Poster Category 3: Genomes and Genome Evolution

PR3.1

Development of DNA Barcodes to Identify Edible Mushrooms

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Basidiomycetes as one of the largest groups of edible mushrooms have become more important in recent times for their medicinal and nutritional properties. For many years, species of this family have been mainly classified by their common phenotypic traits, however, taxonomic identification based solely on morphological features can be misleading and unreliable. In contrast, DNA based identification provides a powerful and reliable method for taxonomic discrimination of fungi, it can be performed at any growth stages using parts of the fruit body, mono- and dikaryotic mycelia, or any other organic fungal. In the current study, three different DNA and c-DNA molecular markers including Internal Transcribed Spacer (ITS) I and II, Intergenic Spacer (IGS) I, and mitochondrial COXI gene were developed to identify mushroom species and individuals. Phylogenetic trees could clearly distinguish the species of Basidiomycetes by showing distinct clades. Species differentiations were re-confirmed by AMOVA analysis, nucleotide divergence, haplotyping and P values. Moreover, the designed primers were perfectly matched with the used species, can be employed in phylogenetic studies of other *Basidiomycetes*. Polymorphism occurred throughout the regions of interest due to insertion-deletion and point mutations, and can be clearly differentiated within the families as well as genera. This study proved that the three developed molecular markers can be used as the consensus DNA and/or c-DNA barcodes for taxonomic identification of *Basidiomycetes*.

PR3.2

Genomic and molecular characterization of a model ascomycete that is ancestral to mutualistic and pathogen-rich fungal lineages (Strain A95 *Sarcinomyces petricola*, Chaetothyriales)

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Melanised micro-colonial fungi (MCF) that colonise bare rock surfaces survive in extreme environments. Phylogenetically, rock-inhabiting MCF are ancestors of lichens as well as important animal and plant pathogens. MCF may thus have been a “stepping-stone” to colonisation of other extreme environments including animals. A meristematic black yeast species *Sarcinomyces petricola* (strain A95) was selected as a model strain. The estimated 29 Mbp DNA sequence is being assembled and annotated. As with all MCF that possess the characteristic stress-tolerant morphology (including thick, melanised cell-walls) disruption of the cellular structure is problematic. Nevertheless an efficient procedure for protoplast formation was developed using various hydrolytic enzymes. Protoplast formation is an important prerequisite for both the development of an appropriate transformation system for A95 and application of other tools to characterise the genome. For example, the number of chromosomes will be ascertained by pulsed-field gel electrophoresis of the protoplasts. Karyotyping A95 in this way will provide an additional check on the estimated genome size (and thus the depth of sequencing required to close the genome) and permit the isolation of mitochondrial DNA and plasmids (if A95 contains them).

PR3.3

Grey mould isolates from commercial strawberry fields show multiple fungicide resistance and represent a novel clade between *B. cinerea* and *B. fabae*

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Botrytis cinerea is a destructive pathogen of many fruit and vegetable crops worldwide, which needs to be controlled by fungicide treatments. Previously, we have observed high frequency occurrence of *B. cinerea* strains with three different multidrug resistance (MDR) types, MDR1-3, in French and German vineyards, and identified the underlying mutations leading to drug efflux transporter overexpression. We have extended our analysis to grey mould populations from German strawberry fields which receive weekly fungicide sprayings during flowering. The strawberry populations carried a combination of fungicide-specific (target site) and nonspecific (MDR type) resistances and showed high frequencies of multiple resistance to currently used fungicides. A stronger variant of the MDR1 phenotype, called MDR1^{h}, was discovered which contributes to reduced control efficiency of two major botryticides. Surprisingly, the majority of German strawberry isolates, called Botrytis group 3, were found be genetically distinct, but otherwise indistinguishable, from *B. cinerea*. Based on sequence comparisons of multiple genes, Botrytis group 3 isolates were identified as a novel clade intermediate between *B. cinerea,* which attacks more than 200 host plants, and *B. fabae*, a species that only infects legume hosts. Population studies revealed that group 3 isolates are almost absent from vineyards, possibly due to reduced sporulation efficiency on grape berries, but common on other fruit and vegetable crops in different European countries. To analyse the genomic basis of evolution, host specificity and biology of the *B. cinerea* species complex and related clades, we are currently performing genome sequencing, interspecific crosses and systematic comparative phenotypic studies.

PR3.4

Similar Is Not The Same: Differences In The Function Of The (Hemi-) Cellulolytic Regulator XlnR (Xlr1/Xyr1) In Filamentous Fungi

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The (hemi-) cellulolytic regulator XlnR (Xlr1/Xyr1) is a major factor in fungal xylan and cellulose degradation as well as in the utilization of D-xylose via the pentose catabolic pathway (PCP).

XlnR homologues are commonly found in filamentous ascomycetes and often assumed to have the same function in different fungi. However, a comparison of the saprobe *Aspergillus niger* and the plant pathogen *Magnaporthe oryzae* showed different phenotypes for deletion strains of XlnR.

In this study wild type and *xlnR/xlr1/xyr1* mutants of six fungi were compared: *Fusarium graminearum*, *M. oryzae*, *Trichoderma reesei*, *A. niger*, *Aspergillus nidulans* and *Aspergillus oryzae*.

The comparison included growth profiling on relevant substrates and detailed analysis of protein profiles of extracellular enzymes and extracellular enzyme activities. The data resulting from this comparison demonstrate significant differences in the influence of XlnR and its orthologs on plant polysaccharide degradation by these fungi. Highlights of the study will be presented.

PR3.5

Twin research in fungi – Phenotype vs. Genotype

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Trichoderma atroviride is a filamentous fungus used widely for biological control of major plant diseases. A strain of this species (LU132) has been developed into a commercial biocontrol agent (BCA) for use in New Zealand. Its broad spectrum of antagonistic activity is linked to a wide range of biological parameters but we have limited knowledge of what specific attributes make this strain particularly effective as a BCA. The development of a molecular marker for LU132 failed, because all known marker genes had identical sequences with *T. atroviride* LU140, a strain that was isolated at the same time from the same paddock but had a different phenotype to LU132. To identify the level of genetic similarity between the two strains, the whole genomes were re-sequenced via Next Generation Sequencing with a surprising result: only 2 Single Nucleotide Polymorphisms (SNPs) could be found between the genomes of LU132 and LU140. In the present study the strains were confirmed to be two different individuals by comparing the phenotypes and confirming the SNPs. Based on those results, five genes were selected whose gene expression was studied. The results are presented here and their impact on the relationship between molecular changes and phenotypic changes in *T. atroviride* are discussed.

PR3.6

Transcriptomics of *Agaricus bisporus* reveals changes in carbon metabolism in different growth stages

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Carbon source utilisation is an important aspect of fungal physiology. Many fungi are exposed to mixtures of carbon sources, which enable them to make choices to use the most favourable substrate. *Agaricus bisporus* is commonly grown on compost, which consists mainly of straw and horse manure. This means that the majority of the carbon source is present as plant-based polysaccharides, which themselves consist of many different monomeric components. The major components of these polysaccharides are glucose, xylose, and arabinose, while smaller amounts of galactose, galacturonic acid, rhamnose and mannose are also present.

In this study we evaluated the expression of genes involved in the catabolism of different sugars during different stages of growth of *A. bisporus*. Clear differences in the expression of genes from different catabolic pathways were observed between mycelium grown on plates, in compost or in casing-soil, and in fruiting bodies, suggesting a high level of specialization.

PR3.7

Homolog searching and RNA-SEQ in *Pleurotus Ostreatus*

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Pleourotus Ostreatus is an edible basidiomycete of great interest in the field of food and bioremediation. In 2009 the genome sequence of the two haplotypes of *Pleurotus* N001 strain were released. The two haplotypes can be individualized permitting the two gene complements of a sexually mature individual to be analyzed simultaneously for first time.

In order to better understanding the mechanisms of ligning degradation and metabolic function of this fungus we realiced three RNA-SEQ experiments in the two haplotypes and the dicariotic strain at three temperatures in liquid and solid grow medium.

For this purpose was of great interest to know the corresponding allele in each haplotype. In each haplotype two sequences were considered alleles if their e-value were less then e-20, their aligment identity percentages were greater than 80% and the aligment covered at least the 80% of each gene. Additional checks were performed, as the best hit was the same in both directions and the positions of both genes corresponded to homologous chromosomes.

The RNA-SEQ experiments were done with SOLID plataform from Applied Biosystems, and then the data were analyzed with the software TopHat and Cufflinks. Read position distribution and the changes in relative isoform abundance determined with Cufflinks were used to perform a genome annotation curation tool.

PR3.8

Genomic context of effector genes in *Fusarium oxysporum* enables prediction of novel effector candidates

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Fusarium oxysporum is a soilborne fungus that causes Fusarium wilt disease in many plant species by colonizing and eventually blocking the host xylem vessels. Virulence depends crucially on effector proteins, which are secreted by the fungus in the xylem sap (Six (Secreted in xylem) proteins). In the tomato wilt strain *F. oxysporum* f. sp. *lycopersici* (*Fol*) Six proteins are encoded on lineage-specific (LS) chromosomes. We analysed the genomic context of *SIX* genes in *Fol* and found that all *SIX* genes harbour a particular transposable element - a mimp (miniature impala) - in their promoters. To investigate the impact of the mimp on *SIX* gene expression, we made partial promoter deletions for two *SIX* gene promoters. The gene products of these two *SIX* genes are recognized by tomato resistance genes. This recognition is abolished in some promoter deletion strains, while in others recognition is still mediated. However, in all promoter deletion strains, *SIX* genes are expressed *in vitro*. We hypothesize that the *SIX* gene promoters are usually in a repressed state and are only transcriptionally released upon plant infection. Partial deletion of the promoters appears to reduce repression of transcription.

Using our current knowledge of *SIX* gene structure and genomic context we developed a bioinformatic pipeline for the prediction of novel effector candidates in *Fol*. We identified 15 of these genes. Currently, we are making knock-out mutant strains to explore whether these genes are important for virulence.

PR3.9

Fungal glutathione transferases:targets for evolutionary innovations

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Glutathione transferases (GST) are detoxification enzymes, which genes are highly duplicated in fungal genomes. These enzymes could display the classical conjugation of glutathione to toxic compounds but also many other activities, making them excellent examples of how multiple gene duplication events has resulted in groups of enzymes with a large panel of functions. The study was here focused on two specific classes Ure2p and GTE, which are extended in wood-decaying fungi. By developing high throughput screening assays in combination with structural data we found that these classes are highly divergent in their enzymatic activities or ligand recognition in spite of strong primary structure homology. The number of Ure2p isoforms varies between *Phanerochaete chrysosporium* and *Phanerochate carnosa* (9 and 20 isoforms respectively) and could be related to different local adaptation rather than genetic background, since both species are taxonomically very close. The GTE class is composed of 5 isoforms. So far homologues of this family were only found in bacteria and are known to degrade the beta-aryl linkage, which is the most abundant intermolecular link in lignin. Functional and structural data revealed various activities and strong wood compounds specificities between duplicates.
Ure2p and GTE could have thus played a major role in fungal adaptation to their environment through their huge versatility. Both classes are microorganism specific and could be excellent markers of fungal diversity. Their capacities of catalytic promiscuity and neo-functionalization make these enzymes excellent sinks of new functions in environmental adaptation process and are excellent models for protein engineering.

PR3.10

Evidence for Extensive Recent Intron Transposition in Closely Related Fungi

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Though spliceosomal introns are a major structural component of most eukaryotic genes and intron density varies by more than three orders of magnitude among eukaryotes, the origins of introns are poorly understood, and only a few cases of unambiguous intron gain are known. We utilized population genomic comparisons of three closely related fungi to identify crucial transitory phases of intron gain and loss. We found 74 intron positions showing intraspecific presence-absence polymorphisms (PAPs) for the entire intron. Population genetic analyses identified intron PAPs at different stages of fixation. We found direct support for extensive intron transposition among unrelated genes. A substantial proportion of highly similar introns in the genome either were recently gained or showed a transient phase of intron PAP. We also identified an intron transfer among paralogous genes that created a new intron. Intron loss was due mainly to homologous recombination involving reverse-transcribed mRNA. The large number of intron positions in transient phases of either intron gain or loss shows that intron evolution is much faster than previously thought and provides an excellent model to study molecular mechanisms of intron gain.

PR3.11

Genotypic Analysis Of *Fusarium* Species Associated With *Allium cepa* In The UK Including Whole Genome Analysis Of *F. oxysporum* f. sp. *cepae*

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This project set out to identify *Fusarium* species associated with onions in the UK and to develop quick molecular methods to identify *Fusarium oxysporum* f. sp. *cepae* (FOC) causing onion basal rot.
More than 500 isolates representing diverse *Fusarium* species, including 171 isolates associated with onions obtained from 12 different sites in the UK were collected. Five species associated with diseased onions have been identified, of these *F. proliferatum, F. solani, F. redolens* and *F. avenaceum* have not previously been reported in association with onion crops in the UK. Assays under controlled conditions confirmed the ability of the *F. proliferatum* isolates to cause disease on onions.

F. oxysporum was by far the most common (143 isolates) and these isolates belong to at least two different genotypes based on sequence comparison of several "housekeeping" genes and overall, appear to be polyphyletic. None of the housekeeping genes studied correlate with pathogenicity. Secreted in xylem (SIX) genes offer more promise for the specific identification of *F. oxysporum formae speciales* (Lievens *et al*., 2009). FOC isolates were screened for the presence of seven SIX genes (SIX1-7). A homologue of SIX7 gene was found in a few FOC isolates which suggests that SIX7 is not necessary for pathogenicity. Whole genome sequencing of a FOC isolate was completed at our university in order to understand pathogenicity and identify novel effector genes. Screening of 11 candidate effector genes suggest that FOC isolates have different gene sets.

References: Lievens *et al*. FEMS Microbiol Lett 300 (2009) 201–215

PR3.12

The evolution of Zygomycetes, the most basal terrestrial fungi: lessons from new genome projects

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Zygomycetes, formerly described as class within the fungal kingdom, are polyphyletic, and therefore, split into five distinct subphyla, which are the Entomophthoromycotina, Mucoromycotina, Mortierellomycotina, Kickxellomycotina and Zoopagomycotina [1, 2]. The former two subphyla contain species, which are human pathogenic causing infections with diverse predisposition and etiologies. They encompass ubiquitously distributed insect-killing, saprotrophic soil- or dead plant material-inhabiting fungi of the orders Entomophthorales and Mucorales, respectively. Human pathogenic species inhabit different growth temperature optima ranging from 33 °C to 42 °C, just members of these two orders are capable of causing diseases, entomophthoromycosis and mucormycosis, in immunocompromised and immuncompetent humans, respectively. Two new genome projects were initiated on *Conidiobolus coronatus* and *Lichtheimia corymbifera* and the results are discussed with respect to the evolution of single genes involved in the development of pathogenicity. The data are embedded in a phylogenomic study comprising all publicly available fungal genomes and EST databases and analysed with Bayesian inference and maximum likelihood approaches [3]. moreover, the novel genome projects are discussed with respect to the 1000 Fungal Genome Project which has been newly launched last year (http://1000.fungalgenomes.org/home/).

1. D.S. Hibbett, M. Binder, J.F. Bischoff. et al., Mycol. Res. **111** (2007), p. 509-547.
2. K. Hoffmann, Voigt K, Kirk PM, Mycotaxon **115** (2011), p. 353-363.
3. Ebersberger I, Matoes Simoes R, Kupczok A, Kothe E, Voigt K, von Haeseler A, Mol. Biol. Evol. (2012), in press.
4. We kindly acknowledge the Fungal Working Group of the International Fungal Barcoding Consortium, the Assembling the Fungal Tree of Life Consortium and the 1000 Fungal Genomes project for integration into their global network. We thank Ingo Ebersberger (CIBIV, University of Vienna, Austria), Rytas Vilgalys and Andrij Gryganski (Duke University Durham, NC, USA) and Conrad Schoch (NCBI, NIH, Bethesda, Maryland, USA) for strain and data share.

PS3.13

Variation in distribution and evolution of fusarin gene cluster in Fusarium

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Fusarins are mycotoxins produced by a wide range of species in the genus Fusarium. The putative fusarin biosynthetic gene (FUS) cluster in the maize pathogen F. verticillioides consists of nine genes (FUS1 – FUS9), including a hybrid polyketide synthase-non ribosomal peptide synthetase gene (FUS1). Here, we examined sequence variation in FUS cluster genes and in regions flanking the cluster in several fusaria from the Fusarium lineages the Gibberella fujikuroi species complex (GFSC), the trichothecene producing clade (TRC), and the F. tricinctum species complex (FTSC). The results indicate that the order and orientation of genes in the FUS cluster are uniform throughout Fusarium, even though the cluster has been lost from most American-clade species of GFSC and from most strains of F. incarnatum-F. equiseti species complex (FIESC). In contrast, genes flanking the cluster differ in most species, indicating the genomic context of the cluster differs in all but the most closely related species. The phylogenetic relationships of FUS genes are correlated with the relationship of Fusarium species in which the genes occur. An exception to this is F. scirpi strain FRC R-06979. Although this strain is part of the TRC, its FUS genes are more closely related to FUS genes in the FTSC. In contrast, the FUS1 gene in other related strains is more similar to FUS1 in other TRC species. These results suggest that the Fusarium lineage that gave rise to R-06979 lost the FUS cluster that it inherited from a TRC ancestor but acquired a FUS cluster from a species within FTSC by horizontal gene transfer.

PR3.14

Alternaria Leafspot Pathogens: Genetics, Evolutionary History And Diagnostics

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Alternaria black spot diseases on pome fruits are economically important in Asia and the Americas. Movement of infected apple and pear material risks establishment of these and other host specific *Alternaria* diseases in Europe. EC countries are obliged to take action on intercepted non-native pathogenic *Alternaria*. However, identification based on morphology is unreliable and conventional genetic loci show little resolution between species. Fast and reliable diagnostics need to be developed to identify *Alternaria* pathogens.

Disease is linked to the presence of an additional small chromosome containing genes responsible for the production of host-specific toxins.

Phylogenetic and morphological studies were performed on the UK culture collection. Genes sequenced were: ITS, EndoPG, mating type genes and three novel loci. Host-specific toxin genes on the pathogenicity chromosome were detected using PCR.

Isolates carrying the pear specific pathogenicity chromosome were phylogenetically distinct. Isolates carrying the apple specific pathogenicity chromosome were not distinct and were polyphyletic. This may indicate that presence of toxin genes is a better molecular marker for pathogenicity than morphology (primarily sporophore shape) or phylogeny. Future work will look for new diagnostics based on more direct indicators of pathogenicity, such as presence of toxin genes and pathogenicity chromosomes.

PR3.15

Recent Developments at the Aspergillus and Candida Genome Databases

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The *Aspergillus* and *Candida* Genome Databases (AspGD, http://www.aspgd.org and CGD, http://www.candidagenome.org/) are freely available, web-based resources for researchers studying the molecular biology of these fungi. The interfaces and navigation functionality of both web sites and databases are recently upgraded, providing streamlined, ortholog-based navigation of the annotation for multiple species concurrently. We have now completed manual curation of the entire published literature about multiple species, including *A. nidulans*, *A. fumigatus*, *A. oryzae*, *C. albicans*, and *C. glabrata*. We also provide resources to foster interaction and dissemination of community information, tools, and data. We collect and provide large-scale datasets with a full-featured genomics viewer to facilitate comparative genomics analysis. AspGD is funded by grant R01 AI077599 from the National Institute of Allergy and Infectious Diseases, and CGD is funded by R01 DE015873 from the National Institute of Dental and Craniofacial Research at the US National Institutes of Health. We welcome, encourage, and appreciate your questions or suggestions, and AspGD and CGD curators can be reached at aspergillus-curator@lists.stanford.edu and candida-curator@lists.stanford.edu, respectively.

PR3.16

Functional diversity of *Trichoderma* mycoparasitism

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Many species of the fungal genus *Trichoderma* (teleomorph *Hypocrea*) are mycoparasites, what is widely used for the biological control of plant's pests. Here we studied common responses of ecologically diverse *Trichoderma* spp. to the presence of a potential fungal prey, using the cosmopolitan soil species and powerful mycoparasites *Trichoderma atroviride* (*Ta,* teleomorph *Hypocrea atroviridis*) and *T. virens* (*Tv,* teleomorph *Hypocrea virens*), and mainly saprotrophic tropical species *T. reesei* (*Tr,* teleomorph *Hypocrea jecorina*). We analyzed transcriptional responses during three stages of interaction with *Rhizoctonia solani* (teleomorph *Thanatephorus* spp.)*, i.e.* prior the contact, during the initial stage of interaction, and shortly after the contact was established. *Tv* completely arrested the growth of the prey while *Ta* did not stop the growth of *R. solani*, but continuously overgrew it. *Tr* did not attack the prey but built up a dense hyphal front, which was not permeable for hyphae of *R. solani.* In total 666, 303 and 424 genes were >2-fold regulated in *Ta, Tv* and *Tr*, respectively. The majority of them was down-regulated in *Ta* and *Tv*, but not in *Tr*. 34 % of the up-regulated genes in *Ta* (in contrast to only 6 % in *Tv*) comprised secreted proteins dominated by subtilisin-like and aspartyl proteases. It suggests that *Ta* mainly acts by a hydrolytic attack. In contrast, the majority of up-regulated genes in *Tv* comprised were those involved in the biosynthesis of gliotoxin and its precursor amino acids. Expression in *Tr* was characterized by a massive up-regulation of ribosomal genes indicative for a strong shift in protein synthesis. In contrast, genes encoding permeases of the major facilitator subfamily, PKS and NRPS and Zn2Cy6 transcription factors were down-regulated in all three species. Our data show that in *Trichoderma* the functional stimulation caused by the prey exhibits diverse patterns, whereas the suppression of gene function in *Trichoderma* mycoparasitism is evolutionary conserved.

PR3.17

The interaction of the plant-pathogen *Verticillium longosporum* and its host *Brassica napus* and insights into the evolutionary origin of the fungal hybrid.

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Verticillium longisporum is a soil-borne fungal pathogen of oilseed rape (*Brassica napus*). Infection is initiated by hyphae from germinating microsclerotia which invade the plant vascular system through penetration of the fine roots. We investigated the reaction of the fungus to xylem sap of the host-plant by differential expression of proteins related to reactive oxygen stress [1]. Knockdowns of the catalase-peroxidase of *V. longisporum* were inhibited in the late phase of disease development. The evolutionary origin of the cruciferous fungal pathogen, *V. longisporum* is still a mystery. It is very closely related to both *V. dahliae* and *V. albo-atrum* but possesses some typical characteristics such as long spores, almost double amount of nuclear DNA content and cruciferous host specificity. *V. longisporum* is an example for an early stage of speciation and we show clear evidences for the origin of the fungus. To clarify the hybrid status, we undertook molecular sequence analyses of the internal transcribed spacer (ITS) and intergenic spacer (IGS) regions of rDNA of putative ancestors of *V. longisporum*. In addition a number of other structural genes were analyzed. We found one gene encoding a putative zinc finger transcription factor with two distinct sequences carrying different markers supporting the hybrid origin detection of the fungus. One of these sequences is almost identical to that of *V. dahliae* and the other is highly similar to the sequence of *V. albo-atrum*. Currently we are sequencing *V. longisporum* to determine which rearrangements occurred during and after the hybridization.

1. S Singh, SA Braus-Stromeyer, C Timpner, O Valerius, Av Tiedemann, P Karlovsky, C Druebert, A Polle, and GH. Braus, Molecular. Plant-Microbe Interactions, accepted (2011), DOI: 10.1094/MPMI-08-11-0217

PR3.18

Using RNA-Seq data to improve the gene structure annotation of Aspergillus species

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The correct structural annotation of genes is fundamental to downstream functional genomics approaches. Genes undetected by gene prediction algorithms, incorrect gene boundaries, misplaced or missing exons and wrongly merged genes can jeopardize attempts to produce a comprehensive catalog of an organism's metabolic capabilities. We are currently working toward generating alternative and improved structural annotation of Aspergillus species relevant to food industry and human health. Our approach consists of reconstituting transcript sequences from RNA-Seq data and aligning those sequences against their respective loci in the reference genome. Potential gene structure modifications are then validated by coding sequence conservation across closely related species. In addition, novel algorithms were developed to deal with the challenges intrinsic to non-strand-specific RNA-Seq, which represent the bulk of data currently available for the Aspergilli; and downstream analyses leveraging the newly defined UTR regions were performed. The improved gene structure annotation of every species associated to this effort will be freely available through the Aspergillus genome database site (http://www.aspergillusgenome.org)

PR3.19

How apomixis shaped the genome of *Arnium arizonense*, a homothallic Sordariomycetes?

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Arnium arizonense is a fungus closely related to the heterothallic fungus *Podospora anserina* but displays several unique features. It is apomictic, *i.e.*dikaryotic croziers are formed inside the perithecia but neither karyogamy nor meiosis take place in the asci, although morphological changes in both chromosomes and spindle pole bodies are reminiscent of those associated with meiosis in heteromictic Pezizomycotina. Instead of meiosis, the two nuclei undergo two mitoses and the resulting eight nuclei are enclosed in uninucleate ascospores, among which four mature normally, and four abort. Arrangement of the two ascospore types in individual asci is random (Mainwaring and Wilson, 1968, Trans Br mycol Soc, 51, 663). Another peculiarity of *A. arizonense* is its low number of chromosomes: the haploid number is two, while most fungi in this group have seven chromosomes. Analysis of the genome sequence reveals that *A. arizonense* contains linked counterparts of the *P. anserina* mating-type genes, a structure that is typical of homothallic life style. Deletion of the mating-type locus results in the loss of perithecium formation, thus confirming the role of the mating-type genes in the fruiting-body development. Mating-type target genes have been recently identified in *P. anserina*(Bidard et al, 2011, PLoS ONE, 6, e21476). Search for their orthologs in *A. arizonense* reveals that some of them are pseudo-genes. The genome of *A. arizonense* is currently under analysis to find candidate mutations for the loss of karyogamy and meiosis, and to determine how apomixis has shaped the genome structure.

PR3.20

Comparative QTL mapping of multiple disease resistance traits in the cultivated mushroom *Agaricus bisporus*

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1. INRA 2. CTC

Biotic stresses lead to severe crop losses in *Agaricus bisporus* cultures. The development of resistant cultivars is the most effective, economical and environmentally friendly approach to manage disease control. The understanding of the genetic architecture of resistance to diseases is a prerequisite for breeding. Bacterial brown blotch (caused by *Pseudomonas tolaasii),* dry bubble (caused by *Lecanicillium fungicola)* and green mould (caused by the fungal competitor *Trichoderma aggressivum)* are the most detrimental disorders affecting yield and quality of the button mushroom throughout the world. Independent studies investigating quantitative trait loci (QTL) of resistance for each of these diseases have been done in our laboratory (Foulongne-Oriol et al. 2011, 2012; Mocquet et al. 1999). The present work proposes, as an integrative approach, to compare the location of these QTL: Are there any evidence for multiple disease resistance loci? Do common or different mechanisms underlie each resistance? Consequences for breeding of multi-resistant cultivar in *A. bisporus* will be discussed. Foulongne-Oriol M *et al.* (2012) Relationship between yield components and partial resistance to *Lecanicillium fungicola* in the button mushroom *Agaricus bisporus* assessed by QTL mapping. AEM (in press) Foulongne-Oriol M *et al.* (2011) QTL for resistance to *Trichoderma* lytic enzymes and metabolites in *Agaricus bisporus*. In: Proceedings of the 7th ICMBMP. Vol 2. pp 17-25. Moquet F *et al.* (1999) A quantitative trait locus of *Agaricus bisporus* resistance to *Pseudomonas tolaasii* is closely linked to natural cap color. FGB 28:34-42

PR3.21

Characterization of farA and other zinc finger transcription factor genes for fatty acid metabolism in *Aspergillus oryzae*

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The zinc finger transcription factor FarA in *Aspergillus nidulans* up-regulates genes required for growth on fatty acids (Hynes et al, 2006). Ctf1 and Por1, orthologs of FarA of *A. nidulans* are required for growth on fatty acids in *Candida albicans* and also for the essential transcriptional activation of genes involved in beta-oxidation and peroxisomal biogenesis in *Yarrowia lipolytica*, respectivel (Ramirez and Lorenz, 2009; Poopanitpan et al, 2010). FarA transcriptional factor is also found in the *Aspergillus oryzae* which has 83% homology of all the amino acid sequences and 97.5% homology of Zn_2Cys_6 motifs with the *A. nidulans.* In this study, *farA* disruptant in *A. oryzae* was characterized and expression levels of genes for fatty acid metabolism were also determined.

Interestingly,*A.oryzae farA* disruptants showed indistinguishable growth in fatty acid sources compared to thewild-type, inconsistent with the growth phenotype of the *A. nidulans* counterpart. In contrast, expressions of some genes for fatty acid metabolism were significantly reduced in the *farA* disruptants. These contradicting results suggested that FarA may act not only the primary transcriptional activator for fatty acid utilization in *A. oryzae* and that another transcriptional factor(s) may regulate other fatty acid metabolic genes which can be accounted to the differences on the number of genes between these two *Aspergilli*. We then proceeded to screen other zinc finger transcription factor gene disruptants from the disruptant library of *A.oryzae* for fatty acid metabolism. Characterization and implication of these disruptants in response to different fatty acid substrates and fatty acid gene expressions are currently underway.

PS3.22

Locus-specific analysis of the *in vivo* spontaneous mutagenesis in the human fungal pathogen *Candida albicans*

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The human fungal pathogen *Candida albicans* displays a very high degree of plasticity, including the types of genomic changes frequently observed with cancer cells, such as gross chromosomal rearrangements, aneuploidy, and loss of heterozygosity. Despite its relevance to every aspect of genetics and evolution of this pathogen, our understanding of the mutation process remains quite limited. This is especially important since *C. albicans* may have evolved mechanisms not only to tolerate but also to generate genetic variation as a means of adaptation. Here, we have estimated the gene-specific mutation frequency of *HIS4*, CEN4 and *EST2* in *C. albicans* by using direct DNA sequencing, indirect gene-specific approaches (*HIS4* reversion) and MA lines. We found that frequency of mutation at these loci is similar to that observed for *S. cerevisiae* at homolog loci following the direct DNA sequencing approach, but was slightly lower at the CEN4. Analysis of the spectrum of spontaneous mutagenesis in both fungi revealed a transition bias. Although absence of the homologous recombination protein Rad52 did not show increased mutation frequency within these loci after 20 generations, an *in vivo* mutational accumulation was observed after 800 generations in *C. albicans*; especially for the *HIS4* locus. This was supported by an increased frequency of *HIS4* reversion in absence of Rad52. However, the fact that no mutation become fixed on these loci after those generations either in absence or presence of Rad52 suggest that *C. albicans* genome is relatively stable in terms of mutational accumulation even in the absence of Rad52.

PR3.23

Evolution and function of Argonaute-proteins in the wheat-pathogen *Mycosphaerella graminicola*

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Important components of the RNA interference (RNAi) machinery are Argonaute-proteins which bind to single-stranded micro RNAs and influence the transcription of complementary messenger RNAs. During defense reactions RNAi fulfills a crucial role in the plant immune system. The role of RNAi in fungal pathogens is poorly understood. We use the fungal wheat pathogen *Mycosphaerella graminicola* to elucidate the role of Argonaute proteins in the evolution of host specificities and virulence. Two Argonaute-proteins, *Mgr 38035* and *Mgr 90232* were identified and selected based on their regulatory behavior *in planta.* We compared sequences of the two genes in different *M. graminicola* isolates to assess patterns of evolution. While *Mgr 35038* was highly conserved at the nucleotide level, the Argonaute gene *Mgr 90232* showed a strong differentiation at the nucleotide level between isolates. The gene has undergone both strong structural changes as well as mutations in the sequence composition. We speculate that the different level of conservation reflects different roles of the two Argonaute genes. We hypothesize that *Mgr 90232* is an evolutionary hotspot in the host-adaption of *M. graminicola* to wheat and plays an important role during infection. Future studies will try to elucidate the function of these genes with a focus on infection behavior via the generation of deletion mutants.

PR3.24

Recent Development In the Taxonomy and Phylogeny of *Aspergillus* and *Penicillium*: implications for Full Genome Sequence Initiatives

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The family of *Trichocomaceae* harbors various economically important genera, such as *Aspergillus*, *Penicillium* and *Paecilomyces*. The importance of these genera is illustrated by the high number of undertaken full genome sequencing projects. Recently, new insights in the taxonomy of *Aspergillus* and *Penicillium* have led to numerous new species and name changes of existing species. In this study, the phylogeny of the full genome sequenced strains is investigated using the protein coding genes *RPB1*, *RPB2*, *Cct8* and *Tsr1*. Furthermore, the impact of recent developments in the taxonomy of these strains is addressed.

Phylogenetic analysis shows a close relationship between the full genome sequenced strains (or representatives of the same species) of *Aspergillus*, *Penicillium sensu stricto*, *Monascus* and *Xeromyces*. *Talaromyces stipitatus* and *Penicillium marneffei* appear to be distantly related to *Aspergillus* and *Penicillium sensu stricto.* As a consequence, *Talaromyces* is re-defined and the combination *Talaromyces marneffei* (= *P. marneffei*) is proposed, leaving *P. chrysogenum* as the sole full genome sequenced species in *Penicillium*. Furthermore, *Talaromyces emersonii* is transferred to the new genus *Rasamsonia* and *Talaromyces thermophilus* will be transferred to *Thermomyces*. The new insights in the relationship among *Aspergillus*, *Penicillium* and related genera will help to interpret the results generated with comparative genomics studies or other studies dealing with evolution of e.g. mating type loci, virulence genes and secondary metabolite biosynthetic gene clusters.

PR3.25

Sequencing of the fungal pathogen *Ramularia collo-cygni,* why and how?

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The fungus *Ramularia collo-cygni* is the major biotic agent involved in Ramularia Leaf Spot (RLS). It was first identified as a pathogen of spring barley in Scotland in 1998 and since then has increased in its importance throughout the whole of the UK. Results from testing of the Rothamsted Hoosfield spring barley archive using quantitative real-time PCR indicated a significant increase in pathogen levels since the 1990's (Fountaine and Fraaije, 2009). RLS has recently been re-classed as a major barley disease in the UK. *R. collo-cygni* is currently classified as a member of the *Mycosphaerella* genera and sequence data derived at SAC suggests a genetic similarity between *R. collo-cygni, Mycosphaerella graminicola* and *M. fijiensis*. These sequences focus primarily on the genes associated with the target sites for fungicides, such as Beta tubulin, Cytochrome *b* and Succinate dehydrogenase, eburicol 14ά-demethylase (CYP51) genes. This paper will highlight previous sequence work and outline a new project using next generation sequencing by the combined approach of illumiina/solexa and Roche/454 sequencing. The use of these combined approaches will help with the assembly of sequence data which can then be used for comparative genetic studies to address the biology of *R. collo-cygni* in areas such as population genetics, fungicide resistance and pathogenicity. These advances should assist in the development of environmentally sound strategies to control this important disease of barley production systems.

PR3.26

Study on Genetic Structure of *Fusarium solani* Populations from Various Host Plants

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One hundred and forty nine *Fusarium solani* isolates were analyzed for their fluorescent AFLP patterns. The isolates were 60 from potato, 30 of *F. s.* f.sp. *cucurbitae*, 29 of *F. s.* f.sp. *phaseoli* and 30 of *F. s.* f.sp. *pisi* collected from different areas of Iran. After DNA extraction, digestion by *Eco*RI and *Mse*I, and ligation of adapters to DNA fragment, preamplification and selective amplification PCR, the products were separated by capillary electrophoresis. Analysis was performed based on 151 polymorphic markers. In general, 92% of observed molecular variance was related to within host populations and 8% variation was found among them. Genetic similarity between four host populations was more than 92%. High degree of similarity between populations may be due to gene flow among them. For determination of phylogenetic relationships, 110 isolates out of 149 isolates were examined based on DNA sequences from rDNA-ITS, a region of the nuclear rDNA-LSU and an intron-rich portion of the translation elongation factor 1 alpha (*TEF*) gene. The analyzed isolates were 59 from potato, 18 of *F. s.* f. sp. *cucurbitae*, 16 of *F. s.* f. sp. *phaseoli* and 17 of *F. s.* f. sp. *pisi.* Phylogenetic trees were inferred from three mentioned loci based on three methods, maximum parsimony, neighbor-joining and UPGMA. Major and minor clades with strong bootstrap, repeated in all trees and confirmed each other. The analyzed DNA sequences similarity of 110 isolates was more than 98%. The results of genomic fingerprinting and DNA sequencing of three loci confirmed each other. Although there are high variations and distinct phylogenetic groups among the Iranian isolates of *F. solani,* high degree of genetic similarity between four host populations presents that these populations are belonged to one clonal lineage.

PR3.27

Diversity of Fusarium Species in the Phytopathogenic Gibberella fujikuroi Complex in Iran

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Gibberella fujikuroi complex (GFC) which contains Fusarium section Liseola, is composed of at least ten reproductively biological species (mating populations) denoted by letters A through J. These Fusarium species causes destructive disease on different agricultural and horticultural plants and also produce harmful mycotoxins and secondary metabolites that contaminate animal and human feed and food worldwide. We examined 46 isolates collected from maize, rice, sugarcane and onion in different areas of Iran, on the basis of morphological and biological characteristics as well as phylogenetic analysis. Based on morphological characteristics, 45 Fusarium isolates identified as F. verticilioides, F. sacchari, F. fujikuroi, F. proliferatum, and F. thapsinum. Mating studies based on crosses of these isolates with tester strains of GFC belonging to mating populations A to I, revealed that the isolates were belonged to mating populations A (F. verticilioides), B (F. sacchari, C (F. fujikuroi), D (F. proliferatum) and F (F. thapsinum). The results presented accordance between morphological and biological characteristics. We sequenced portions of translation elongation factor 1 alpha (tef) protein coding gene as well as internal transcribed spacer (ITS) of the nuclear ribosomal genes of 22 isolates out of 46 isolates. Molecular sequence analysis revealed that 22 isolates representing all five species identified on the basis of morohological and biological analysis. There were an example of discordance between species identified based on phylogenetic analysis and morphogical/biological analysis. Isolates belonged to F. verticilioides from sugarcane identified as F. andiyazi and isolates belonged to F. verticilioides and F. proliferatum from rice identified as F. fujikuroi based on phylogenetic analysis. This study represents, six Fusarium species from GFC are already identified in Iran. In contrast with previous reports, F. fujikuroi is the only species that causes foot rot on rice in Iran.

PR3.28

Analysis of the lignocellulose-degrading transcriptome and systematics of the white rot basidiomycete *Phlebia radiata*

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Phlebia radiata Fr. is a saprobic basidiomycete species belonging to the family Corticiaceae in the class Agaricomycetes. It is able to cause a white rot type of decay both in dead hardwood (conifers) and softwood (angiosperms). The species is capable of degrading wood lignin and lignocellulose by secreting a number of lignin-modifying enzymes - class II heme-including peroxidases and laccases. Our molecular systematic analyses indicate that *P. radiata* is more near related to *P. acerina* than to most of the other species allocated in the genus *Phlebia*. As a result of the ongoing whole genome sequencing of the Finnish *P. radiata* isolate 79, we have generated over 1.7 million expressed sequence tags (ESTs) from cDNA constructed from malt extract liquid culture mycelia of *P. radiata*. ds-cDNA was made from purified polyA$^+$ RNA and ESTs were sequenced with 454 sequencer using GS FLX Titanium series reagents, and assembled using gsAssembler (v2.6) (454 Life Sciences). The 10 875 contigs and 6 064 isotigs were subjected to Galaxy Megablast analysis. The Blast results with E-value cutoff of 1x e^{-03}, we found hits to protein coding sequences of gene transcripts functioning in the plant cell wall biodegradation, i.e. to several laccases, manganese peroxidases, lignin peroxidases and cellobiohydrolases. Our data indicate that *P. radiata* expresses a versatile array of oxidoreductases and polysaccharide-degrading enzymes already when growing on complex liquid medium.

PR3.29

Ensembl Fungi: an integrative resource for genome-scale data from fungal species

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Ensembl Fungi (http://fungi.ensembl.org) is a portal offering access to genome-scale data from fungal species, using the Ensembl Genome Analysis system. Access to the data is provided through a common set of interfaces (shared with non-fungal species also represented in the Ensembl system) including a web-based genome browser, a Perl API, a public MySQL server and a query orientated data warehouse (BioMart). Core data provided for all species includes genome sequence, sequence patterns, annotation of protein and non-coding genes and functional annotation imported from direct curation, UniProt and InterPro. The platform also supports integration of additional information including regulation, variation and comparative analysis. The current releaseprovides access to 23 fungal genomes across 9 different taxonomical orders. Extensive comparative analyses are performed across all the fungal species, and across the wider taxonomy. Protein alignments are used to reconstruct evolutionary trees and infer homology relationships, while pairwise alignments between DNA sequences are performed between closely related species. Ensembl Fungi also provides access to polymorphism data in the context of reference genome sequences for *Saccharomyces cerevisiae* and the phytopathogen *Gibberella zeae*. Ensembl Fungi gives access to the manually curated data from the fungal model organisms *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, which are imported from Saccharomyces Genome Database and Pombase respectively, while genes from plant pathogen data is being integrated with information about infectious (derived from PHI-base (http://www.phibase.org) through a new targetted resource PhytoPath (http://www.phytopathdb.org).

PR3.30

Comparative analysis of Aspergilli to facilitate novel strategies in fungal biotechnology

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Aspergillus is not only one of the most important fungi in biotechnology, it is also one of the most commonly found groups of fungi in environments worldwide and one of the most severe opportunistic human pathogens. Due to its relevance for industry and medicine, and their use as model systems for fungal biology, *Aspergillus* has one of the largest research communities in the fungal field. Indeed, this has resulted in this genus being one of the most intensively studied fungi with respect to genomics, with genome sequences for eleven species publicly available. The availability of this set of genomes in combination with the tools developed for *Aspergillus* genomics (e.g. AspGD and CADRE) enables comparative genomics at a highly detailed level.

In 2011 the JGI approved sequencing of an additional 8 Aspergilli and Penicillia, to further advance comparative genomics in this group of fungi. These species include additional industrial species, but also species that are distantly related to those for which genome sequences are already available.

A large consortium of researchers has been established to perform comparative genomics on the newly generated and already available Eurotiales genomes (32 in total). In addition, this analysis will be supported with experimental data to validate the differences found through bioinformatics. Details on the project and current status will be presented.

PR3.31

Sequencing Of The *Taphrina betulina* Genome

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Taphrina are plant pathogenic yeasts, which cause the growth of tumors on mostly woody plant species. *Taphrina* are early diverging Ascomycetes, related to the model organism Schizosaccharomyces *pombe* and the human pathogen *Pneumocystis jirovecii*. There are a variety of *Taphrina* species reflecting the wide host range of this genus. Notable among them are the economically important peach leaf curl causing pathogen *T. deformans,* and the pathogen of the model tree poplar, *T. populina*. Also, *T. betulina* is a pathogen of birch trees and the causative agent of witch's broom that remodels the birch into tumorous brooms to create its living environment. Indeed, all of these *Taphrina* cause leaf curling and other tumor like developmental changes in their hosts. *Taphrina* are biotrophic plant pathogens, i.e. they depend upon evading defence responses in living host cells for their survival. We are developing the interaction between *T. betulina* and Birch (*Betula pendula*) into a model pathosystem. In order to discover genes for molecular work the genome of *T. betulina* is being sequenced. Our preliminary characterization of *Taphrina* species and analysis of the *T. betulina* draft genome will be presented.

PR3.32

The use of transposons as molecular tools for random mutagenesis in filamentous fungi

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The mobile and mostly also repetitive genetic elements named transposons are found in all eukaryotic genomes. Their impact on gene expression as well as their ability to cause major mutations within the genome or single genes makes them an interesting tool for random mutagenesis. Using transposable elements in filamentous fungi would aid gene characterization for instance in pathogenic or biotechnological important strains (1, 2, 3). At current we are employing two types of transposons, i.e. *Restless* from *Tolypocladium inflatum* (4) and *Vader* from *Aspergillus niger* (5). Each transposable element was inserted between the promoter and the open reading frame of the hygromycin phosphotransferase (*hph*) gene and transformed into *Neurospora crassa* or Aspergilli, respectively. We use hygromycin selection to identify transposition events and observed a *Vader* excision frequency of about 1 in 2.2 x 10^5 spores. All colonies analyzed showed an excision event on the DNA level. *Vader* footprints were found and after performing TAIL-PCRs the reintegration sites of 21 independent excision events were determined. *Vader* mostly integrates within or very close to genes, thus it appears to be a useful tool for transposon-mediated mutagenesis in *A. niger* (6). At current we try to improve the *Vader* tool and analyze its function in the heterologous host *A. nidulans.* Furthermore we analyse the performance of *Restless* in *N. crassa*.

1. Kempken F, Kück U (1998) *BioEssays* 20:652-659
2. Kempken F (2003) In: Arora DK, Khachatourians GG (eds) Applied Mycology and Biotechnology, Vol. 3 Fungal Genomics, Elsevier Science Annual Review Series, pp83-99
3.Pöggeler S, Kempken F (2004) In: Kück U (ed) The Mycota II, Genetics and Biotechnology, 2nd edition, Springer Verlag, Heidelberg, New York, Tokyo, pp165-198
4.Kempken F, Kück U (1996) *Mol Cell Biol* 16:6563-6572
5. Braumann I, van den Berg M, & Kempken F (2007) *Fungal Genet Biol* 44(12):1399-1414.
6. Hihlal E, Braumann I, van den Berg M, Kempken F (2011) *Appl Environment Microbiol*, 77: 2332–2336

PR3.33

Phytopath – A new bioinformatics resource for plant pathogens

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PhytoPath (http://phytopathdb.org), a new bioinformatics resource launched in 2012, integrates genome scale data from important plant pathogenic species with literature-curated information about the phenotypes of host infection. It provides access to complete genome assemblies, gene models, supporting alignments and genomic polymorphism from priority species of fungi and oomycete pathogens of crop and model plants (using the Ensembl genome browser interface). This information is directly linked to the peer-reviewed information about infectious phenotypes curated within the PHI-base (Pathogen-Host Interactions) database. Nine fungal and four oomycete pathogen genomes are available in the first release including Mycosphaerella graminicola, Puccinia graminis f. sp. tritici and Phytophthora infestans. New releases will be made at 2 monthly intervals. Comparative analysis is provided at many levels, including DNA and protein sequence alignments (between pathogens themselves, and with other fungal species) and the differential role of genes involved in pathogenesis in different hosts.

The PhytoPath project and the PHI-base National Capability are supported by grants from the Biotechnology and Biological Sciences Research Council (BBSRC).

PR3.34

Annotation of 8 Aspergillus genomes derived by the multi-genome Gnomon pipeline

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NCBI

Recent advances in genome sequencing technologies allow generating whole genomes for many organisms in a fast and cost-effective way. Accurate annotation of the genomes is still a challenge. Large-scale sequencing projects usually provide additional experimental data (EST, full-length cDNA) that can be utilized in the annotation process to improve the quality of gene models. More recently sequencing efforts are concentrated on pathogens and model organisms from Fungi and Protozoa organism groups and are focused on sequencing of genomes of closely related organisms for evolution, genetics and comparative studies. These genomes are relatively small but often lack additional transcript or protein data. Using comparative multi-genome approach can greatly improve the accuracy of gene prediction compared to single genome method. The multi-genome Gnomon approach allows utilizing the transcript and protein data from closely related organisms in a single multi-genome annotation run. This method starts from a single genome Gnomon gene prediction and then uses a comparative analysis among multiple genomes to gradually improve the annotation through an iterative process. At each iteration the best models are selected and used as a training set and evidence for the next step. Transcript and protein alignments are used to guide gene model predictions. The most recent version of Gnomon can utilize RNA-Seq data giving more support to the splice junctions. Eight *Aspergillus* genomes have been annotated simultaneously using this method. Four of these genomes have RNA-Seq data available. It was found that the multi-genome approach successfully used the indirect RNA-Seq data to improve the annotation of genomes without such data. The resulting annotation has proven to be more consistent across the genomes than the annotation of the individual genomes.

PR3.35

Fungal subtilisin-like serine proteases in a novel division into subfamilies

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Subtilisin-like serine proteases (subtilases) are a superfamily of enzymes covering different essential activities from removing the start methionin from a peptide sequence to degrading substrate proteins extracellularly. Due to the wide variety of roles, it is difficult to predict a function based on an amino acid sequence. Subtilases are widespread in fungi, and the growing number of sequenced fungal genomes makes it important to have better tools to analyze these enzymes. Here, we apply PPR, a new non-alignment based analytical approach defining protein subfamilies. It allows both prediction of function based on comparative analysis of sequence and the design of PCR primers that can be used to discover novel members of such subfamilies (Busk and Lange, 2011). We have assembled over 4000 subtilase sequences of various organisms and defined subfamilies that do not correspond to published, alignment-based subtilase subfamilies. We are currently investigating, whether fungi of specific physiological life forms are characterized by a specific set of subtilase PPR-subfamilies. Here, the focus lies on Basidiomycota, in particular wood rotting and mycorrhiza basidiomycetes. Our hypothesis is that such sets of subtilases will unravel functional information about PPR-subfamilies. As a consequence, determining the subfamilies of novel subtilisin-like serine proteases will facilitate their annotation, enabling a short cut to a functionally targeted experimental discovery strategy.

Busk P.K. and Lange L. (2011) A novel method of providing a library of n-mers or biopolymers. Patent application EP11152232.2.

PR3.36

Can mechanisms of host specificity in smut fungi be inferred from genome data?

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Smut fungi parasite more than 4000 flowering plants, including important crop plants like maize, sugar cane, sorghum grass and barley. Typically, smut fungi have a narrow host range, and only one host species is infected. In addition to the two published genomes of *Ustilago maydis* and *Sporisorium relianium* 5-1, both parasitizing maize, three additional smut genome sequences have been determined and manually annotated (*U. hordei* parasitizing barley, *S. reilianum* H2 parasitizing sorghum and *S. scitamineum* parasitizing sugar cane). The narrow host range and the genome availability of closely related species make smut fungi a particularly interesting model to study host specificity.

To identify putative genes involved in host specificity, we follow a computational approach relaying on two strategies. One strategy follows the idea that genes unique for each species (so called orphans) are involved in host specificity. The other idea is to build families of homologous proteins and to scan these families for signatures of species-specific positive selection. This is based on the idea that proteins serving as pathogenicity factors on a particular host will show high rates of adaptive substitutions, since they are involved in a co-evolutionary arms race between parasite and host. To identify candidates for both strategies, we built gene families and alignments based on similarity criteria and clustering techniques. The members of each family of interest were subsequently analyzed with regards to positive selection, ontology categorization, or potential secretion, respectively.

PR3.37

Fusion of two divergent fungal individuals led to the recent emergence of a new widespread pathogen species

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In genome sequences of five individuals of the ascomycete fungus *Zymoseptoria pseudotritici,* a close relative of the wheat pathogen *Z. tritici* (synonym *Mycosphaerella graminicola*), we observed peculiar diversity patterns. On aligned chromosomes long fragments up to 100 kilobases without variation alternate with similarly long fragments of high variability. The variable segments in the genome alignment are organized into two main haplotype groups, ~3% divergent from each other. The genome patterns in *Z. pseudotritici* are consistent with a hybrid speciation event resulting from a cross between two divergent haploid individuals, and the resulting hybrids formed the new species without backcrossing to the parents. The segments without variation is the result of a strong population bottleneck following the hybridization. We observe no variation in 54% of the genome in the five individuals and estimate a complete loss of variation for at least 25% of the genome in the entire species. Variable segments in the *Z. pseudotritici* genome represent the two haplotypes contributed by the parental individuals. From our previously estimated recombination map of *Z. tritici* and the size distribution of regions with two haplotypes we estimate that the hybridization occurred ~675 sexual generations ago. We show that the amount of variation lost is explained by genetic drift during the bottleneck, and by natural selection as evidenced by the correlation of variation presence/absence with gene density and recombination rate. The successful spread of this new reproductively isolated pathogen highlights the accelerating potential of hybridization in the evolutionary emergence of pathogen species with sexual reproduction.

PR3.38

Characterization of Fumonisin B_2 biosynthetic gene cluster in *A. niger* and *A. awamori*.

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A set of 43 *Aspergillus niger* and *A. awamori* strains isolated from grapes cultivated in Mediterranean area was tested to evaluate genetic differences in fumonisin B_2 (FB_2) biosynthetic gene cluster between toxin producing and non-producing strains.

The ability to produce FB_2 was related to *fum* genes occurrence, evaluated by PCR assyas, using primer sets designed to amplify fragments of *fum1*, *fum3, fum6, fum7, fum8, fum10, fum13, fum14, fum15,* and some relative intergenic regions.

A.niger and *A. awamori* FB_2 producing strains and *A. niger* FB_2 non-producing strains arose amplicon for all tested *fum* genes, while the *A. awamori* FB2 non-producing strains arose amplicon only for few of tested *fum* genes.
Maximum parsimony analysis based on the calmodulin gene sequences indicated that the presence/absence of *fum* genes in the isolates is not correlated with phylogenetic relationship among strains. This is the first report correlating the presence of multiple fumonisin biosynthetic genes with fumonisin production in *A. niger and A. awamori*. The results suggest that the absence of FB_2 production in *A. awamori* can result from the absence of at least one gene of the cluster, while in *A. niger* it should involve other regulator gene/s probably out of the cluster, or concern variations in a regulatory sequence essential for cluster expression.

PR3.39

Pooled-segregant Whole-genome Analysis Reveals Genetic Basis of High Thermotolerance in Yeast

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Thermotolerance of *Saccharomyces cerevisiae* has long been studied not only because of its industrial application potential, but also its nature as a complex trait that can be influenced by different genomic loci and strain backgrounds. In this study we have performed a genetic mapping of thermotolerance by using pooled-segregant whole-genome sequence analysis. A mixture of genomic DNA from a total of 58 thermotolerant segregants selected from a cross between a highly thermotolerant yeast strain and the control lab strain BY4742 has been sequenced by illumina technology. The ratio of SNPs that represents the abundance of the thermotolerant strain DNA versus the control strain DNA in this segregant population has been plotted against the SNP position along the chromosomes. Afterwards genotyping of the individual thermotolerant segregants has been performed at the loci that show a clear biased deviation from 50% in the plot, and based on this, an FDR statistic analysis has been applied which confirmed that four loci have a significant linkage to the phenotype. A previously identified gene (*MKT1*) located in the locus with the strongest linkage was also identified in this study as having a contribution to high thermotolerance by reciprocal hemizygosity analysis. In addition and surprisingly, one locus with a preference for BY4742 DNA has been confirmed as showing significant linkage. This implies that besides a possible genome dosage effect, the higher thermotolerance of the diploid compared to the haploid parents (thermotolernt haploid and BY4742) may be due to a contribution of its non thermotolerant parent (BY4742).

PR3.40

The FUNG-GROWTH database: linking growth to genome

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Fungal genome sequences demonstrate the potential to utilize a variety of different carbon sources. Natural carbon sources for many fungi are based on plant biomass and often consist of polymeric compounds, such as polysaccharides. They cannot be taken up by the fungal cell and are extracellularly degraded by a complex mixture of enzymes. Plant polysaccharide degrading enzymes have been studied for decades due to their applications in food and feed, paper and pulp, beverages, detergents, textile and biofuels. These enzymes have been classified based on amino acid sequence modules (www.cazy.org).

Based on the hypothesis that fungal genomes have evolved to suit their ecological niche, we have performed a comparative study using >120 fungal species. In this study we have compared growth profiles on 35 different carbon sources (consisting of mono-, oligo- and polysaccharides, lignin, protein and crude plant biomass) to the CAZy annotation of the genomes to identify correlations between growth and genomic potential.

Highlights of these comparisons will be presented as well as the public database in which the growth data is stored and the developments of the database anticipated for the next two years.

PR3.41

***Colletotrichum acutatum*: a model system for studing evolution in filamentous fungi**

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Colletotrichum acutatum is an important pathogen causing economically significant losses of crops and an interesting organism in its own right. *C. acutatum* has a wide plant host range in both crops and natural ecosystems, and its capability to infect different types of host, such as insects, has also been described. *C. acutatum* is able to develop three different types of interaction with plant host: covering biotrophic, necrotrophic and hemibiotrophic infections. It is also capable of growing as a non-pathogen. The life styles of *Colletotrichum* species can include sexual (both homothallic and heterothallic [teleomorph Glomerella]) and asexual states. Furthermore, sexual behaviour in Glomerella is more complicated than in most ascomycetes and strains within the same species do not show a typical MAT1-1/2 system. Globally, *C. acutatum* populations display considerable genotypic and phenotypic diversity. Our previous results suggest the existence of *C. acutatum* populations potentially undergoing speciation processes, related to their reproductive behavior and host association patterns
All this evidence and complexity suggest *C. acutatum* is a suitable system for studying evolution and speciation process trhough whole genome comparisons. One isolate has been sequenced by NGS and more are planned.

PR3.42

The role of cell wall degrading enzymes during the evolution of *Zymoseptoria tritici*

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Plant cell wall degrading enzymes (PCWDEs) of plant pathogens are receiving increasing interest for their potential to trigger plant defense reactions. In an antagonistic co-evolutionary arms race between host and pathogen, PCWDEs could be under strong selection. In a first population genetic study, we tested the hypothesis that PCWDEs in the fungal wheat pathogen *Zymoseptoria tritici* have been positively selected by analyzing ratios of non- synonymous and synonymous nucleotide changes in the genes encoding these enzymes. Analyses of five PCWDEs demonstrated that one (ß-xylosidase) has been under strong positive selection and experienced an accelerated rate of evolution. In contrast, PCWDEs in the closest relatives of *Z. tritici* collected from wild grasses did not show evidence for selection or deviation from a molecular clock. Since the genealogical divergence of *Z. tritici* from these latter species coincided with the onset of agriculture, we hypothesize that the recent domestication of the host plant and/or agricultural practices triggered positive selection in ß-xylosidase and that this enzyme played a key role in the emergence of a host-specialized pathogen. Using an extended second approach based on comparative genomics, we assessed molecular patterns of adaptation and/or selection of all orthologous PCWDEs on a population genomic scale between *Z. tritici* and its closest relatives.

PR3.43

Comparative molecular evolution of *Trichoderma* chitinases

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Certain fungi from the anamorphic genus *Trichoderma* are known for their ability to antagonize plant-pathogenic fungi and are therefore used as biocontrol agents in agriculture. In *Trichoderma*, whole-genome sequencing reveal between 20 and 36 different family 18 glycoside hydrolase (GH18) genes, and several of these genes have been shown to be induced during the mycoparasitic attack. Sequences of *Trichoderma* GH18 chitinase genes *chi18-5*, *chi18-13*, *chi18-15* and *chi18-17*, that all exhibit specific expression during mycoparasitism-related conditions, were determined from up to 13 different taxa and studied with regard to their evolutionary patterns. *Chi18-13* contained two codons that evolve under positive selection and seven co-evolutionary site networks. Regions of high amino acid variability were preferentially localized to substrate- or product side of the catalytic cleft. *Chi18-15* displayed a unique codon-usage and contained five codons that evolve under positive selection and three co-evolutionary site networks. Regions of high amino acid variability were preferentially localized to coil-regions adjacent to certain alpha-helixes, suggesting structural adaptations of enzyme architecture. In addition, differences in amino acid variability/conservation patterns, indicative of type 1 functional divergence, were observed between *Trichoderma* chi18-15 orthologs and a bacterial ortholog, *Streptomyces* chiJ. These observations show that *Trichoderma* chitinases *chi18-13* and *chi18-15* evolve in a manner consistent with rapid co-evolutionary interactions and identifies putative target regions involved in determining substrate-specificity and structural modifications of the family 18 chitinase TIM-barrel structure. Our results suggest that fungal/fungal interactions can drive adaptive changes in enzymatic properties as a response to specific ecological contexts of different *Trichoderma* species.

PR3.44

Decifering the mechanisms of aflatoxin formation through functional genomics in *Aspergillus flavus*

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Sequencing of *A. flavus* NRRL3357 showed that its 36-Mb genome contains 13,488 genes including predicted 55 secondary metabolite gene cluster. We sequenced cDNA fragments obtained from Poly(A)-enriched total RNA samples extracted from mycelium grown under 3 conditions: (i) PMS medium, 30 C, 24h, no toxin; (ii) GMS medium, 30 C, 24h, make toxin; and (iii) GMS medium, 37 C, 24h, no toxin. Two cDNA libraries from each treatment were sequenced using the Illumina (SOLEXA) short-read technology. Over 5 Million 100 nt reads were sequenced for each cDNA prep, which were combined to generate a powerful high resolution map of the *A. flavus* transcriptome. The analysis detected expression in at least 50 % of the genes for each condition and contributed to our understanding of the genetic basis of the aflatoxin regulation. This study demonstrates that the aflatoxin pathway gene cluster consisting of 30 genes are tightly regulated. High temperature turns down aflatoxin gene transcription by turning down transcription of the two regulatory genes, the *aflR* and *aflS* (old name: *aflJ*). Further, the change in gene expression ratio of *aflS* to *aflR* renders *aflR* non-functional for activation of aflatoxin pathway gene transcription.

PR3.45

Sequencing and assembly of a fungal genome for less than $1000?

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Next Generation Sequencing technology has opened up a whole way of looking at organisms, and it is now possible for a small lab to have the genome of a fungus completely sequenced, and the genes predicted for less than a couple of thousand dollars; however, access to high performance computing facilities as well as some biocomputational skills are required. The results of a project for sequencing the genomes of several ascomycetes is presented, as well as the use of the data to uncover mating type genes and infer the presence of sexual reproduction in populations.

PR3.46

Heterochromatin influences the secondary metabolite profile in the plant pathogen *Fusarium graminearum*

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Chromatin modifications and heterochromatic marks have been shown to be involved in the regulation of secondary metabolism gene clusters in the fungal model system *Aspergillus nidulans*. We examine here the role of HEP1, the heterochromatin protein homolog of *Fusarium graminearum*, for the production of secondary metabolites. Deletion of *Hep1* in a PH-1 background strongly influences expression of genes required for the production of aurofusarin and the main tricothecene metabolite DON. In the *Hep1* deletion strains AUR genes are highly up-regulated and aurofusarin production is greatly enhanced suggesting a repressive role for heterochromatin on gene expression of this cluster. Unexpectedly, gene expression and metabolites are lower for the trichothecene cluster suggesting a positive function of *Hep1* for DON biosynthesis. However, analysis of histone modifications in chromatin of AUR and DON gene promoters reveals that in both gene clusters the H3K9me3 heterochromatic mark is strongly reduced in the *Hep1* deletion strain. This, and the finding that a DON-cluster flanking gene is up-regulated, suggests that the DON biosynthetic cluster is repressed by HEP1 directly and indirectly. Results from this study point to a conserved mode of secondary metabolite (SM) biosynthesis regulation in fungi by chromatin modifications and the formation of facultative heterochromatin.

PR3.47

Effectors in fruit scab fungi: *Venturia inaequalis* and *V. pirina*: a comparative genomics approach

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The hemi-biotrophic fungi, *Venturia inaequalis* and *V. pirina,* cause scab disease on apples and pears respectively. The pathogens are host-specific, with non-overlapping host ranges: *V. inaequalis* is limited to infecting hosts within the Maloideae; and *V. pirina* infects pear (*Pyrus* spp). The genetics of the finer interactions between these fungi and their respective host cultivars follow the gene-for-gene model; effectors (pathogen proteins required for infection) are presumably secreted into the plant/pathogen interface during the infection cycle where a subset can be recognised by plant resistance gene products to induce a hypersensitive response. Whole genome sequencing of *V. inaequalis* and *V. pirina* isolates has been performed. Orthologues of several fungal effector genes were identified in both genomes; including *Ecp6*, *AvrLm6* and *Avr-Pita*. *AvrLm6* is represented by an expanded family of over 25 genes in both *V. inaequalis* and *V. pirina* (*Leptosphaeria maculans* has 2 orthologues). The *Venturia* AvrLm6 orthologues share up to 32% amino acid sequence identity with *L. maculans* AvrLm6. Most of these orthologues are also represented in the *V. inaequalis* transcriptome, with eight upregulated by more than two fold during infection. These, and other effector candidates, are currently being functionally characterized using gene silencing/disruption and GFP fusions. The secretomes, assessed using a proteomics and Illumina sequencing approach, of both pathogens are being compared to reveal *Venturia*-specific elicitors as well as species-specific elicitor candidates that may determine host range. Sequencing of the closely related *V. nashicola* as well as *formae speciales* of *V. inaequalis* from loquat is also planned.

PR3.48

The *Didymellaceae*: insights into the genomes of key pathogens of legume crops

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The *Didymellaceae* family includes some of the most important pathogens of legume crops: the causal agents of Ascochyta blight in chickpea, pea, lentil, and faba beans among others [1]. Despite their substantial economic impact, little is known about the molecular aspects of pathogenicity in these closely related species. At this meeting, the first genome assemblies of *Ascochyta rabiei*, *Peyronellaea pinodes* (syn. *A. pinodes*), and *Phoma medicaginis* (pathogen of the model legume *Medicago truncatula*) will be presented. Untrained *in silico* annotation resulted in the identification of 9,000-12,000 genes per species, of which 91-97% are complete gene models. The sequence data served to: pinpoint areas of synteny and clusters of genes associated with reproduction and adaptation; identify pathogenicity-related gene-candidates through proteomic, transcriptomic and *in silico* comparative analyses; and design DNA makers for diagnostics and studies in population structure. Among the pathogenicity related genes, we have identified potential necrotrophic effectors that seem to operate similarly to those found in *Stagonospora nodorum* [2]. Detailed understanding of the molecular mechanism involved in fungal adaptation in general, and pathogenicity in particular, is facilitating the development of novel tools and strategies in crop protection.

1. Aveskamp, M.M., et al. (2010) Highlights of the *Didymellaceae*: A polyphasic approach to characterise *Phoma* and related pleosporalean genera. Studies in Mycology **65**(1): p. 1-60.
2. Oliver, R. (2009) Plant breeding for disease resistance in the age of effectors. Phytoparasitica **37**(1): p. 1-5.

PR3.49

Intersterility loci of *Heterobasidion occidentale* and *H. irregulare* has increased recombination rates and affects virulence, speed of growth and wood decay

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The closely related basidiomycete species *H. occidentale* and *H. irregulare* are the major pathogens on industrial forestry of conifers in North America. Although being separate, intersterile species, a degree of interfertility has been observed. This is controlled by a set of five intersterility loci. Mutual + alleles in at least one of these loci are required for compatible mating between *Heterobasidion* mycelia to occur. Using a dense genetic linkage map, the positions for three intersterility loci have been determined. Cross-comparisons with other analyzed traits suggest these regions to be fundamental for the fungal life cycle, as they also carry QTLs for virulence, wood decay and speed of growth on agar medium. The linkage map further reveals that these regions have an increased recombination rate, between 10 and 20 times higher than the genome wide average. As has been described for other species, this could indicate that these regions are in fact flanked by spots for high recombination rate, to prevent recombination and disruption within the crucial regions themselves.

PR3.50

Host specialization in the *Rhynchosporium* genus – a genomics approach

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Based on phylogenetic criteria fungi of the *Rhynchosporium* genus were recently assigned to four species, each exhibiting a specific host spectrum. *R. secalis* grows on rye (*Secale cereale*) and triticale, *R. commune* on *Hordeum* species including cultivated barley and on brome grass (*Bromus* spp.), and *R. agropyri* on couch grass (*Agropyron* spp.). Previously, these morphologically indistinguishable species were all referred to as *R. secalis*. *R. orthosporum* constitutes the fourth species, which is characterized by a different spore shape and its host species, cocksfoot (*Dactylis glomerata*). DNA sequencing of five isolates from the four *Rhynchosporium* species yielded mitochondrial genomes of 69 kb (*R. orthosporum* 49 kb) and nuclear genomes of 50-55 Mb carrying about 12,000 genes. Following a comparative genomics approach, we are now aiming at identifying the factors that underlie host colonization and specialization. Using molecular and proteomics techniques along with targeted deletion we are currently focusing on genes encoding fungal effector proteins as well as polyketide synthesizing enzymes.

PR3.51

The *Hyaloperonospora arabidopsidis* species complex – a new model system for investigating the evolutionary and ecological genomics of plant pathogens

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The *Hyaloperonospora arabidopsidis* species complex contains about 10 phylogenetically and physiologically distinct lineages of obligate biotrophic oomycetes. These include H. arabidopsidis, the downy mildew pathogen of *Arabidopsis thaliana* and *H. cardaminopsidis*, which is parasitic to *Arabidopsis arenosa*. The genomes of both *Arabidopsis thaliana* and *H. arabidopsidis* are available, and this pathosystem has recently become a model system for investigating the molecular basis of plant-oomycete interaction, in which several resistance proteins and some corresponding effectors with avirulence activity have been described. However, little is known about the evolution of the pathosystem on biogeographic scale, and even less about the functional radiation of pathogens on unrelated hosts or their coevolution with closely related hosts.

Here we propose the *Hyaloperonospora arabidopsidis* species complex as a model to dissect the functional evolutionary dynamics of plant pathogens on a landscape and biogeography level.

During the past four years, extensive collections of plants and pathogens throughout the distribution range of *Arabidopsis thaliana* and the annual Brassicaceae *Microthlaspi perfoliatum* have been done, encompassing several hundred populations. The ongoing studies show positive selection comparing known effector genes from the two species, as well as intraspecific variation in both pathogens and hosts with respect to several traits, including pathogenicity and resistance, respectively.

Genome projects for both *H. thlaspeos-perfoliati* and *Microthlaspi perfoliatum* are underway, and first results already helped to reveal some pattern related to effector evolution that will be shortly discussed.

PR3.52

'Exploring the genome of Fusarium fujikuroi and related species with emphasis on secondary metabolism gene clusters'

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The fungi of the *Gibberella fujikuroi* species complex synthesize a vast array of secondary metabolites and specifically infect a broad spectrum of hosts.

In this work we present the fully annotated genome sequence of *Fusarium fujikuroi*. Due to the availability of other new sequenced genomes, representing every geographic subclade of the species complex, an extensive comparative approach with regards on unique features of the single species and subclades is possible. To determine the factors that contribute to host specificity we explored the make-up of PKS, NRPS, terpene and isoprenoid based secondary metabolite clusters.

Using DNA chip and ChIPseq experiment data we studied the expression of gene clusters under different environmental conditions as well as the influence of chromatin modifications. In addition we revealed transcription factor based regulation in predicting cluster specific transcription factor binding sites. Beyond these key players in plant-pathogen interaction other unique features like secreted proteins or unique transcription factors of the genomes are of interest.