, which had an RMSE of around 12 between true and inferred ages. Currently, we are preparing the modern WGBS datasets to use them as the basis for the simulation of ancient data, which allows us to establish the error distributions expected on real ancient data. The next step is to use real ancient data with known ages to benchmark the used clocks. Once we have the clocks working, we will apply our tool to a series of population- wide palaeogenomic datasets and try to obtain demographic information from extinct populations, which would revive the field of paleodemography.

Münster Evolution Meeting

LÄNGER, Zoe Marie

Poster number: 37

Proteomic and experimental approaches to the identification of Bacillus thuringiensis spore culture supernatant components associated with immune priming in *Tribolium castaneum*

Pathogens often impose strong selection pressures on their hosts, favouring the evolution of elaborate immune defences. There is even rising evidence for forms of immune memory within the insect immune system. Such 'immune priming' results in enhanced survival after a second encounter with a pathogen or pathogen-derived cues. To gain deeper understanding of the evolution of such alternative forms of immune memory, we need to know more about the cues that are used to launch a protective immune response. In the host-pathogen model of *Tribolium castaneum* and *Bacillus thuringiensis* bv. tenebrionis (Btt), the bacteria-derived cues the red flour beetle is confronted within the context of oral priming are unexplored. To identify candidate proteins, we utilized mass spectrometry-based proteomics to compare the supernatant composition of Btt cultures, which induce immune priming, with those of another non-pathogenic *Bacillus thuringiensis* (Bt) strain, Bt407-, which does not induce immune priming. Proteins exclusively identified in Btt filter-sterilized spore culture supernatants were screened for their potential to induce oral immune priming. Specifically, we identified species-specific metabolites and spore-coat associated proteins which have a high potential to induce a specific immune response. Moreover, traces of the bacteria's main crystal toxin, Cry3Aa were found. To further understand the role of Cry3Aa in the induction of immune priming, *T. castaneum* larvae were primed with supernatants of Btt cured of the Cry3Aa carrying plasmid (Btt-) and Bt407 artificially expressing Cry3Aa (Bt407+). Bt407+ primed larvae showed a trend of higher survival after a subsequent Btt spore infection, whereas a loss of priming efficiency for Btt- supernatant was observed. This indicates Cry3Aa is involved in the induction of immune priming.

2ND Münster Evolution Meeting - 2023 schedule

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	03	Audrey Proenca
	04	Carsten Kemena
	05	Dana Lauenroth
	06	Ekin Deniz Aksu
	07	Enrique Celemin
	08	Fatemeh Zebardast
	09	Ian Dewan
	10	Jos Kramer
	11	Katharina Natascha Meyer zu Riemsloh
	12	Léonore Wilhelm
	13	Maide Nesibe Macit
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