



COST Action SUSTAIN

Evolutionary Genomics of Plant Pathogens

Summer Workshop
26th August - 28th August 2015
Kiel, Germany

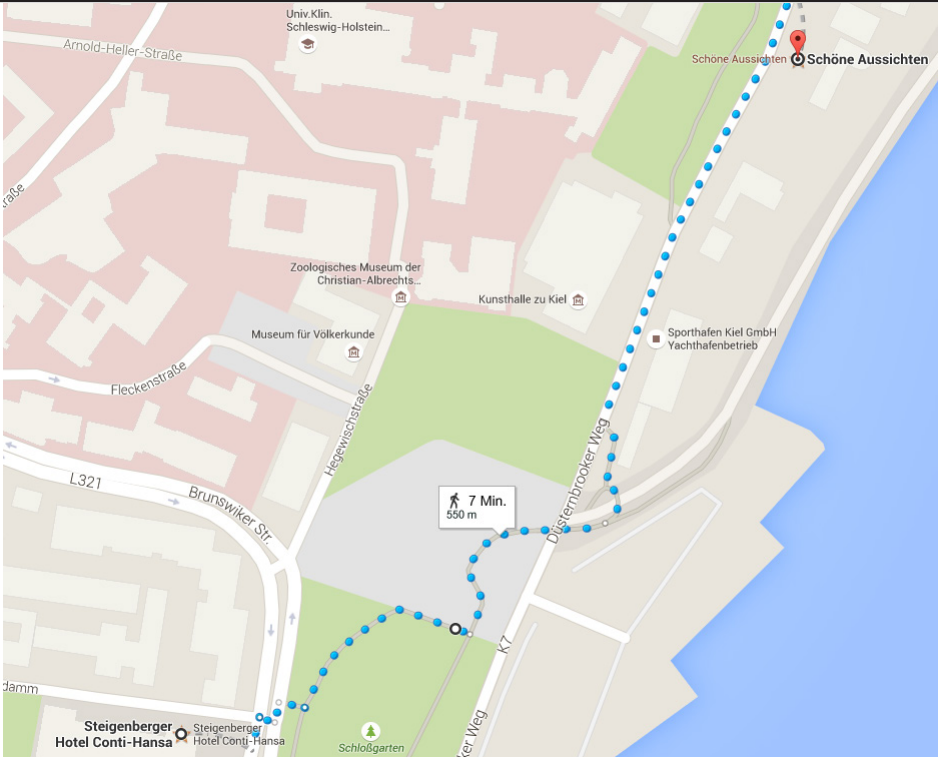


Christian-Albrechts-Universität zu Kiel

CONTENTS

Programme	5-11
Abstracts	12-60
Information, Maps, Addresses & Links	64-69
Presenter Guidelines	70-71
Participants	72-76

PROGRAMME



Detailed map of walk from the hotel to the restaurant for the welcome dinner

17.30 - 19.00 Registration in the lobby of the hotel „Steigenberger“

19.00 Meeting in the lobby for a walk to the restaurant
see previous page for detailed map

19:00 - 22.00 Welcome dinner at the restaurant “Schöne Aussichten”

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- 8.30 Pick-up in the hotel lobby for the bus transfer to the ZMB
- 9.00 - 9.15 Eva Stukenbrock (University of Kiel/MPI Plön, Germany)
Welcome
- Session I Population genomics** (Chairs: Bart Thomma & Ronny Kellner)
- 9.15 - 9.45 Thomas Bataillon (Aarhus University, Denmark)
Can we infer the mechanisms underlying rapid adaptation ?
- 9.45 - 10.05 Eran Bosis (Tel Aviv University, Israel)
Identification of New Type III Effector Proteins of *Xanthomonas* by a Bioinformatic Approach
- 10.05 - 10.25 Jonathan Grandaubert (University of Kiel/MPI Plön, Germany)
Transposable elements promote adaptive evolution in the genome of the fungal wheat pathogen *Zymoseptoria tritici*
- 10.25 - 10.55 Daniel Croll (ETH Zurich, Switzerland)
Drivers of genome evolution in highly diverse populations of the fungal wheat pathogen *Zymoseptoria tritici*
- 10.55 - 11.25 Coffee break
- 11.25 - 11.45 Fabrizio Menardo (University of Zurich, Switzerland)
Hybridization of powdery mildew strains gives rise to pathogens on novel agricultural crop species
- 11.45 - 12.05 Antoine Persoons (INRA Champenoux, France)
Identification of candidate effectors in the poplar rust fungus *Melampsora larici-populina* through a population genomics approach
- 12.05 - 12.35 Alice Guidot (INRA Toulouse, France)
Multihost experimental evolution of the pathogen *Ralstonia solanacearum* unveils genes involved in adaptation to plants

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- 12.35 - 14.00 Lunch, Botanical Garten, CAU
- 14.00 - 14.20 Ralf Koebnik (Institut de Recherche pour le Développement, Montpellier, France)
Evolution of Pathogenicity Beyond Type III Effectors in *Xanthomonas*
- 14.20 - 14.40 Fanny Hartmann (ETH Zurich, Switzerland)
Genome-wide association study of the wheat pathogen *Zymoseptoria tritici* identifies genetic variation linked to pathogenicity on wheat
- 14.40 - 15.00 Michelle Hulin (East Malling Research, Kent, UK)
Exploring the genetic basis of host specificity in *Pseudomonas syringae*
- 15.00 - 15.30 Pierre Gladieux (INRA Montpellier, France)
Genomic Sequencing Reveals Demographic, Historical, and Selective Factors Associated with the Diversification of the Fire-Associated Fungus *Neurospora discreta*
- 15.30 - 17.00 Poster Session (Groundfloor, ZMB)
- 17.00 Pick-up on groundfloor of ZMB for bus transfer to hotel

Evening Session at the hotel Steigenberger

- 18.20 - 18.35 Thomas Kroj (INRA Montpellier, France)
COST Action Sustain
- 18.35- 19.30 John Taylor (University of California, Berkeley, USA)
How fungal population genomics informs developmental biology.
- 19.30 Dinner

8.30 Pick-up in the hotel lobby for the bus transfer to the ZMB

Session II Comparative genomics (Chairs: Alice Guidot & Daniel Croll)

9.00 - 9.30 Eric Kemen (MPI Cologne, Germany)
Hub microbes shape the hologenome of their host

9.30 - 9.50 Ronny Kellner (Sainsbury Laboratory, Norwich, UK)
Adaptive evolution of expression level of the EPIC1 protease inhibitor in the *Phytophthora infestans* lineage

9.50 - 10.20 Bart Thomma (Wageningen University, NL)
Evolution of virulence in the vascular wilt pathogen *Verticillium dahliae*

10.20 - 10.50 Coffee break

10.50 - 11.20 Etienne Danchin (INRA Sophia-Antipolis, France)
Comparative genomics of root-knot nematodes provides clues to their parasitic success despite asexual reproduction.

11.20 - 11.40 Gabriel Schweizer (MPI Marburg, Germany)
Host specificity in smut fungi: Insights from evolutionary comparative genomics

11.40 - 12.00 Thomas Mathers (The Genome Analysis Centre, Norwich, UK)
tba

12.00 - 12.30 Marie-Agnes Jacques (INRA Angers, France)
Evolutionary history of the plant pathogenic bacteria *Xanthomonas* spp.

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- 12.30 - 14.00 Lunch, Botanical Garten, CAU
- 14.00 - 14.30 **John Jones** (James Hutton Institute, Scotland, UK)
Genomic adaptations for plant parasitism by nematodes.
- 14.30 - 14.50 **Sebastian Eves-van den Akker** (James Hutton Institute, Scotland, UK)
Effector gene birth in plant-parasitic nematodes: Glutathione synthetase effectors
- 14.50 - 15.20 **Johannes Helder** (Wageningen University, NL)
Evolution of Plant Parasitism in the Phylum Nematoda
- 15.20 Pick-up on groundfloor of ZMB for bus transfer to hotel

Afternoon & Evening Event

- 16.40 Meeting in the hotel lobby for Kiel waterfront excursion
- 19.15 Dinner: Restaurant Louf

8.30 Pick-up in the hotel lobby for the bus transfer to the ZMB

9.00 - 9.20 Remco Stam (TUM, Friesing, Germany)
Coevolution of plant pathogens and wild tomato species

Session III Two-speed genomes (Chairs: Pierre Gladieux)

9.20 - 9.50 Sophien Kamoun (Sainsbury Laboratory, Norwich, UK)
Effector evolution following host jumps

9.50 - 10.10 Martijn Rep (University of Amsterdam, NL)
Effector genes in the cloud: the pangenome of *Fusarium oxysporum*

10.10 - 10.30 Luigi Faino (Wageningen University, NL)
Genome plasticity mediated by transposable elements drives the evolution of virulence in the vascular wilt pathogen *Verticillium dahlia*

10.30 - 11.00 Coffee break

11.00 - 11.20 Like Fokkens (University of Amsterdam, NL)
The three-speed genome of *Fusarium oxysporum*.

11.20 - 11.40 Xiaoqian Shi (Wageningen University, NL)
The occurrence of chromosomal rearrangements in the fungal genus *Verticillium*

11.40 - 12.10 Michael Freitag (Oregon State University, Corvallis, USA)
"Chromatin in fungi - perhaps not as conserved as we thought "

PROGRAMME

FRIDAY 28TH

12:10 - 12.20 Eva Stukenbrock
Closing of meeting

12.20 - 14.00 Lunch, Botanical Garten, CAU

12.20 & 14.00 Pick-up on groundfloor of ZMB for bus transfer to train station

ABSTRACTS

CAN WE INFER THE MECHANISMS UNDERLYING RAPID ADAPTATION ?

WED 26TH
09:15 - 09:45

Adaptation is generated by natural selection sieving through heritable variation. Examples of adaptation are available from the fossil record and from extant populations. Genomic studies have supplied many instances of genomic regions exhibiting footprint of natural selection favouring new variants. Despite ample proof that adaptation happens, we know little about beneficial mutations– the raw stuff enabling adaptation. Is adaptation mediated by genetic variation pre-existing in the population, or by variation supplied de novo through mutations? We know even less about what factors limit rates of adaptation. Answers to these questions are crucial for Evolutionary Biology, but also for believable quantifications of the evolutionary potential of populations. Population genetic theory makes predictions and allows in principle to make demographic and selective inference from the patterns of polymorphism within species and divergence between species. Yet models specifying the fitness effects of mutations are often missing. I will show how Fitness landscape models can be mobilized to fill this gap and develop methods for inferring the distribution of fitness effects and factors governing rates of adaptation. I will show how Insights into the processes underlying adaptation can be gained from experimental evolution and population genomics data. The applicability of insights gained from experimental evolution to comprehend adaptation in nature will be discussed.

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WED 26TH
09.45 - 10.05

IDENTIFICATION OF NEW TYPE III EFFECTOR PROTEINS OF *XANTHOMONAS* BY A BIOINFORMATIC APPROACH

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Bacteria belonging to the genus *Xanthomonas* can cause severe diseases in a wide variety of plant species. Type III effectors, injected into the host cells by the type III secretion system, play an important role in the pathogenicity and host specificity of *Xanthomonas* species. Significant efforts were made in the recent years to identify the repertoire of type III effectors of *Xanthomonas*, resulting in the identification of more than 50 different effector families. Many of these effector families were identified based on homology to known type III effectors of other phytopathogenic bacteria. It is likely that the complete repertoire of type III effectors includes additional proteins that are unique to *Xanthomonas*. In this work, we apply a bioinformatic approach to identify new type III effectors. Our approach does not rely on sequence similarity to known type III effectors. Instead, it is based on prediction of the N-terminal type III secretion signal and identification of regulatory elements such as the plant-inducible promoter box-like sequence and the -10 box-like sequence. We combine this information with additional features that help to distinguish effectors from noneffectors. Finally, we rank the candidate proteins according to the likelihood that these are indeed type III effectors. The methodology presented here would be applicable to all *Xanthomonas* species carrying a type III secretion system. Identifying the entire repertoire of type III effectors would help us to better understand the mechanisms of bacterial virulence and host defense.

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TRANSPOSABLE ELEMENTS PROMOTE ADAPTIVE EVOLUTION IN THE GENOME OF THE FUNGAL WHEAT PATHOGEN *ZYMOSEPTORIA TRITICI*

WED 26TH
10.05 - 10.25

Fungal pathogens display a broad diversity of mechanisms enabling them to rapidly evolve under selection pressure and to overcome host defense systems. Unraveling the genetic and genomic basis of these mechanisms is a challenge and requires the integration of functional and evolutionary approaches. Our studies focus on the prominent wheat pathogen *Zymoseptoria tritici* that has co-evolved and spread with its host since wheat domestication more than 10,000 years ago. We applied population genomics approaches to unravel the genetic and genomic basis of adaptive gene evolution in *Z. tritici*. Twelve genomes were sequenced, de novo assembled and aligned to a reference genome. More than 1.5 million SNPs were identified from the 31-Mb genome alignment. The SNP map was used to assess genome wide patterns of linkage disequilibrium and generate a high-resolution recombination map. Gene alignments were analysed with models of sequence evolution in order to identify the patterns and strengths of selection along the genome. In agreement with predictions of background selection, a significant negative correlation was found between the strength of purifying selection and both recombination rate and gene density. Among the 11,839 predicted genes in *Z. tritici*, 873 show signatures of positive selection. Significantly higher dN/dS ratio values were found for genes encoding secreted proteins potentially involved in pathogenicity. These genes are located close to transposable element-rich heterochromatic regions suggesting a role of the latter in adaptation of putative pathogenicity-related genes. These results bring new insights in the genomics of rapid adaptive evolution of fungal pathogens.

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WED 26TH
10.25 - 10.55

DRIVERS OF GENOME EVOLUTION IN HIGHLY DIVERS POPULATIONS OF THE FUNGAL WHEAT PATHOGEN *ZYMOSEPTORIA TRITICI*

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Fungal pathogen populations show extraordinary evolutionary potential to adapt to changes in the environment, host genotypes or chemical control agents. The main drivers of rapid evolution are frequent sexual reproduction, high dispersal capabilities and extensive standing genetic variation. Despite the ubiquity of evidence for rapid turnover occurring in fungal populations, little is known how the structure of the genome influences the evolution of genetic variation. In particular, chromosomal rearrangements and variations in recombination rates are expected to significantly affect the evolutionary potential of loci. We aimed to comprehensively study the fate of chromosomal structural variants in fungal populations of the ascomycete wheat pathogen *Zymoseptoria tritici*. We used re-sequencing data of 130 field isolates from a global collection to identify the population-level frequency of structural variants. Comparative analyses showed that a substantial proportion of the polymorphism in the species is due to structural variation in chromosomal sequences. Integrity of chromosomal structure is strongly influenced by recombination, as the occurrence of at least one crossover event per chromosome promotes proper homologous segregation. We analyzed high-density genetic map data to identify chromosomal regions with either increased recombination rates or cold spots of recombination. We found that recombination rates were heterogeneous and highly polymorphic chromosomes tended to exhibit large tracts devoid of crossovers. The presence of segregating structural variants within populations and variations in recombination rates provide an important genomic context to predict the evolutionary potential of virulence loci in a pathogen genome.

HYBRIDIZATION OF POWDERY MILDEW STRAINS GIVES RAISE TO PATHOGENS ON NOVEL AGRICULTURAL CROP SPECIES

WED 26TH
11.25 - 11.45

During the history of agriculture, many new crop species (polyploids or artificial hybrids) were introduced to diversify products or to increase yield. However, little is known on how such new crops impact the evolution of new pathogens and diseases. Triticale is an artificial hybrid of wheat and rye which was introduced into commercial agriculture in the 1960ies. For many years it was resistant to powdery mildew (*Blumeria graminis*), but in 2001, powdery mildew growth was first described on triticale and it has become a major disease since then. We sequenced and compared the genomes of 46 isolates of powdery mildew covering several *formae speciales* which are specialized on cereal crops like triticale, rye, tetraploid and hexaploid (bread) wheat. We found that *B.g. triticales* growing on triticale and wheat is a hybrid between wheat powdery mildew (*B.g. tritici*) and the mildew form specialized on rye (*B.g. secalis*). The genomes of *B.g. triticales* isolates are composed of about 12.5% of *B.g. secalis* genomes and about 87.5% of *B.g. tritici* genomes. Thus, our data show that the hybrid of the two mildews specialized on two different hosts can infect the hybrid plant species originating from those two hosts. Furthermore, nucleotide diversity analyses showed that mildew of bread wheat itself originated from an earlier hybridization event, probably after widespread cultivation of bread wheat as a new host approximately 10,000 years ago. We conclude that hybridization between mildew pathogens specialized on different species is a major mechanism of host expansion and adaptation to new cereal crop species originating from human activities.

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WED 26TH
11.45 - 12.05

IDENTIFICATION OF CANDIDATE EFFECTORS IN THE POPLAR RUST FUNGUS *MELAMPSORA LARICI-POPULINA* THROUGH A POPULATION GENOMICS AP- PROACH

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The outcome of host-pathogen interactions depends on a complex molecular dialogue between the protagonists. Among the molecules involved, effectors released by the pathogen are critical for the success of infection, as they interfere with host metabolism, signaling and defense responses and allow expression of the disease. Effector proteins reported so far in rust fungi exhibit common features (e.g. secreted, small, cysteine-rich) and candidate effectors most likely reside among fungal secreted proteins. *Melampsora larici-populina* is a fungal pathogen responsible for the foliar rust disease on poplar trees, causing severe damage in plantations. Almost all the resistances (R) released so far have been overcome, with the latest major breakdown event in 1994 (R7). The genome of the virulent 7 isolate 98AG31 has been sequenced using a whole genome shotgun strategy, revealing a large genome of 101 megabases containing 16,399 predicted genes including 1184 small secreted proteins. A population genetics study based on 600 isolates was performed to finely determine the impact of this breakdown on the demographic history of *M. larici-populina*. The genomes of 80 poplar rust isolates, distributed among three genetic groups, were sequenced using Illumina technology to understand the effect of the R7 breakdown at the genetic scale. More than 300,000 polymorphic sites (SNPs) were uncovered across isolates, indicating a remarkable level of polymorphism. In order to understand the emergence of the virulence 7, we performed a genome scan analysis based on SNP data using differentiation and selection indices, taking into account the demographic history. We found several genomic regions related to the virulence 7 that bear genes encoding small secreted proteins. This study demonstrates the benefit of population genomics in the search for candidate effector genes. Functional validation of the most promising candidates is underway.

MULTIHOST EXPERIMENTAL EVOLUTION OF THE PATHOGEN *RALSTONIA SOLANACEARUM* UNVEILS GENES INVOLVED IN ADAPTATION TO PLANTS

WED 26TH
12.05 - 12.35

Ralstonia solanacearum, the causal agent of a lethal bacterial wilt plant disease, infects an unusually wide host range, including original (susceptible) hosts and distant (asymptomatic) hosts. Moreover, this pathogen is able to adapt to many plants as supported by field observations reporting emergence of strains with enlarged pathogenic properties. To investigate the genetic bases of host adaptation, we conducted evolution experiments by serial passages of a single clone of the pathogen on three original and five distant hosts over 300 bacterial generations and then analyzed the whole-genome of 50 evolved clones. Phenotypic analysis of the evolved clones showed that the pathogen can increase its fitness both on original and distant hosts although the magnitude of the adaptive process appeared more important on distant hosts. Only few genomic modifications were detected in evolved clones compared to the ancestor but parallel evolutionary changes in two genes was observed in independent evolved populations. A regulatory gene, *efpR*, was mutated in clones originating from six independent populations evolved on four different plant species. Reverse genetic approaches confirmed that these mutations were associated with fitness gain on bean plants. This work provides a first step towards understanding the within-host evolutionary dynamics of *R. solanacearum* during infection and identifying bacterial genes subjected to *in planta* selection. The discovery of EfpR as a determinant conditioning host adaptation of the pathogen illustrates how experimental evolution coupled with whole-genome sequencing is a potent tool to identify novel molecular players involved in central life-history traits.

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WED 26TH
14.00 - 14.20

EVOLUTION OF PATHOGENICITY BEYOND TYPE III EFFECTORS IN *XANTHOMONAS*

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Pathogenic bacteria in the genus *Xanthomonas* collectively cause disease on an extensive set of hosts, including tomato, pepper, bean and cannabis. We sequenced the genomes of 13 strains of three *Xanthomonas* species that were able to cause symptoms on pepper and/or tomato, including two strains that were originally isolated from symptomatic hemp plants. Comparative genome analyses suggest that the two cannabis isolates belong to the same species as a recently isolated bean-pathogenic strain. Interestingly, the cannabis isolates do not possess a type III secretion system (T3SS) nor type III effectors (T3Es), which are found in most *Xanthomonas* spp.; meanwhile the corresponding genes are present in the phylogenetically close bean pathogen. Most notably, all genomes contained genes encoding the T3SS regulators HrpG and HrpX, which are known to induce T3E genes. Promoter prediction of HrpX-regulated genes suggests that HrpX regulates pathogenesis beyond the T3SS. Indeed, transcript profiling confirmed that HrpX induces the expression of type II-secreted hydrolytic enzymes, which appear to constitute an ancient pathogenicity arsenal, which likely predates the acquisition of the T3SS and its T3Es. We propose a stepwise evolution of pathogenicity in *Xanthomonas* spp. where strains acquired: 1) a basic repertoire of non-type III pathogenicity factors and their regulators, 2) the pathogenicity regulators HrpG and HrpX, 3) the T3SS, and 4) T3E genes. In parallel, genes evolved cis-regulatory elements that brought pathogenicity factors under control of HrpX. We will provide hypotheses about the molecular interplay between the cannabis, pepper and tomato pathogens and their host and non-host plants.

GENOMEWIDE ASSOCIATION STUDY OF THE WHEAT PATHOGEN *ZYMOSEPTORIA TRITICI* IDENTIFIES GENETIC VARIATION LINKED TO PATHOGENICITY ON WHEAT

WED 26TH
14.20 - 14.40

The wheat leaf blotch fungus *Zymoseptoria tritici* undergoes rapid adaptive evolution to evade control methods such as the use of fungicides and cultivar resistance, hence causing major economic losses. The fungus is a model species for genomic studies in Dothideomycetes due to its compact, haploid genome and the ability to perform genetic crosses. However, the genetic basis of many important life history traits of the fungus including virulence is poorly understood. A large number of functional studies of candidate loci failed to show a role in virulence. We performed a genome-wide association study (GWAS) to identify genetic variation linked to pathogenicity on wheat, fungicide resistance to demethylase inhibitors (DMIs) and temperature sensitivity. GWAS is a powerful tool to investigate the genetic architecture of a trait in natural populations. The main drawback of GWAS is that spurious associations due to population structure or relatedness among isolates need to be controlled. We sequenced and phenotyped 130 isolates originating from four geographical locations: Australia, Israel, Switzerland and the United States (Oregon). Using Illumina whole-genome sequencing, we obtained 767'211 high-quality SNPs with a genotyping rate superior to 90% and a minor allele frequency >5%. Of these markers, 95% were polymorphic in at least two populations. We did not find strong associations for temperature sensitivity and fungicide resistance. However, we identified several locations in the genome associated with pathogenicity on two spring wheat cultivars differing in resistance to *Z. tritici*. Analyses to characterize associated loci are ongoing.

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WED 26TH
14.40 - 15.00

EXPLORING THE GENETIC BASIS OF HOST SPECIFICITY IN *PSEUDOMONAS SYRINGAE*

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The globally important phytopathogen, *Pseudomonas syringae*, includes pathovars that infect over 180 plant species. Individual pathovars only infect one or a few hosts. However, despite this specialisation, host jumps have occurred frequently within *P. syringae*. It is believed that effector repertoires are linked to host range and therefore genetic alteration of these repertoires may enable host range expansion or host jumping events. This topic was explored using three divergent clades that have convergently evolved to cause bacterial canker on *Prunus* species: *P. syringae* pv. *morsprunorum* (*Psm*) (which is differentiated into two races) and *P. syringae* pv. *syringae* (*Pss*). A *P. syringae* strain isolated from *Aquilegia vulgaris* (only distantly related to *Prunus*) is closely related to *Psm* R2, suggesting a host jump event. The genomes of *Psm* R1, R2, *Pss* and the *Aquilegia* strain (a total of 19 genomes) were sequenced using 2x250bp Illumina reads. Comparative genomics of the *Prunus* strains has revealed highly divergent effector repertoires within and between the different clades infecting *Prunus* species. They also differed in the presence of phytotoxin genes and those associated with survival in woody plant tissue. This analysis indicates that they may utilise different virulence mechanisms to cause similar disease outcomes. Further bioinformatics and cloning approaches are underway in order to find genes contributing to host specificity and pathogenicity.

GENOMIC SEQUENCING REVEALS DEMOGRAPHIC, HISTORICAL, AND SELECTIVE FACTORS ASSOCIATED WITH THE DIVERSIFICATION OF THE FIRE-ASSOCIATED FUNGUS *NEUROSPORA DISCRETA*

WED 26TH
15.00 - 15.30

Background. Delineating microbial populations, discovering ecologically relevant phenotypes, and identifying migrants and admixed individuals has long proved notoriously difficult, thereby limiting our understanding the evolutionary forces at play during the diversification of microbial species. However, recent advances in sequencing and computational methods have enabled an unbiased approach whereby incipient species and the genetic correlates of speciation can be identified by examining patterns of genomic variation within and between lineages.

Results. We present here a population genomic study of a phylogenetic species in the *Neurospora discreta* species complex, based on the resequencing of full genomes (ca. 37Mb) for 52 fungal isolates from 9 sites in three continents. Population structure analyses revealed two distinct lineages in Southeast Asia, three lineages in North America/Europe with a broad longitudinal and latitudinal range, and limited admixture between lineages. Genome scans for selective sweeps and comparisons of the genomic landscapes of diversity and recombination provided no support for a role of linked selection on genomic heterogeneity in levels of divergence between lineages. However, demographic inference indicated that the observed genomic heterogeneity in divergence was generated by varying rates of gene flow between lineages following a period of isolation.

Conclusion. Many putative gene transfer events between phylogenetically divergent fungal lineages have been discovered, and our work highlights the quantitative importance of genetic exchanges between more closely related taxa to the evolution of fungal genomes. Our study also supports the role of allopatric isolation as a driver of diversification in saprobic microbes.

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WED 26TH
18.35 - 19.30

HOW FUNGAL POPULATION GENOMICS INFORMS DEVELOPMENTAL BIOLOGY.

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The powerful tool of genome wide association (GWA), pioneered in studies of human disease, can also be applied to fungi. GWA aims to correlate genetic variation with phenotypic variation in a freely interbreeding population. A necessary prerequisite of GWA studies of wild organisms is a thorough population genomic study to identify the truly interbreeding populations. Pioneering GWA studies in fungi have found genes associated with plant pathogenesis (Dalman et al., 2013) and complex developmental traits (Palma-Guerrero et al., 2013). These initial studies showed that associations can be made with fewer than 50 individuals and that hypothesized associations can be tested by gene deletion studies. Here, we present a study in progress of the thermotolerant yeast, *Kluyveromyces marxianus*, chosen because efficiency of biofuel, ethanol fermentation would be increased at 40°C. We isolated multiple strains of the fungus from composting sugarcane bagasse, the byproduct of sugar extraction. Population genomics identified two clades of *K. marxianus*, the larger of which has 22 individuals. GWA for traits that are variable and important to biofuel fermentation has found significant correlation with genetic variation in the form of single nucleotide polymorphisms. Research is underway to develop means of testing the hypothesized associations.

HUB MICROBES SHAPE THE HOLOGENOME OF THEIR HOST

THU 27TH
9.00 - 9.30

Plant-associated microorganisms critically affect host physiology and performance. A pathogen infecting a host therefore not only faces the host immune system but also a broad range of microbe-microbe interactions. While there is a deep understanding in co-evolutionary processes between host and pathogen, there is hardly any knowledge in evolutionary processes between pathogens and host microbial communities or pathogen and its own associated microbial communities.

We have addressed this knowledge gap by simultaneously studying three major groups of *Arabidopsis thaliana* phyllosphere microorganisms (bacteria, fungi, and oomycetes) following *Albugo laibachii* infection, the causal agent of white rust on *Brassicaceae*. We were using a systems biology approach in combination with metagenomics. We evaluated multiple potential factors of microbial community control: we sampled wild *A. thaliana* populations (location and sampling time), performed field plantings (host genotype) and implemented successive colonization experiments under lab conditions (both abiotic and host genotype control of pathogen colonization).

Our results indicate that both abiotic factors and host genotype interact to affect plant colonization by all three groups of microbes. Considering microbe-microbe interactions, however, uncovered a network of inter-kingdom interactions with significant contributions to community structure. Within this network we could identify *A. laibachii* as a major "hub", relevant for transmission of biotic or abiotic factors to the microbial community. Comparisons to closely related pathogens such as *Hyaloperonospora arabidopsidis* revealed that "hub" status, however, is not a prerequisite for all pathogens.

We are currently in the process of identifying genomic signatures of "hub" organism compared to "non-hub" microbes. Parallels to human microbiome "keystone" pathogens open new avenues of interdisciplinary research that promise to better our understanding in the identification of environmental factors and microbe-microbe interactions that shape pathogen genomes.

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ADAPTIVE EVOLUTION OF EXPRESSION LEVEL OF THE EPIC1 PROTEASE INHIBITOR IN THE *PHYTOPHTHORA INFESTANS* LINEAGE

THU 27TH
9.30 - 9.50

Ronny Kellner¹
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Throughout evolution, opportunistic interactions between pathogens and nonhost plant species occasionally give rise to novel pathogen lineages. One example is *Phytophthora mirabilis*, a sister species of the Irish potato famine pathogen *Phytophthora infestans*. Comparative genomics revealed that after the host jump many *P. mirabilis* genes, including the gene encoding the protease inhibitor PmEPIC1, have rapidly diverged and exhibit signatures of positive selection. Adaptive evolution has shaped PmEPIC1 biochemical activity towards higher specificity for a protease of its host plant *Mirabilis jalapa*. In addition, comparative transcriptomics revealed higher expression of PmepiC1 in planta compared to its *P. infestans* ortholog. In this project, we examined the dynamics in gene expression of PmepiC1. We hypothesized that increased expression of PmepiC1 is an adaptive trait in *P. mirabilis* to cope with *Mirabilis* protease contents and/or to compensate for a reduced number of paralog gene copies. We surveyed gene expression over a time course of infection using *P. infestans* isolates on potato and *P. mirabilis* isolates on *M. jalapa*. Preliminary results showed that PmepiC1 is expressed at high levels whereas epiC1 of *P. infestans* is only induced during biotrophy. Next, we will test the hypothesis that polymorphisms in the cis-regulatory region of PmepiC1 drive higher levels of expression. This study could potentially highlight changes in effector gene expression level as an adaptive trait during pathogen adaptation following a host jump.

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EVOLUTION OF VIRULENCE IN THE VASCULAR WILT PATHOGEN *VERTICILLIUM DAHLIAE*

THU 27TH
9.50 - 10.20

The soil-borne fungal pathogen *Verticillium dahliae* causes vascular wilt disease on hundreds of plant species, and disease control is challenging because resistance in plants is relatively rare. Moreover, *V. dahliae* has a flexible genome allowing it to escape host immunity and maintain aggressiveness. So far, knowledge on mechanisms governing this genomic flexibility remains limited. Through comparative population genomics we have started to unravel mechanisms to establish the genomic diversity that is essential for adaptive genome co-evolution during the continued arms race with host plants. To this end, two *V. dahliae* genomes were assembled from telomere-to-telomere using long-read sequencing technology and optical mapping, and compared these to the genomes of other *Verticillium* spp., revealing a pre-speciation genome duplication event. Comparative genomics using the two finished *V. dahliae* genomes furthermore revealed recent segmental duplications that established lineage-specific regions. Interestingly, these regions are enriched for in planta-expressed effector genes encoding secreted proteins that enable host colonization, and thus contribute to the evolution of virulence. Our evidence suggests that error-prone homology-dependent DNA repair has caused genomic rearrangements, leading to extensive structural variations. Re-sequencing of additional strains showed that independent losses of genetic material favored the escape of host recognition and, likely, host specificity. We propose that evolution of *V. dahliae* is linked to segmental genome duplications mediated by improperly repaired DNA breaks. In addition to genome evolution, we also study the role of epigenetic modifications on virulence of *V. dahliae* and the biological functions of effector proteins. Collectively, these research lines provide insight in mechanisms that make this fungus such a successful broad host range pathogen.

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THU 27TH
10.50- 11.20

COMPARATIVE GENOMICS OF ROOT-KNOT NEMATODES PROVIDES CLUES TO THEIR PARASITIC SUCCESS DESPITE ASEQUAL REPRODUCTION.

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Root-knot nematodes show an intriguing diversity of modes of reproduction from obligatory sexual reproduction to fully asexual reproduction. Surprisingly, the most damaging species to the world agriculture are those that reproduce without meiosis and without sex. This observation seems to contradict the evolutionary advantages of sex and genetic exchanges. To disentangle this parasitic success despite asexuality, we have compared the genomes of root-knot nematodes with different modes of reproduction. We have sequenced and assembled the genomes of 3 obligatory asexual species, *Meloidogyne incognita*, *M. arenaria* and *M. javanica*, unable to do meiosis. We have compared these genomes to those of the facultative asexual *M. hapla* and the obligatory meiotic asexual *M. floridensis*. Our comparative genomics analysis shows that the genomes of the ameiotic asexual root-knot nematodes are composed of duplicated regions that show a high average nucleotide divergence of ~10% within a species. Phylogenomic analysis of the genes present on duplicated blocks suggest that these divergent regions result from multiple independent hybridization events. Average nucleotide divergence at the coding portion averages 6% between duplicated regions. This may allow functional divergence and provide plasticity despite the absence of sexual recombination. In contrast, mitochondrial genome divergence between the three ameiotic asexuals is very low (avg. ~0.17% in coding sequences) and suggests that these putative hybrids share a common or closely related maternal donor lineage. We also observed high variations in TE contents. The genomes of two obligatory asexuals, *M. arenaria* and *M. javanica* show a high proportion of TE (~50%) while the third asexual, *M. incognita* has a lower TE content (~25%), closer to the proportion in the facultative sexual *M. hapla* (~20%). Proliferation of TE following hybridization might have taken place in these asexuals. The intriguing parasitic success of mitotic root-knot nematodes despite absence of sex could be partly explained by the presence of several genomes in one nucleus as a result of hybridization events.

HOST SPECIFICITY IN SMUT FUNGI: INSIGHTS FROM EVOLUTIONARY COMPARATIVE GENOMICS

THU 27TH
11.20 - 11.40

Smut fungi are biotrophic plant pathogens infecting mainly grasses. Their typically narrow host range, the availability of five annotated genomes of closely related species (*Ustilago hordei*, *U. maydis*, *Sporisorium scitamineum*, *S. reilianum* f. sp. *zeae*, *S. reilianum* f. sp. *reilianum*) and their accessibility to genetic manipulations make them a particularly interesting model to unravel mechanisms underlying host specificity. We used a computational approach to identify genes with an interesting evolutionary history: species specific genes and genes showing signs of positive selection. A deletion mutant of a gene under positive selection in *S. reilianum* f. sp. *zeae* (sr10529) shows a clear reduction in virulence symptoms upon maize infections. Whether this phenotype can be better complemented with the endogenous allele compared to the orthologous allele of the close relative *S. reilianum* f. sp. *reilianum*, a pathogen of sorghum grass, is currently investigated. Since the orthologous gene in *U. maydis*, *um01375* (Pit2), has been characterized as inhibitor of salicylic acid-induced cysteine proteases, we currently assess whether the two alleles of *S. reilianum* show differences in interactions with cysteine proteases using a comparative yeast-2-hybrid approach.

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THU 27TH
11.40- 12.00

TBA

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EVOLUTIONARY HISTORY OF THE PLANT PATHOGENIC BACTERIA *XANTHOMONAS* SPP.

THU 27TH
12.00 - 12.30

Deciphering mechanisms shaping bacterial diversity should help to build tools to predict the emergence of infectious diseases. Xanthomonads are plant pathogenic bacteria responsible for major socio-economic impact worldwide. As a whole the genus *Xanthomonas* gather strains that are responsible for diseases on a large number of plants. However, each strain displays a narrow host range that is referred to in the pathovar concept. We address the question of the nature of the evolutionary processes – geographical, ecological and pathological speciation – that shaped the diversity for two species of this genus, namely *X. axonopodis* and *X. arboricola*. We assembled large collections of strains that were isolated over a long period, over continents, and from various plants, some strains being pathogenic on those plants, the other being not pathogenic on their host of isolation. We performed population genetic analyses using coalescent and genealogy approaches, we studied the diversification of the pathogens, and we analyzed the evolution of the virulence-associated gene repertoires in these strains. For *X. axonopodis*, the suggested evolutionary scenario involves a first step of generalist diversification that spanned over the last 25 000 years. A second step of ecology-driven specialization occurred during the past two centuries. Eventually, secondary contacts between host specialized strains probably occurred as a result of agricultural development and intensification, allowing genetic exchanges of virulence-associated genes. These transfers may have favored the emergence of novel pathotypes. We showed that the species *X. arboricola* presents an epidemic structure. The three highly aggressive pathovars (*juglandis*, *pruni* and *corylina*) represent epidemic clones, which emergence was linked to the acquisition of type three effectors. Nonpathogenic strains and strains from minor pathovars of this species represent the recombinant network within which loss of virulence-associated factors from the common ancestor is evidenced for non-pathogenic strains. Altogether, these study highlighted that evolutionary history of xanthomonads is tightly linked to their biological environment and involved horizontal gene transfer for pathoadaptation.

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THU 27TH
14.00- 14.30

GENOMIC ADAPTATIONS FOR PLANT PARASITISM BY NEMATODES.

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For many years studies aimed at understanding the mechanisms by which plant-parasitic nematodes infect plants lagged behind those on other plant pathogens due to the difficulties of working with these obligate biotrophic pathogens. However, genomics has shed light on the mechanisms used by nematodes to exploit plants and has provided information on the effectors that nematodes deploy during infection. Horizontal gene transfer from bacteria and fungi has played a key role in the evolution of plant parasitism in all nematode groups where plant parasitism has evolved. While the presence of a variety of cell wall degrading and modifying proteins has been known for some years, more recent findings indicate that genes have been acquired that are important in suppressing host defences and in allowing nematodes to exploit plants as a food source. However, other than these horizontally acquired genes, there is almost no overlap between the effectors deployed by different groups of plant-parasitic nematodes. In cyst nematodes, expanded gene families of effectors are present that may be adapted for various functional roles. In addition, cyst nematodes possess many effectors that can suppress host defence responses, including a local suppression of those activated as a result of the detection of an avirulence gene product by a resistance gene. This talk will explore the evolutionary pressures on cyst nematodes in contrast to those of root-knot nematodes and will discuss how the ability to suppress effector triggered immunity may be related to host range and to how cyst nematodes reproduce.

EFFECTOR GENE BIRTH IN PLANT-PARASITIC NEMATODES: GLUTATHIONE SYNTHETASE EFFECTORS

THU 27TH
14.30 - 14.50

Plant parasitism in the phylum nematoda has arisen multiple times independently. Within the Tylenchida, it is generally accepted that a linear evolutionary progression occurred from migratory ecto-parasites to migratory endo-parasites, and subsequently from migratory endo-parasites to the highly specialised sedentary endo-parasites. Yet, surprisingly little is known about the genes specifically required for each evolutionary transition. We demonstrate that at least two successive expansions of glutathione synthetase genes occurred during these transition events. The first major expansion increased the reactive oxygen detoxification capacity of the intestine and co-occurred with the evolution of migratory endo-parasitic species. The second major expansion resulted in the evolution of numerous secreted glutathione synthetase effectors, and co-occurred with the evolution of prolonged plant-nematode biotrophic interactions in cyst and reniform nematodes. Despite a diversification in biological function, we demonstrate that glutathione synthetase effectors have retained their biochemical function in glutathione biosynthesis, yet must act in co-operation with abundant plant precursors during the biotrophic interaction. Consistent with this, we detect an accumulation of glutathione within the feeding site during parasitism. Finally, we simultaneously undermine the collective function of all glutathione synthetase effectors by inhibiting the production of their precursors *in planta* at discrete levels by two independent means. This reveals the feeding site specific glutathione apoptotic threshold and provides an insight into glutathione synthetase effector function. Glutathione synthetase effectors represent a complete description of effector evolutionary origin and a novel mechanism to modulate host redox state and facilitate a sustained biotrophic interaction.

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THU 27TH
14.50- 15.20

EVOLUTION OF PLANT PARASITISM IN THE PHYLUM NEMATODA

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Nematodes constitute a fairly speciose ($\approx 27,000$ documented species) and trophically diverse group of metazoans positioned close to the base of the superphylum Ecdysozoa. Bacterial feeding is thought to be the ancestral feeding type of nematodes from which a multitude of lineages with other food preferences arose. At least four independent major lineages of plant parasites have evolved, and in at least one of these major lineages plant parasitism arose independently multiple times. Ribosomal DNA data, sequence information from nematode-produced, plant cell wall-modifying enzymes, and the morphology and origin of the style(t), a protrusible piercing device used to penetrate the plant cell wall, all suggest that facultative and obligate plant parasites originate from fungivorous ancestors. Data on the nature and diversification of plant cell wall-modifying enzymes point at multiple horizontal gene transfer events from soil bacteria to bacterivorous nematodes resulting in several distinct lineages of fungal or oomycete-feeding nematodes. Phylogenetic patterns arising from a ribosomal DNA framework with sequence data from over 2,700 nematode taxa will be compared with diversification patterns of nematode effectors such as cellulases, venom allergen-like proteins, and members of the SPRYSEC family.

Molecules involved in plant pathogen interactions are thought to be under selective pressure due to coevolution between plants and their pathogens. Plant resistance genes (NLRs) and pathogen effectors are thought to be the main interacting molecules and thus exhibit such selective patterns. A number of studies have confirmed long-term evolutionary relationships of NLRs and have shown that different mechanisms might have been in place to accomplish such great NLR diversity as can be observed today. Evolution and population theory predicts that also in short term interactions genes that play such an important role in the plant-pathogen interaction should be under certain selective pressure. Limited data suggests that in the inbreeding plant *Arabidopsis thaliana* NLRs show signs of positive selection and certain classes of pathogen effectors are under selective pressure too. However, very little data exists for complex ecological systems or outbreeding plant species.

As proof of principle we assess NLR diversity in a complex, wild tomato species. Using Rgene Enrichment Sequencing (RENSeq) we sequence R-genes in multiple populations, from known geographical locations and assess short term evolutionary changes. We can use these data to test and improve coevolutionary models and to understand plant and pathogen population dynamics. Pathology assays will link this information back to the molecular biology of NLRs and present effectors in different pathogen species. Understanding short-term evolution of R-genes and effectors in different habitats and under different pathogen pressures could provide new insights that could be used for durable resistance breeding in crops.

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FRI 28TH
9.20 - 9.50

EFFECTOR EVOLUTION FOLLOWING HOST JUMPS

[Sophien Kamoun](#)

Many plant pathogens, including those in the lineage of the Irish potato famine organism *Phytophthora infestans*, evolve by host jumps followed by specialization. Accelerated gene evolution is a hallmark of pathogen adaptation following such host jumps. In many cases, particularly with regards to effectors that are recognized by plant immune receptors, the genetic determinants and the nature of the adaptive mutations are known. However, the biochemical basis of adaptation and specialization of effectors to new host targets remains largely unknown. In this talk, I will review this concept and discuss how plant pathogens are great model systems to study rapid evolutionary adaptations. I will also introduce the next phase of research on this topic

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EFFECTOR GENES IN THE CLOUD: THE PANGENOME OF *FUSARIUM OXYSPORUM*

FRI 28TH
9.50 - 10.10

As a general rule, effector genes reside in non-essential or accessory regions of the genomes of pathogens. This is also true for the vascular wilt pathogen *Fusarium oxysporum*, in which all genes for small, in planta secreted proteins reside on accessory chromosomes or chromosomal subregions that are highly enriched in transposable elements as well as histone modifications associated with silencing. In tomato-infecting strains, the genes for thirteen out of fourteen identified effector genes reside on a single accessory chromosome. This 'pathogenicity' chromosome can be transferred between strains. It can also be lost spontaneously, leading to complete loss of pathogenicity but no other immediately obvious phenotypes. Through comparative genomics involving strains from various host-specific forms, we found that the suite of effector genes in a strain can be used to predict its host specificity, despite the polyphyletic nature of host-specific forms within the *F. oxysporum* species complex. Effector genes form only a small portion of the very large accessory pangenome of *F. oxysporum*. It appears that the evolutionary history of this accessory pangenome is different from the core genome and is influenced by horizontal transfer, duplications, deletions and a very high rate of reorganisation.

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GENOME PLASTICITY MEDIATED BY TRANSPOSABLE ELEMENTS DRIVES THE EVOLUTION OF VIRULENCE IN THE VASCULAR WILT PATHOGEN *VERTICILLIUM DAHLIA*

FRI 28TH
10.10 - 10.30

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Verticillium dahliae is a soil-borne pathogen that aggressively colonizes hundreds of host plants, including high-value crops such as tomato and potato, leading to the formation of vascular wilt disease. Resistance factors in the host population exert selective pressure on the pathogen forcing the rapid evolution of adaptive traits to successfully participate in the arms race with the host. By comparative genomics on a *V. dahlia* population, we recently revealed extensive genomic rearrangements that facilitate the gain and loss of genetic material and the establishment of highly dynamic lineagespecific (LS) regions. LS regions are enriched for transposable elements (TEs) and in planta-induced effector genes encoding secreted proteins that significantly contribute to aggressiveness towards the host, and thus have been hypothesized to contribute to the genome plasticity required for adaptive genome evolution. However, factors that drive genome plasticity in *V. dahliae* remain enigmatic. Using long-read sequencing technologies, we re-sequenced two *V. dahliae* strains and analyzed the previously identified genomic rearrangements in unprecedented detail, revealing multiple genomic breakpoints at the nucleotide level. We established that genomic breakpoints are flanked by multiple TEs, suggesting that these elements play essential roles in their formation. Moreover, we established that invasion and subsequent proliferation of specific retrotransposons lead to the diversification several species within the *Hypocreomycetidae* subclass. In summary, we highlight the profound role of TEs on the evolution of virulence in the vascular wilt pathogen *V. dahliae*.

THE THREE-SPEED GENOME OF *FUSARIUM OXYSPORUM*.

FRI 28TH
11.00 - 11.20

Many plant pathogens have a two-speed genome in which genes that are involved in pathogenicity, cluster with transposable elements (TEs), which is thought to enhance the evolvability of a species. We study the evolution of genome organization in *Fusarium oxysporum*, a versatile pathogen with a very dynamic genome. Previously, comparative genomics between *Fusaria* revealed that *F. oxysporum* has 11 conserved core chromosomes and 4 lineage specific (LS) chromosomes. Here we use whole genome alignments of the reference genome of *Fusarium oxysporum* (that of Fol4287) with 32 other *F. oxysporum* strains to compare sequence conservation and synteny between different genomic regions. We find that the three smallest core chromosomes (chr 11, 12 and 13) change at an intermediate rate to the large core and the LS chromosomes: *F. oxysporum* has a three-speed rather than a two-speed genome. These chromosomes resemble the core in terms of gene- and repeat density, but are under similar epigenetic control as the LS chromosomes. Moreover, they are enriched in genes that are upregulated during infection. Further functional analyses demonstrate that the three speeds correspond to different levels of interaction with the host. The fastest evolving LS regions encode many putative secreted effector proteins that function 'behind enemy lines'. The fast evolving core chromosomes are enriched in proteins involved in metabolism and transport: they need to adapt to the host but are not in the forefront of the host-pathogen arms-race. Finally, the slow evolving core chromosomes encode genes that are generally not directly involved in host interactions

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FRI 28TH
11.20 - 11.40

THE OCCURRENCE OF CHROMOSOMAL REARRANGEMENTS IN THE FUNGAL GENUS *VERTICILLIUM*

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Based on a recent comparative population genomics study, extensive chromosomal rearrangements between strains of the plant pathogenic species *V. dahliae* have been found (de Jonge et al., 2013). The rearrangements result in the occurrence of lineage-specific genomic regions that appear to be greatly enriched for in planta-expressed genes that encode virulence factors that enable host colonization. Thus, it is speculated that genomic rearrangements foster evolution of aggressiveness in the asexual pathogen *V. dahliae* (de Jonge et al., 2013). In this project, we aim to investigate the occurrence and regulation of chromosomal rearrangements in the genus *Verticillium* that comprises plant pathogenic (*V. dahliae*, *V. longisporum*, *V. albo-atrum*, *V. alfafa*, *V. nonalfalfae*), and saprophytic and weakly pathogenic (*V. tricorpus*, *V. zagamsianum*, *V. nubilum*, *V. isaacii* and *V. klebahnii*) species. We hypothesize that chromosomal rearrangements occur in the virulent plant pathogens and are not observed in the weak pathogens and saprophytes. To investigate this hypothesis, the genomes of multiple strains of each of the species within the *Verticillium* genus were sequenced and assembled to investigate chromosomal structures within and between each of the species. Furthermore, we investigated chromosomal size polymorphisms based on karyotyping. Collectively, our data show that inter-chromosomal rearrangements are not confined to pathogenic *Verticillium* spp

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“CHROMATIN IN FUNGI - PERHAPS NOT AS CONSERVED AS WE THOUGHT”

FRI 28TH
11.40 - 12.10

Fungi are excellent models for comparative studies of chromatin biology. To illustrate this point, I will touch on two major aspects of recent work: (1) interactions between kinetochore proteins and centromeric DNA, and (2) patterns of heterochromatin distribution and regulation in various fungi. We found that the centromeric chromatin of *Neurospora* and *Fusarium* is heterochromatic (i.e. largely transcriptionally silent) and marked by histone H3 trimethylation at lysine 9 (H3K9me3), but this is not the case in *Zymoseptoria*. Similarly, the three genera show differences in the distribution of two commonly studied histone marks associated with gene silencing, H3K9me3 and H3K27me3. In *Fusarium*, the absence or presence of H3K27me3 controls the expression of more than 25% of all genes. This discovery resulted in novel ways to express the “cryptic genome”, regions that are usually transcriptionally silent when *Fusarium* strains are grown under lab conditions and not under environmentally conducive conditions (e.g. in planta or in competition with other microbes). This work has implications on the study of secondary metabolite gene clusters and pathogenicity of fungi on animals and plants. Overall, studies with several taxa show that histones may be extremely conserved but how individual histone modifications are wired into regulatory circuits appears to be different. Conversely, kinetochore proteins serve conserved roles but show much variation in primary sequence. A complete picture of chromatin regulation in fungi will only emerge if several species are examined in detail.

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GENOMIC AND PHENOTYPIC CHARACTERIZATION OF *DICKEYA* SP. CAUSING SOFT ROT OF *PHALAENOPSIS ORCHIDS*

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Bacterial soft rots are a serious limitation to the production of economically important crops and ornamental plants. Bacteria isolated from diseased tissues of *Phalaenopsis* orchids in a commercial production in Slovenia were identified as *Dickeya* spp. based on morphological characteristics, partial sequencing of 16S rDNA and selected biochemical tests (API 20E). Partial sequencing of *fliC* gene assigned isolates to previously observed unassigned lineages UDL-3 and UDL-4 (Van Vaerenbergh *et al.* (2012)). Genomes of two *Dickeya* spp. isolates that were representative of the observed diversity of bacteria in rotted tissues were sequenced using Ion Torrent technology, which has resulted in two draft genomes currently composed of 59 and 61 contigs. Based on average nucleotide analysis (ANI) of the obtained genomes with the publicly available genomes of *Dickeya* spp., our isolates, along with strains MK 7 and NCCPB 3274 (isolated from water and an ornamental plant, respectively) potentially represent a new species within the *Dickeya* genus. To further examine the taxonomic and phylogenetic position of the potential novel *Dickeya* spp. the phenotypic analysis and comparative genomics analysis are underway.

CHARACTERISATION OF LINEAGE SPECIFIC REGIONS IN ONION BASAL ROT PATHOGEN *FUSARIUM OXYSPORUM* F.SP. *CEPAE*

POSTER #2

Fusarium oxysporum is a soil-borne pathogen of global significance. It causes crown and root rots as well as vascular wilt on a broad range of hosts. Host adaptation is observed in these pathogens, allowing the separation of over 120 'formae specialis' on the basis of host specificity. The onion (*Allium cepa* L.) pathogen *F. oxysporum* f. sp. *cepae* causes basal rot of onion bulbs and leads to annual losses of £11M the UK alone. Phasing out of soil fumigants is increasing reliance upon the natural resistance found in onion varieties. We performed whole genome sequencing on eight *F. oxysporum* f. sp. *cepae* isolates. Comparison of these strains to publicly available sequence data from tomato pathogens has revealed regions of the genome that are lineage specific to onion pathogens. Comparative genomics of the multiple onion strains has revealed differences in gene complements between pathogenic and non-pathogenic isolates. Known SIX gene homologs have been identified, as well as novel RxLR-containing genes implicated in pathogenicity. Research is ongoing, but identification of pathogenicity genes in lineage specific regions provides new targets for molecular diagnostics to detect infection and infestation by *F. oxysporum* f. sp. *cepae* as well as putative R gene targets. Ultimately, effector-informed breeding strategies will be implemented that reflect understanding of disease and resistance mechanisms in this pathosystem.

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POPULATION OF *PHYTOPHTHORA INFESTANS* IN THREE DIFFERENT REGIONS OF POLAND IN YEARS 2010 - 2012

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Phytophthora infestans (Mont.) de Bary belongs to Oomycetes and it causes late blight, which is the most destructive potato disease worldwide. This pathogen originates from Mexico but it has spread wherever potatoes are grown. *P. infestans* populations are diversified, sexual or asexual and their composition may be affected by climate changes, different methods of cultivation, potato cultivar and migrations. Mating type, mitochondrial haplotype, Simple Sequence Repeats (SSR) markers, sensitivity to metalaxyl and virulence were evaluated to monitor changes in Polish *P. infestans* population.

Samples of potato leaflets with single late blight lesions were collected from fields located in three regions of Poland: Młochów, Boguchwała and Siedlce, in three years 2010, 2011 and 2012. In region Młochów large commercial and intensively protected fields were dominating. Mainly small gardens and experimental fields with insufficient chemical protection predominated in region Boguchwała. In region Siedlce early and starch potatoes were cultivated. Total number of isolates tested was 365. Mating type, mitochondrial haplotype and SSR were evaluated using a PCR method. Sensitivity to metalaxyl was tested on rye A agar media. Virulence was tested on detached potato leaflets.

Polish *P. infestans* population is diverse. We do not observe major clonal lineages. A1 mating type (69%) and Ia mitochondrial haplotype (73%) dominated. Most of the isolates were sensitive to metalaxyl (66%). We observed differences in population composition between the regions, which indicated that cultivation system and numerous fungicide applications had an impact on the population of *P. infestans*.

COMPARATIVE TRANSCRIPTOMIC ANALYSIS AMONG PLANT PARASITIC NEMATODE SPECIES

POSTER #4

Sedentary plant parasitic nematodes (SPPN) are notorious pathogens, causing significant economical losses worldwide. It is generally assumed that, to successfully manipulate the host plant cells, SPPN depend on a set of genes coding for certain effectors, some of which are species-specific while others are more common among the various nematode species. Categorizing these subsets among the various SPPN can shed light on the mechanism and evolution of nematode parasitism. The availability of several nematode transcriptomes made it possible to analyze our *Heterodera schachtii* transcriptome by comparative genomics. Therefore, putative secretomes of each tested species were predicted and functionally annotated. The putative secretome dataset was then further analyzed to detect enriched Gene Ontology terms (GO) using Gene Set Enrichment Analysis (GSEA). Our results show that commonly enriched GO terms include carbohydrate binding, and carbohydrate metabolic process. Furthermore, we identified a subset of *H. schachtii* putative effectors that have their homologs exclusively in PPN species, which we called PPN specific Putative Effector (PPE) as a result of comparing them to sequences from NEMBASE4. Interestingly, we found that PPEs showed a higher level of enrichment of the previously mentioned GO terms in comparison with secretome subset. Genes of interest, included within the enriched GO terms, were experimentally analyzed. Analyzing the results of these comparisons have enabled us determining and categorizing putative effectors onto species-specific or common sequences among the tested PPN species.

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THE DRAFT GENOME SEQUENCE OF *FUSARIUM LANGSETHIAE*, A T-2/HT-2 MYCOTOXIN PRODUCER

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Fusarium langsethiae is a widespread pathogen of oats in Northern Europe, contaminating the grain with the highly toxic cytotoxin and immunosuppressive Type A trichothecene T-2/HT-2. In an effort to better understand the molecular basis for this fungus' plant infection mechanisms and toxin production, we present here a draft genome sequence of *F. langsethiae* isolated from oats in Norway. De novo assembly of this complex asexual genome generated 1586 scaffolds, using a combination of Illumina GaIIX and Roche 454 paired end sequencing. The size of the genome is around 37.5 MB, GC content ~48%, and the genome have around 12200 predicted protein-coding genes. Our focus so far has been on secondary metabolites genes and chemicals. We found 13 polyketide synthases and 13 non-ribosomal peptide synthetases, some unique compared to sequence databases. *F. langsethiae* produces an amazing number of secondary metabolites on YES agar media, with Type A trichothecenes dominating. Interestingly is the production of glycosylated HT-2 toxin (Glu-HT-2) which has been hypothesized to be done in plants. We did not find any indication of Glu-T-2 toxin, nor Glu-DAS nor Glu-NEO. We did however find a Glu-hydroxytrichodermol, further suggesting that the biosynthesis of the Glu-HT-2 is done by the fungus. We will present results from the genome sequence as well as comparative analysis of secondary metabolite genes, and chemical profiles on growth media and in barley and oats.

TEMPORAL AND SPATIAL EXPRESSION OF *VERTICILLIUM ALBO-ATRUM* CANDIDATE EFFECTOR GENES DURING INFECTION OF HOP (*HUMULUS LUPULUS*)

POSTER #6

The soil-borne hemibiotrophic ascomycete *Verticillium albo-atrum* (Vaa) secretes a complex repertoire of effector proteins to modulate the hop (*Humulus lupulus*) immune response system. From 9.269 Vaa gene models we predicted 181 candidate effectors using the GenCloud pipeline. We ranked them based on different properties (e.g., *in planta* expression, differential expression in RNAseq, no sequence homology, pathotype-specific, no PFAM domains or effector-specific domains, transposable elements, homology on PHI database and genes with positive selection) and selected the 9 best ranked effector candidates.

Before deletion mutation of the 9 selected candidate effectors, we wanted to check their expression pattern during Vaa colonization in hop. We validated reference genes for RT-qPCR beforehand.

We tested six reference genes described in the literature on Vaa-infected hop samples (roots and stems of susceptible and resistant plants). Compared to fungi quantification in infected hop material, the two best reference genes were shown to be topoisomerase and splicing factor 3a2, which was also confirmed by the Genorm tool. The selected reference genes will be used in further expression analysis. We have already carried out RT-qPCR to evaluate the expression profiles of the 9 selected effector candidate genes at 6, 12 and 18 days post infection of hop by a highly virulent strain of Vaa. The effector expression profiles and some deletion mutants will be presented.

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COMPREHENSIVE TRANSCRIPTOME PROFILING OF ROOT-KNOT NEMATODES DURING PLANT INFECTION AND CHARACTERIZATION OF SPECIES-SPECIFIC TRAITS

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Root-knot nematodes (RKN) are obligate endoparasites that maintain a biotrophic relationship with their hosts over a period of several weeks. They infect roots as microscopic vermiform second-stage juveniles (J2) and migrate between cells to reach the plant vascular cylinder. To further develop and molt into a pear-shaped female that will release hundreds of eggs on the root surface, J2s need to successfully establish and maintain specialized feeding structures called "giant-cells" from which they withdraw water and nutrients. Our project aims to identify RKN genes specifically involved in plant parasitism with an emphasis on genes encoding new secreted effectors. Using Illumina RNA-seq technologies, we compared transcriptomes of *Meloidogyne incognita* during its life cycle and identified genes over-expressed in early parasitic stages as compared to pre-parasitic juveniles (J2s), eggs, females and males. Once the over-expression of selected genes in parasitic stages was confirmed by RT-qPCR, *in situ* hybridizations were carried out to localize the candidates in the nematode secretion organs. Furthermore, siRNA soaking was used to silence these genes and study their role in pathogenicity. In parallel, we are also comparing the transcriptomes of *M. incognita* with those of another RKN species that reproduces by obligatory parthenogenesis, *M. enterolobii*. This nematode represents a new threat for the agriculture worldwide because of its ability to reproduce on the majority of known RKN-resistant plants. This comparison will allow us to identify, not only the common set of effectors, but also those specific to one of the other RKN species and possibly involved in host range differences.

MOLECULAR CHARACTERIZATION OF FUNGICIDES RESISTANCE OF *BOTRYTIS CINEREA* ON PEPPER AND HOST -RESISTANCE OF SELECTED BREEDING MATERIALS

POSTER #8

Pepper (*Capsicum annuum* L.) is a species of *Solanaceae* family. Turkey ranks third at the list on production of pepper in the World. Antalya is the province with the highest yield by 236,552 tons, covering 59.7% of the total greenhouse production in our country. *Botrytis cinerea* causing gray mold disease, is a polyphagous fungal pathogen, which limits pepper cultivation in greenhouses. Many fungicides are used to control of *B. cinerea*, nowadays however; it shows resistance to fungicides. Obtaining information on the pathogen is of importance for effectively pest management. In this study, virulence and fungicide resistance of the collected pathogens and reference isolate (B05.10) on pepper were classified based on molecular markers within isolates. Host-resistance of breeding materials was assessed. The surveys have been conducted in fields where greenhouse cultivation is intensively done. A total of fifty-one *B. cinerea* isolates were collected. Morphological and molecular (ITS1- ITS4) identification of isolates and degrees of virulence were determined *in-vitro*, *in-vivo* and *Bos1* and β -tubulin gene analysis to evaluate resistance that would develop to fungicides. We determined resistance on 2 isolates showing the highest virulence and reference isolate to seven tested fungicides. 39 SSR primers, 20 ISSR primers and 50 SRAP primer combinations were employed for assessing genetic diversity within isolates. The data showed remarkable genetic diversity within the isolates. Also, five accession lines of each banana, capia, bell and long-type pepper of BATEM genepool were tested and reactions against to *B. cinerea* was determined. Bell-type pure line was more susceptible than the others.

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COMPLETE GENOME OF *RALSTONIA SOLANACEARUM* UY031 AND ITS TRANSCRIPTOME DURING THE INTERACTION WITH THE WILD POTATO *SOLANUM COMMERSONII*

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Ralstonia solanacearum is the causative agent of the bacterial wilt disease on a large number of plant species and is considered as one of the most devastating bacterial plant pathologies worldwide. Due to its agronomical impact, it is important to study specific plant-pathogen interactions in order to better understand how the pathogen modulates the outcome of the infection at a genetic level. In this work, we studied the interaction of *R. solanacearum* UY031, a very aggressive strain isolated from potato, with the wild potato *Solanum commersonii*. To this end, we sequenced the genome of strain UY031 and present here the first fully closed genome of a phylotype IIB1 strain and the fourth of *R. solanacearum*. We describe the Type III Effector (T3E) repertoire of UY031 and compare it to other completely and partially sequenced strains. Furthermore, we have used RNAseq to study the transcriptome of bacteria grown in rich medium and that of bacteria growing inside plant tissues. In the latter case, bacterial transcripts were obtained after deep sequencing of total RNAs isolated from infected and non-infected potato tissues and in silico read selection. With this information, we provide new insights in the understanding of *R. solanacearum* mode of infection, and define genes that are necessary and specific for a successful interaction.

THE ROLE OF HOST SPECIFIC INTERACTIONS AND HOST ADAPTATION OF *PARASTAGONOSPORA NODORUM* UNDER NORWEGIAN FIELD CONDITIONS

POSTER #10

Leaf blotch diseases in wheat can cause yield losses above 30 %. The necrotrophic fungus *Parastagonospora nodorum* is the dominating leaf blotch pathogen in Norwegian spring wheat. It has been well documented at the seedling stage that the pathogen produces necrotrophic effectors (NEs) which induces cell death in plants carrying susceptibility genes (*Snn*), allowing the necrotroph to enter. However, the role of these interactions under field conditions is less researched.

In this study, we conducted field experiments with bi-parental and association mapping populations of spring wheat, to investigate the role of NE/*Snn* in adult plant resistance. The populations have been genotyped with the Illumina 90 K SNP chip, *P. nodorum* has high genetic diversity and both sexual and asexual reproduction, but the actual adaptation of the pathogen population to cultivars with different levels of resistance is not well studied. We are screening a collection of Norwegian isolates from known host sources to look for differences in NE-frequencies and haplotype distribution.

The mapping populations are also inoculated and infiltrated with culture filtrates from single isolates on the seedling stage. Isolates involved in novel interactions will be deep-sequenced in order to look for candidate effector genes. Potential effector proteins will be purified by LPC and HPLC to confirm their role in disease development.

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GENOMICS PAVE THE WAY FOR STUDIES OF NON-MODEL ORGANISMS AS
BLUMERIA GRAMINIS

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The obligate biotrophic fungus *Blumeria graminis* f.sp. *tritici* (Bgt) infects the economically important crop species wheat (*Triticum* spp.). Recently, genome sequencing (Wicker et al., 2013) has given a picture of the *Blumeria* genome and sequence-based molecular markers were used to construct a genome-wide genetic map. While the combination of physical and genetic mapping helped identifying pathogenicity genes (Bourras and McNally et al., submitted, Parlange et al., submitted) the number and structure of chromosomes as well as the position of structural elements like centromeres and telomeres are still unknown. Pathogenicity genes have been shown to locate to telomere ends in fungal pathogens (Schmidt and Panstruga, 2011). In this project we built a consensus map based on two mapping populations, this allowed us to estimate the chromosome number and linkage group size. We are currently attempting to map *Blumeria's* telomeres, this will finally resolve chromosome number, and also enable association between position, function and evolution of pathogenicity genes. Furthermore, genetic mapping uncovered a surprisingly low recombination rate within the two mapping populations, confirming the observation of low recombination between natural isolates by Wicker et al. (2013). A low recombination rate could be a major factor in genome evolution and pathogenicity of Bgt. We plan to run comparative analysis with the sister-*formae speciales* *B.g. f.sp. hordei*, which has also been sequenced and mapped genetically (Spanu et al., 2010; Pedersen et al., 2002) and has a considerable higher recombination rate to identify potential determinants of such a recombination rate variation between the two *formae speciales*.

A COMBINED TRANSCRIPTOMIC AND PROTEOMIC APPROACH TO IDENTIFY AND COMPARE EFFECTORS FROM ECONOMICALLY IMPORTANT APHID SPECIES

POSTER #12

Aphids are phloem-feeding insects that cause significant damage to agriculture. Aphid effector studies have shown that these insects, like plant pathogens, deliver effectors inside their host. While some aphid species have a wide host range, many are restricted to a few host species. We are interested to determine the extent to which effector repertoires vary among different aphid species and whether they contribute to governing host range. In order to investigate this, three economically important aphid species, with varying host ranges were selected for RNAseq analysis. Sequencing was conducted on head and separately body (not including nymphs) samples. Differential expression analysis was conducted to identify transcripts that are significantly up-regulated in heads, of these, those that encoded a signal peptide, but no transmembrane domain, were defined as putative effectors. Saliva, which contains the effectors, was collected from over 60 000 aphids per species and subjected to proteomic analysis. Comparative transcriptomic analyses were used to gain an insight into the diversity within and between aphid species and also to identify conserved transcripts. We identified a core set of putative effectors common to all aphids, completely novel (pioneer) putative effectors and diversifying effector repertoires. A better understanding of the complexity of aphid effector repertoires may provide novel insights into the roles of these proteins in plant-aphid interactions.

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UNRAVELLING POTATO WART DISEASE; DETERMINATION OF THE DRAFT GENOME OF THE OBLIGATE BIOTROPHIC FUNGUS *SYNCHYTRIUM ENDOBIOTICUM*

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Potato wart disease (PWD), caused by the soil-borne obligate parasitic fungus *Synchytrium endobioticum*, is a quarantine disease of potato with high economic impact. Over the last decades a multitude of new pathotypes emerged that break resistance; we hypothesize this is the result of an arms race between *S. endobioticum* avirulence (Avr) genes and potato resistance (R) genes.

To better understand the obligate parasitic lifestyle of the fungus and its interaction with potato, we set out to determine the *S. endobioticum* genome. As this pathogen cannot live without its host generating DNA for this fungus free from its host and other contaminants proved to be impossible. Based on a *S. endobioticum* specific TaqMan (van Gent-Pelzer et al., 2010) DNA extracts with the highest *S. endobioticum* concentration per ng DNA were selected for sequencing. Sequence data was generated from *S. endobioticum* pathotype 1(D1) winter spores using Roche 454, Illumina HiSeq (paired-end and RNAseq), and PACBIO. After removal of sequences derived from its host, a de novo assembly combining 454 and HiSeq reads resulted in >2000 contigs originating from *S. endobioticum* but also from other fungal and bacterial contaminants. Mapping reads of 16 additional HiSeq datasets derived from DNA of winterspores, covering 11 PWD strains and at least three pathotypes, on these contigs managed to reduce the draft genome to 808 contigs with a total size of 21 Mbp (zoo-approach). PACBIO reads were used to validate the assembled draft genome, and comparative genomics showed low levels of polymorphism between the genomes of 11 *S. endobioticum* strains.

A first round of gene prediction resulted in \approx 8000 genes, but evidence-based structural and functional annotation of the genome is currently ongoing. We will predict the secretome and hope to identify putative effectors, which will be tested for their ability to trigger cell-death in the host, and ultimately to identify Avr genes whose products induce effector triggered immunity (ETI).

ANALYSIS OF GENETIC DIVERSITY AND POPULATION STRUCTURE OF A NORWEGIAN *DRECHSLERA TERES* POPULATION AND IDENTIFICATION OF MOLECULAR MECHANISMS IN THE *D. TERES* – BARLEY PATHOSYSTEM

POSTER #14

Net blotch is a major barley disease in Norway caused by the necrotrophic fungus *Drechslera teres* leading to yield losses of up to 40%. At present, resistance of Norwegian cultivars is insufficient. The pathogen secretes necrotrophic effectors (NEs) which act as virulence factors in order to gain entry into and nutrients from the host (Liu et al., 2014). NEs cause a hypersensitive response in the presence of corresponding dominant host susceptibility factors.

In this study we examine the potential role of NEs and host receptors in explaining susceptibility to net blotch in Norwegian barley. This knowledge together with an understanding of the genetic background of the Norwegian net blotch population will be utilized to speed up resistance breeding.

365 Norwegian *D. teres* isolates collected from various regions and years, together with a selection of globally collected isolates, will be RADtag genotyped in order to obtain GBS markers to study the genetic diversity, genomic evolution and population structure of the current Norwegian fungal population and to compare it to pathotypes from other countries. Additionally, this data will allow us to perform Genomewide Association Studies (GWAS) to identify potential novel NE genes.

Selected isolates and their culture filtrates will be screened for specific reactions against an association mapping panel of ca. 200 mostly Norwegian barley lines and a biparental mapping population (both genotyped with the Illumina barley 9K chip) to characterize novel NE-host susceptibility interactions and to map the corresponding sensitivity loci. Effector protein candidates will be purified and further analysed to verify their effect on disease development.

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EVOLUTIONARY GENOMICS AND FUNCTIONAL CHARACTERIZATION OF THE FUNGAL GRASS PATHOGEN *ZYMOSEPTORIA BREVIS*

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The hemibiotrophic fungal wheat pathogen *Zymoseptoria tritici*, the causal agent of Septoria tritici blotch (STB), is one of the most damaging threats for wheat production worldwide (Ponomarenko, Goodwin & Kema 2011). The close relation of this high specialized fungus to the fungal wild grass pathogens *Z. ardabilae* and *Z. pseudotritici* and the recently identified *Z. brevis* and their recent divergence make this set of species to an excellent model system for the analysis of speciation, species evolution and host specialization (Stukenbrock et al. 2012). The wild grass pathogen *Z. brevis* sampled from *Phalaris* species in Iran (Quaedvlieg et al. 2011) has so far been poorly characterized. We here aim to use *Z. brevis* to investigate the functional and evolutionary relationship of *Z. brevis* to *Z. tritici*. Preliminary characterization of chromosomal structure using Pulsed Field Gel Electrophoresis (PFGE) and population genomic analyses show a high level of structural variation in *Z. brevis*. A recent genome annotation of *Z. brevis* identified 10.557 protein coding genes (Grandaubert, Bhattacharyya & Stukenbrock in review). We aim to identify genes under positive selection using maximum likelihood analysis. The comparison between gene sets under positive selection in *Z. brevis* and *Z. tritici* will provide insight into different genetic components important for host specialization in the two species.

SHORT REGIONAL CENTROMERES OF CORE AND ACCESSORY CHROMOSOMES
ARE NOT DIFFERENT IN THE PLANT PATHOGENIC FUNGUS *ZYMOSEPTORIA*
TRITICI

POSTER #16

B chromosomes, or accessory chromosomes are present in the genomes of many organisms. The plant pathogenic fungus *Zymoseptoria tritici* has up to eight accessory chromosomes. These have a high content of repetitive DNA, low gene density and are transcriptionally mainly silent. Among individuals of *Z. tritici* the accessory chromosomes show dramatic length and presence/absence polymorphisms. We set out to investigate whether meiotic instability of the accessory chromosomes is due to a different centromeric organization of these chromosomes.

We used ChIP-seq of DNA associated with CenH3, to identify centromeric DNA and find that the centromeres are small, ranging from 6 to 14 kb and mostly located near telomeres. Centromeres of core and accessory chromosomes have overall similar sequence composition; they do not contain conserved domains or motifs and are, to a large extent, composed of non-repetitive DNA. Remarkably, when correlating centromeric DNA with gene predictions and RNA-seq data we find 25 expressed genes in the relatively short centromeric regions, suggesting that centromeric DNA is accessible to the transcription machinery. We furthermore show none of the histone modifications we have tested (H3K4me2, H3K9me3 and H3K27me3) completely co-localize with CenH3.

Unlike those of most fungi, centromeres of *Z. tritici* do not cluster into a single chromosome during interphase. The findings presented here reveal a novel way to organize short regional centromeres.

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COMPARATIVE MICROSCOPY AND TRANSCRIPTOME ANALYSIS OF THE FUNGAL PATHOGEN *ZYMOSEPTORIA TRITICI* (SYN. *MYCOSPHAERELLA GRAMINICOLA*) DURING WHEAT INFECTION

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The ascomycete *Zymoseptoria tritici* (Zt) is a global pathogen of wheat with a complex and poorly understood hemibiotrophic lifestyle. Field isolates of Zt exhibit a striking degree of inter-species variation. We find high numbers of SNPs, individual sets of chromosomes, diverse *in-vitro* colony types, and different levels of stress tolerance. We show that plant infections involve individual virulence phenotypes and differences in temporal disease development between isolates. By confocal laser scanning microscopy (CLSM) we have identified and characterised four distinct core infection stages of Zt on wheat. Development and infection morphology during these stages however differ dramatically between isolates. Our aim is to better understand the underlying traits of host infection of distinct Zt isolates. Based on individual sampling schedules we have collected infected leaves of three isolates during all infection stages. From these samples we sequenced full transcriptomes and relate fungal gene expression to microscopy data of the corresponding infection time points. We find that isolates have different transcriptional programs during host infection reflecting the isolate-specific infection development. Detailed analysis of differentially expressed genes among the infection stages shows significant enrichment of putative effector genes involved in virulence. We conclude that a fine-tuned regulation of diverse virulence related genes plays an essential role for successful wheat infections and hemibiotrophic development. Our combined comparative analysis of host infections by CLSM and RNAseq provides a novel perspective on the diverse range of host-pathogen interactions among isolates of this important pathogen.

THE IMPACT OF HISTONE METHYLATION ON GENOME STABILITY SHOWN BY EXPERIMENTAL EVOLUTION OF *ZYMOSEPTORIA TRITICI*

POSTER #18

Zymoseptoria tritici is a plant pathogenic fungus of wheat (*Triticum aestivum*). The genome of *Z. tritici* consists of 21 chromosomes of which the eight smallest chromosomes are classified as accessory (Goodwin et al., 2011). These chromosomes are highly unstable during meiosis and show an increased rate of rearrangements and repetitive elements as well as an enrichment of heterochromatic histone marks (Schotanus et al., in review). To gain insight into the mechanisms involved in stability of the accessory chromosomes we combined epigenetic and evolutionary analyses. Based on studies in *Schizosaccharomyces pombe* and cancer cells, we hypothesized that the methylation of specific histone tails and the resulting changes in chromatin structure could play a role in chromosome stability in *Z. tritici* (Allshire et al., 1995; Kondo et al., 2008). We therefore deleted the methyltransferases KMT6 and KMT1 in *Z. tritici* to study the importance of the histone modifications H3K27me3 and H3K9me3 on genome stability over several mitotic cell divisions. We conducted an evolution experiment to analyze the influences of prolonged growth in axenic culture on the genotype and phenotype of the $\Delta kmt6$ and $\Delta kmt1$ mutant strains in comparison to the WT. We sequenced the genomes and epigenomes of the progenitor strains and the evolved WT and mutants and analyzed the phenotypes and karyotypes of evolved wild type and mutant strains. Prolonged growth of the $\Delta kmt6$ mutants led to a dramatic extent of accessory chromosome losses and rearrangements, while the loss of H3K9me3 in the $\Delta kmt1$ mutants affected the stability and structure of both core and accessory chromosomes. Based on our results we conclude that the absence of H3K27me3 over a longer period leads to genome instability in particular affecting the small repeat rich chromosomes and that loss of H3K9me3 has a genome wide effect on chromosome stability.

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REPEAT-RICH HETEROCHROMATIC AREAS ARE FAVORABLE GENOMIC ENVIRONMENTS FOR PUTATIVE EFFECTOR-GENES IN *ZYMOSEPTORIA TRITICI*

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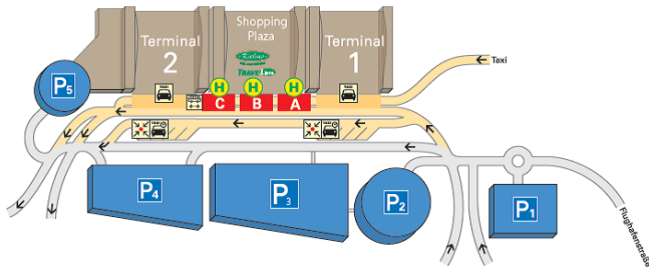
The Dothideomycete *Zymoseptoria tritici* (synonym *Mycosphaerella graminicola*) is a pathogen of wheat (*Triticum aestivum*) and has a hemibiotrophic life style suggesting a fine-tuned regulation of gene expression. The genome of *Z. tritici* comprises 21 chromosomes including up to eight conditionally dispensable chromosomes (CDCs) containing a considerably higher proportion of transposable elements (TEs) compared to core chromosomes (Goodwin et al., 2011). Evidence is accumulating that genes involved in host-pathogen interaction, such as effector-genes, can be located in TE-rich, heterochromatic genomic areas. In *Z. tritici*, while CDCs are TE-rich, they harbor few effector-genes (Grandaubert et al., 2015). We however hypothesized that putative effector-genes are not randomly located on the core chromosomes. In order to investigate their location with respect to the chromatin structure, a genome-wide histone map was established using ChIP-sequencing (Schotanus et al., in revision) and correlated with TE, effector-gene location and transcriptomic data *in vitro* and *in planta*. TE-rich regions are enriched in histone modifications typical of heterochromatic domains. Analysis of the location of putative effector-genes highlighted a significant association with TE and heterochromatic domains on the core chromosomes. We further investigated the effect of this location on the regulation of expression of putative effector-genes during two stages of the wheat infection. We conclude that TE-rich regions of the core chromosomes define favorable genomic environments for putative effector-genes and that histone modifications may control their expression.

TRAVEL INFORMATION

By plane

The nearest airport is Hamburg Airport. To travel from the airport to Kiel we recommend the coach service called „Kielius“, which brings you to Kiel main train station (Hauptbahnhof). We recommend to buy a „Kielius-Kombi-Karte“, which includes a taxi service from the train station to your final destination (www.bahn.de/autokraft/view/angebot/kielius/en-kielius-ueb.shtml). You will have to keep tickets for reimbursement. The coach service starts directly in front of the terminal at ground level (indicated with B on the schematics of the airport terminal below)

In case of late arrival, you may also take shuttle taxi service called “KIELEXX” which has to be booked at least 50 min prior to the departure (+49 (0) 431 77080). The “KIELEXX” will take you directly to your destination in Kiel (approx. 31€ one way). The shuttle taxi is **only eligible for reimbursement by COST when taken after 10 pm or before 7 am**, due to late or early departure or arrival.



By train

You will arrive at Kiel main station. By exiting the train station on the left hand side you will find the bus lines 61 or 62 departing from platform B2, (www.kvg-kiel.de/en/timetable/route-maps/). These will take you to the Hotel Steigenberger (bus stop "Schloßgarten"). Taxis (leaving in front of the building) are **only eligible for reimbursement when taken after 10 pm or before 7 am**, due to late or early departure or arrival.

Accommodation

We have reserved rooms for all participants of the workshop at the hotel "Steigenberger", Schloßgarten 7, 24103 Kiel (<http://en.steigenberger.com/Kiel/Steigenberger-Conti-Hansa>). Accommodation costs for your stay at the hotel (including breakfast) are covered by your registration fee.

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 web: www.louf.de/kontakt-anfahrt.html

Restaurant „Schöne Aussichten“
 Düsternbrooker Weg 16, 24105 Kiel
 phone: +49 431 2108585
 web: www.schoene-aussichten-kiel.de/anfahrt.html

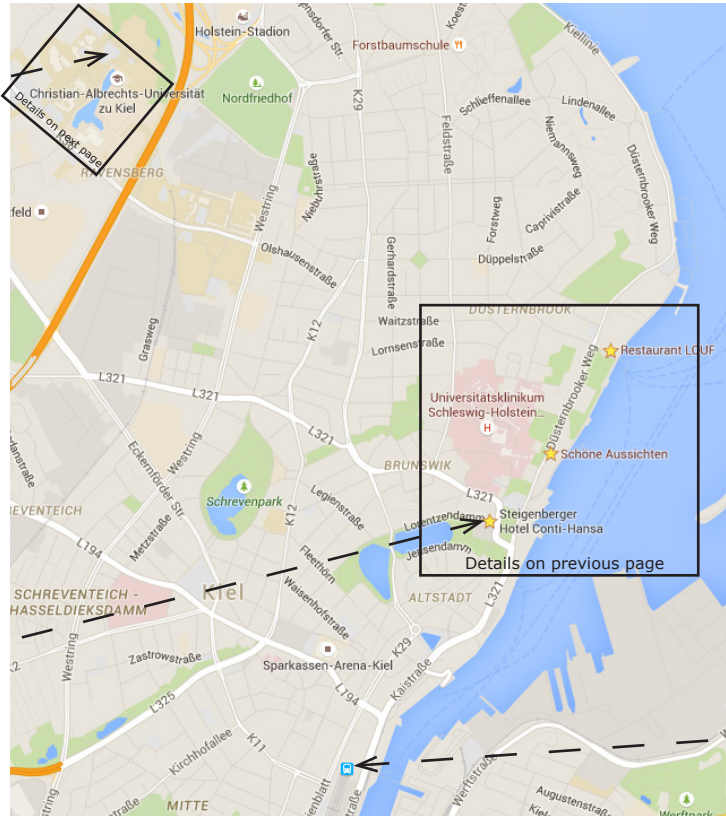
Hotel „Steigenberger“
 Schlossgarten 7, 24103 Kiel
 phone: +49 431 5115-0
 web: <http://en.steigenberger.com/Kiel/Steigenberger-Conti-Hansa>



detailed map for hotel and restaurants

MAPS

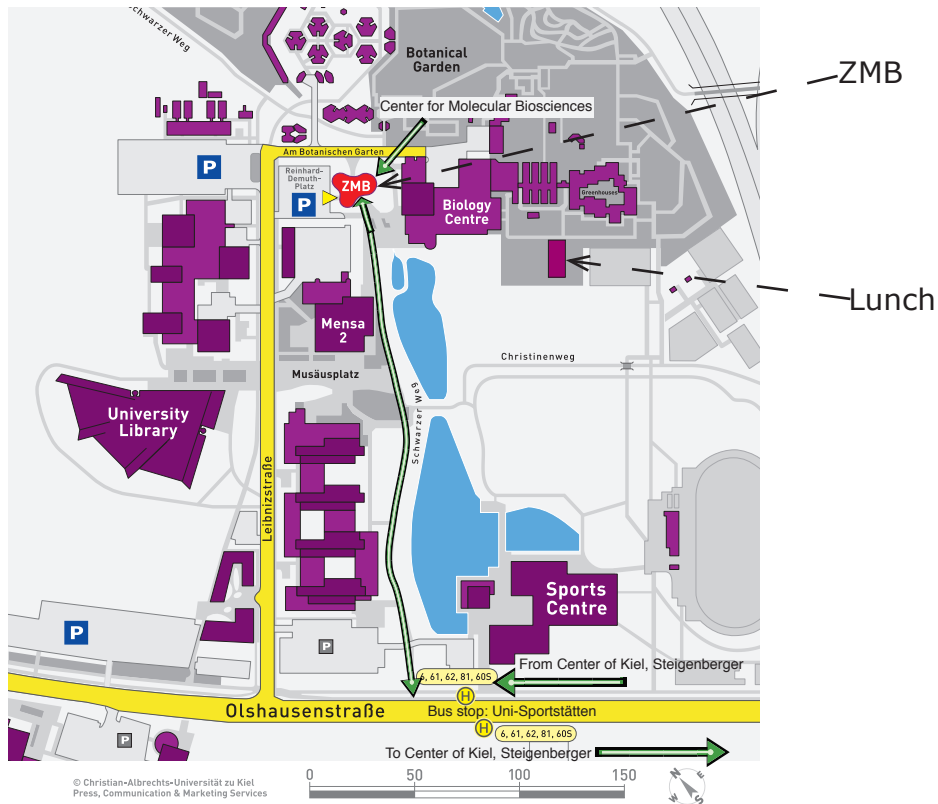
University Campus
Workshop Venue:
ZMB
Am Botanischen Garten 11
Kiel



Hotel „Steigenberger“

Train station

Overview map of the inner city of Kiel



ZMB

Lunch

Detailed map of the University campus

Venue

All conference sessions will take place at the Christian-Albrechts University of Kiel, Center for Molecular Biosciences (ZMB) seminar room 4th floor. The address is Am Botanischen Garten 11, 24118 Kiel, Germany. Transport from and to the hotel has been organized.

Center for Molecular Biosciences (ZMB)



Lunch and dinner will be provided from Tuesday (25th) evening to Friday (28th) at lunch time. All meals will be covered by your registration fee. Please inform us in advance if you have any dietary restrictions.

Wifi Internet Access

There will be WiFi at the Center for Molecular Biosciences, 4th floor and other places at Kiel University. The login is based on eduroam.

Social media

To share your comments and impressions from the meeting on social media use the hashtag: #evolpathkiel

PRESENTER GUIDELINES

Oral Presentations

Format

- All presentations should be in Powerpoint format (.pptx or .ppt)
- Presentations must be loaded onto the common laptop in advance of your session. Be sure to double check in advance that your slides display as planned.
- If your presentation includes additional files, such as video files embedded in your PPT, please make sure that the files work properly before your presentation. There is no support planned for laptop audio.
- Do not rely on having an internet access available during your talk.

Timing

- Talks of invited speakers are 30 minutes in length (including discussion)
- Talks of selected speakers are 20 minutes in length (including discussion)

Poster Presentations

Format

- Posters must be vertically oriented (portrait)
- Dimensions: International A0 Size (84 x 118 cm, 33 x 46 in)

Display

- All posters will be on display for the duration of the meeting. Please mount yours as soon as you arrive (ideally before the first oral session). Posters must be up by the beginning of the first coffee break.
- Pushpins for hanging posters will be provided.
- All posters and poster boards will be assigned a number, which can be found in the hard copy of the program book.

All poster presenters are required to be with their posters during the poster session on Wednesday, the 26th between 15.30 to 17.00.

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NOTES



Photo: Michael Habig