PS3.1

Evolution of Obligate Parasitism in the White Rust Pathogen of *Arabidopsis thaliana*

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Biotroph evolution and molecular mechanisms of biotrophy in eukaryotic plant pathogens are poorly understood. To address evolutionary and mechanistic aspects of biotrophy we sequenced the genome of the obligate biotroph oomycete *Albugo laibachii*. Comparisons with other oomycete plant pathogens revealed independent loss of biosynthetic pathways while other proteins like HSP90 show an unexpected expansion. Biotrophic organisms require “effectors” to suppress host defence; we revealed RXLR and Crinkler effectors shared with other oomycetes, and furthermore discovered a novel class of effectors, the “CHXCs”.

A requirement for a biotrophic life style is an intimate complex haustorial interface with parasitized cells. Beside its role in nutrient uptake, little is known about structural features, protein content and protein trafficking. We used proteomics and cytological approaches to dissect the haustorial interface and revealed the accumulation of a HSP90 subclass within the extrahaustorial matrix.

We hypothesize that evolution of biotrophy involves a series of steps: step 1, involving progressively more effectors to suppress defence that might evolve from conserved proteins step 2, attenuated activation of defence by reduction in the inventory of cell wall hydrolyzing enzymes, resulting in, step 3, weak selection to maintain certain biosynthetic pathways if the products of the pathways can be obtained from the host. This results in progressively more comprehensive auxotrophy and culminates in irreversible biotrophy.

PS3.2

Harnessing Natural Genetic Variation to Elucidate the Relationship Between Genotype and Phenotype in *Saccharomyces paradoxus*

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A more thorough understanding of the causative relationship between genotype and phenotype is of great interest to many fields of biology. In the interests of improving our understanding of this relationship, we have pursuing several lines of research that have revealed specific phenotypic consequences resulting from natural genetic variation and divergence in the wild yeast *Saccharomyces paradoxus*. In one study, we use gene expression and population genetics tests to investigate the effects of natural selection on a group of co-regulated membrane protein genes in a European population of *S. paradoxus.* We provide evidence for expression level divergence of these membrane protein genes relative to other *Saccharomyces* species, and suggest that this divergence has been partially driven by the heightened selective pressures that we find to be acting on the promoters of these genes. In a second study, we investigate the genetic variants that underlie the invasive growth and flocculant phenotypes that are displayed by several members of this same *S. paradoxus* population. We find that these phenotypes are driven by distinct genetic variants that have arisen independently in several isolates from the population and represent a case of convergent evolution. These findings illustrate the potential for harnessing natural variation within and between wild populations to elucidate the relationship between genotype and phenotype.

PS3.3

Evolutionary Genomics Of Accessory Chromosomes In *Mycosphaerella graminicola*

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Fungal genomes evolve very rapidly through the acquisition of foreign genes, hybridization events and ectopic recombination during meiosis. One of the most striking aspects of genomic diversity in fungi is the presence of accessory chromosomes (also termed supernumerary or dispensable). Accessory chromosomes are defined as chromosomes that are specific to a subset of isolates from one species. Accessory chromosomes were found to follow separate evolutionary trajectories due to horizontal transfer and extensive rearrangements during meiosis. A growing number of pathogenic fungi are recognized to carry accessory chromosomes and harbor genes involved in virulence on these chromosomes. The epidemic threat of an emerging pathogen may, hence, depend on the evolutionary dynamics of its accessory chromosomes. To better understand these processes, we studied *Mycosphaerella graminicola*, a major leaf pathogen of wheat harboring the highest known number of accessory chromosomes. We performed whole-genome resequencing of a *M. graminicola* population to identify polymorphisms among the eight known accessory chromosomes. All accessory chromosomes showed significant length variation due to large segmental deletions, including complete absence of certain chromosomes. Furthermore, we performed PCR assays at <100kb intervals along the chromosomes to assess presence-absence of chromosomal segments in a global collection. We found population-specific patterns in segmental deletions and differences in frequencies of accessory chromosomes among populations. We extended the genome re-sequencing and PCR assays to progeny from several controlled crosses and found that meiosis generated a substantial proportion of population-level variation. High degrees of sexual reproduction likely maintain the enormous plasticity in accessory chromosomes of *M. graminicola*.

PS3.4

Genome sequence of Shiitake mushroom *Lentinula edodes* and comparative mushroom genomic analyses

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Lentinula edodes, Shiitake mushroom, is one of the most important cultivated mushrooms and wood-degrading fungi. We have been performing genomic analyses of the *L. edodes* monokaryon L54A. The L54 genome was sequenced using Roche 454 and ABI SOLiD sequencing platforms. There are 13,382 predicted protein-coding genes. We constructed a high-density genetic linkage map using the high-quality genetic variations among a mapping population of haploid basidiospores of dikaryon L54. For computational analysis of mushroom genomes, we compiled the genome sequences of *L. edodes* and other fungi into a mushroom genome analysis platform. Comparative mushroom genomic analyses revealed conserved genes in mushroom genomes, including putative regulators, protein-binding proteins and transcription factors. BTB, Fbox, paracaspase and RING domain proteins expanded in mushroom-forming basidiomcyetes. The unique composition of plant biomass-degrading enzymes in *L. edodes* genome was revealed. There are both laccases and peroxidases for lignin degradation. Multiple polysaccharide-degradation enzyme families also expanded, including glycoside hydrolase families which target beta-glucans and pectin. Our works provide insights into the molecular mechanism of mushroom development. The *L. edodes* genome sequence, genetic map and comparative genome analysis platform are key resources for the mushroom research community.

PS3.5

Comparative genomics of *Fusarium pseudograminearum* and other cereal fungal pathogens

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Fusarium pseudograminearum is a pathogen widely associated with crown rot in wheat and barley. We have sequenced the genome of an *F. pseudograminearum* isolate using paired-end short read technology at 180 fold coverage. Comparison of the predicted proteins to those of the genomes of a range of other cereal pathogens, dicot pathogens and saprophytes was undertaken using a reciprocal BLASTp analysis pipeline. This revealed genes that have strong orthologues only in specific cereal pathogens, and suggests multiple horizontal transfer events may have occurred in the evolution of cereal pathogens. One example gene encodes a putative amidohydrolase enzyme with orthologous sequences only detected in *F. pseudograminearum* and *Phaeosphaeria nodorum,* cause of glume blotch disease, but not in any other fungal genome, with the next closest matches all from bacteria. Deletion of this gene from *F. pseudograminearum* resulted in a reduction in virulence on barley and virulence could be restored by expression of the wild type coding sequence, indicating this is a novel virulence gene. Population surveys suggest the gene has been present in both *F. pseudograminearum* and *Phaeosphaeria* lineages for a long time and most probably was independently acquired by both species, possibly from bacteria. Its presence in these two otherwise unrelated pathogens suggests a role for this gene in a common pathogenesis mechanism that targets an important defence pathway in cereals. This study demonstrates that a comparative genomics approach can identify novel events that have influenced the evolution of fungal pathogenesis on cereal crops

PS3.6

Comparative genomics of basidiomycetes telomere and subtelomere regions.

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Telomeres are complex nucleoprotein structures found at chromosome ends and essential for their physical integrity. Telomeres are formed by a species-specific number of repetitions of a conserved sequence unit, a distal domain containing tandem repeated motifs and a proximal domain containing less repeated sequences and clusters of related genes. Because of their structural characteristics, telomeres are underrepresented in most assembled genomes and they must be characterized using dedicated molecular and bioinformatics strategies.

We are interested in determining which genes are preferentially placed at the subtelomeric regions, to study the effect of this position on their expression and to correlate it with the physiological and ecological characteristics of the organism. For this purpose, we have analyzed the genomes of several basidiomycetes involved in environmental and other biotechnological processes sequenced by the JGI.

We found that the basic telomere repetitive unit as well as its copy number varied among species. Gene density at the subtelomeric regions ranged from less than 0.20 to 0.49 genes per Kbp. Synteny analysis of these regions using *Pleurotus ostreatus* as a reference, revealed that seven of the 12 *P. ostreatus* chromosomes harboured gene models also found in the subtelomeric regions of other basidiomycetes, the subtelomeric chromosome regions were statistically enriched in specific gene sets, and that a mosaic of modules of subtelomeric genes described in other basidiomycetes was identified at *P. ostreatus* chromosome 4. These facts suggest that the ecologic niche of a species could be the responsible for the movement of subtelomeric chromosome specific genes to core chromosome locations.

PS3.7

Comparative genomics of Cochliobolus cereal pathogens: the core and pan genome

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Cochliobolus is a species-rich genus of taxa that have caused devastating losses to US agriculture. The superpathogens, *Cochliobolus heterostrophus* (host/corn), *Cochliobolus carbonum* (corn), *Cochliobolus victoriae* (oats), *Cochliobolus sativus* (cereals), and *Cochliobolus miyabeanus* (rice), form a tight phylogenetic group, suggesting a progenitor gave rise, over a short period, to this series of distinct biotypes, each distinguished by unique pathogenic capability to particular plants. Working with the Joint Genome Institute and its Fungal Genomics Program, we have sequenced, assembled, and compared genomes of all of these species. The close relationship has allowed characterization of a core and pan-genome, consisting of species-unique and conserved regions. We have inventoried genes for secondary metabolism and secreted proteins, in particular, as these are candidate virulence effector molecules. Included in these categories are secondary metabolite and protein host selective toxins (HSTs), the calling-card of Dothideomycete necrotrophs, known to confer hypervirulence on susceptible hosts. This project provides insights into how unique genomic regions contribute to virulence, and thus how new pathogens emerge.

PS3.8

A Dynamin-like Protein Affects Both RIP and Premeiotic Recombination

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Repeat-induced point mutation (RIP) and premeiotic recombination affect gene-sized duplications in many filamentous fungi. RIP causes G:C to T:A transition mutations while premeiotic recombination can result in loss of repeated DNA segments (J. Galagan and E. Selker, 2004). Both processes occur after fertilization but prior to meiosis and can be very efficient, in some cases mutating and/or deleting the duplication in essentially every nucleus. At least in *Neurospora crassa*, RIP has countered the expansion of gene and transposon families (E. Selker, 1990), suggesting that genome streamlining and protection from transposition events may yield long-term benefits to Neurospora populations. We employ genetic approaches to elucidate the mechanism of premeiotic recombination and RIP. Here we report the successful identification of semi-dominant mutations that affect both of these processes by using UV mutagenesis, followed by a screen for reduced RIP of linked duplications of *hph* and *pan-2*. Classical genetic mapping and complementation tests revealed that a mutation in the histone H3 gene,*hH3*$^{dim-4}$, is responsible for greatly reduced RIP of one mutant. We identified two additional mutations by bulk segregant analysis and high-throughput Illumina sequencing. Single point mutations were found in the same gene, encoding a novel dynamin-like long GTPase, albeit in different conserved domains. Both premeiotic recombination and RIP frequencies are affected, supporting the idea that these processes are mechanistically linked. To investigate this further, we are screening the Neurospora single gene deletion collection for mutants that show RIP defects, starting with deletion mutants that are known or expected to affect recombination pathways.

Sunday 1 April

Parallel session 4: Organismic Interactions

PS4.1

ß-1,3-glucan synthase of the maize anthracnose fungus *Colletotrichum graminicola* is essential at specific stages of pathogenesis

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Covalently cross-linked β-1,3-glucan and chitin are the most prominent and morphogenetically relevant carbohydrate polymers of fungal cell walls. In most filamentous fungi several chitin synthases, but only a single β-1,3-glucan synthase contribute to forming the glucan-chitin core. While the role of individual chitin synthase genes in pathogenic development has been analyzed in several plant pathogenic fungi, functional analyses of β-1,3-glucan synthase genes, encoding the catalytic subunit of the β-1,3-glucan synthase complex, is lacking.

We investigated the role of the β-1,3-glucan synthase gene (*GLS1*) in infection structures of the maize pathogen *Colletotrichum graminicola*. Infection assays with a *GLS1*:*eGFP* replacement strain, in combination with aniline blue fluorochrome-staining, showed that massive β-1,3-glucan synthesis occurs in conidia, appressoria and necrotrophic hyphae, but, surprisingly, not in biotrophic hyphae. As targeted deletion of *GLS1* was lethal, we employed RNA interference (RNAi) to generate transformants gradually differing in *GLS1* transcript abundance. Appressoria of RNAi strains had reduced turgor pressure, elastic, inefficiently melanized cell walls, and many of these infection cells exploded spontaneously. Due to loss of appressorial adhesion, penetration of intact maize leaves did not occur and normally shaped biotrophic primary hyphae formed on the maize cuticle. In wounded leaves, only necrotrophic hyphae were found, as indicated by strains carrying biotrophy- and necrotrophy-specific promoters controlling expression of *eGFP*. Necrotrophic hyphae formed by RNAi strains in the host tissue were severely distorted, hyper-melanized, and unable to cause spreading disease. Our studies suggest that *GLS1* is essential in appressoria and fast growing necrotrophic, but not in biotrophic hyphae of *C. graminicola*.