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Key words: adaptation, forestry, genome evolution, genomics, woody plant.

Fungal and oomycete genes galore

Fungal Genomics workshop: Plant and Animal Genome XV Conference, San Diego, CA, USA January 2007

The popular Plant and Animal Genome Conference (http://www.intl-pag.org/) has long been relatively well attended by microbiologists, in part because funding agencies often hold their awardee meetings concurrently (one instance is the NSF/USDA-CSREES Microbial Genome Sequencing awardees workshop). However, since the now defunct Agricultural Microbe Genomes meeting, last held in 2001 (Kamoun & Hogenhout, 2001), there have been few sessions in the program devoted to microbial genomics. This is no longer the case, with the Plant and Animal Genome Conference now hosting the new Fungal Genomics workshop. Although the workshop was scheduled as the last session of the conference (following the Host–Microbe Interactions workshop and just before the banquet and dance party), it kicked off with a roar, as mycologists now have access to dozens of genome sequences, and mycology has, in effect, moved into the postgenomics era (Galagan *et al.*, 2005).

The objective of the new workshop is to go beyond generating the sequences to discuss what can be done next, particularly in comparative genomics and global functional analyses. Fungi have more sequenced genomes than any other eukaryotic kingdom, and unlike those of most other eukaryotes, many fungal genomes also are being finished, resulting in a complete accounting of every base. Thus, the new workshop is timely and will hopefully provide a broad forum for the exchange of ideas on how these genomic resources can advance our understanding of fungal biology. For the purpose of the workshop, 'fungus' was defined broadly to include oomycetes and other fungal-like organisms. This first collection of talks, highlighted here, succeeded in addressing the objective of illustrating how genome sequences can be exploited to further our understanding of fungal biology. A list of the organisms under discussion is shown in Table 1.

'... oomycetes may have acquired fungal genes through horizontal gene transfer ...'

Oomycetes

In a somewhat ironic twist, the opening talk focused on oomycetes, a group of organisms that are not 'true fungi' but are phylogenetically related to brown algae and diatoms. However, oomycetes share some superficial traits with fungi, such as filamentous growth and heterotrophic lifestyle, and the oomycete research community has long interfaced closely with the fungal community. Recent findings from Nick Talbot's lab (University of Exeter, UK) suggest that oomycetes may have acquired fungal genes through horizontal gene transfer (Richards *et al.*, 2006), so perhaps the affinity between fungi and oomycetes is more than superficial.

Sophien Kamoun (Ohio State University, Wooster, OH, USA) provided an update on the five oomycete genomes that have been sequenced to date (Tyler *et al.*, 2006) (Table 1). The *Phytophthora* species sequenced are among the most notorious plant pathogens. *Phytophthora capsici*, *P. infestans*, and *P. sojae* infect diverse crops, such as pepper, potato, and soybean, respectively. *Phytophthora ramorum* affects native woody plants, resulting in environmental damage, and the downy mildew, *Hyaloperonospora parasitica*, a pathogen of *Arabidopsis thaliana*, figures prominently in research on this model plant. Kamoun focused on one class of oomycete effectors, the so-called RXLR genes that are delivered inside plant cells to manipulate host defenses (Kamoun, 2006). Using computational approaches, genome-wide catalogs of the RXLR effectors were produced, unravelling a complex and divergent set of hundreds of candidate effector genes. Recent work has involved testing for selection among families of recently duplicated paralogous genes and this has provided evidence for positive selection in more

Table 1 List of fungal and oomycete genomes discussed at the Fungal Genomics workshop and their associated web resources

Species	Taxonomy	Genome size (Mbp)	Estimated number of genes	Resources
Alternaria brassicicola	Ascomycetes, Pleosporales	30.3	–	Washington University (http://genome.wustl.edu/genome.cgi?GENOME = Alternaria%20brassicicola)
Aspergillus flavus	Ascomycetes, Eurotiales	36.8	12 497	http://www.aspergillusflavus.org/)
Aspergillus oryzae	Ascomycetes, Eurotiales	36.7	12 735	http://www.bio.nite.go.jp/dogan/MicroTop?GENOME_ID = ao)
Botrytis cinerea B05.10	Ascomycetes, Helotiales	38.8	16 448	Broad (http://www.broad.mit.edu/annotation/genome/botrytis_cinerea/Home.html)
Botrytis cinerea T4	Ascomycetes, Helotiales	–	–	Genoscope, in progress with expected release during 2007)
Fusarium graminearum	Ascomycetes, Hypocreales	36.2	11 640	Broad (http://www.broad.mit.edu/annotation/genome/fusarium_graminearum/Home.html)
Fusarium verticillioides	Ascomycetes, Hypocreales	41.7	14 179	Broad (http://www.broad.mit.edu/annotation/genome/fusarium_verticillioides)
Hyaloperonospora parasitica	Oomycetes, Peronosporales	75	NA	Washington University (http://genome.wustl.edu/pub/organism/Fungi/Hyaloperonospora_parasitica/assembly/draft/Hyaloperonospora_parasitica-2.0/)
Laccaria bicolor	Basidiomycetes, Agaricales	64.9	20 614	JGI (http://genome.jgi-psf.org/Lacbi1/Lacbi1.home.html)
Mycosphaerella fijiensis	Ascomycetes, Dothideales	73.4	–	JGI (in progress with expected release during 2007)
Mycosphaerella graminicola	Ascomycetes, Dothideales	41.9	11 395	JGI (http://genome.jgi-psf.org/Mycgr1/Mycgr1.home.html)
Nectria haematococca	Ascomycetes, Hypocreales	52.4	16 237	JGI (http://genome.jgi-psf.org/Necha1/Necha1.home.html)
Phanerochaete chrysosporium	Basidiomycetes, Aphyllophorales	30	10 048	JGI (http://genome.jgi-psf.org/Phchr1/Phchr1.home.html)
Phycomyces blakesleeanus	Zygomycetes, Mucorales	55.9	14 792	JGI (http://genome.jgi-psf.org/Phybl1/Phybl1.home.html)
Phytophthora capsici	Oomycetes, Peronosporales	65	12 011	JGI (in progress with expected release during 2007)
Phytophthora infestans	Oomycetes, Peronosporales	240	NA	Broad (http://www.broad.mit.edu/annotation/genome/phytophthora_infestans/Home.html)
Phytophthora ramorum	Oomycetes, Peronosporales	65	15 743	JGI (http://genome.jgi-psf.org/Phyra1–1/Phyra1–1.home.html)
Phytophthora sojae	Oomycetes, Peronosporales	95	19 027	JGI (http://genome.jgi-psf.org/Physo1–1/Physo1–1.home.html)
Pichia stipitis	Ascomycetes, Saccharomycetales	15.4	5841	JGI (http://genome.jgi-psf.org/Picst3/Picst3.home.html)
Sclerotinia sclerotiorum	Ascomycetes, Helotiales	38	14 522	Broad (http://www.broad.mit.edu/annotation/genome/sclerotinia_sclerotiorum/Home.html)
Stagonospora nodorum	Ascomycetes, Pleosporales	37.1	16 597	Broad (http://www.broad.mit.edu/annotation/genome/stagonospora_nodorum/Home.html)

–, not complete or NA, not available.

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than two-thirds of the examined RXLR effector families. This positive selection has, for the most part, targeted the C-terminal region of these effectors consistent with the view that this domain functions inside plant cells and may have been involved in a coevolutionary arms race with host factors. Kamoun also reported on functional analyses, namely applying high-throughput screens to discover effectors with avirulence activities, and this offered Kamoun the opportunity to contribute 'effectoromics' to the ever-expanding list of 'omics' terms.

Mycosphaerella

Gert Kema (Plant Research International, Wageningen, the Netherlands) moved the focus of the workshop on to the 'true fungi' by introducing the plant pathogens *Mycosphaerella fijiensis* and *M. graminicola*, members of the phylum ascomycota. *M. fijiensis* is the causal agent of Black Sigatoka disease, the most serious constraint to banana production worldwide, resulting in this pathogen receiving the highest fungicide application of all pathogens globally. Similarly *M. graminicola* causes septoria tritici blotch, one of the most economically important diseases of wheat worldwide and the largest target for fungicides on this crop. Both of these fungi are in the class Dothideomycetes, a group that includes several other important fungi with sequencing projects, for example *Stagonospora nodorum*, *Pyrenophora tritici-repentis* and *Alternaria brassicicola*. The *M. graminicola* genome assembly was greatly enhanced by an exhaustive genetic map comprising more than 2200 markers, almost 2000 of which have been sequenced, on 23 linkage groups. Strains of this fungus bear from 18 to 21 chromosomes, among the highest numbers reported among ascomycetes, and also carry some of the smallest chromosomes. However, these are not dispensable as they undergo regular meiosis and do not appear to carry an excessive amount of repetitive DNA. The high-quality genome assembly is close to completion, with the few remaining gaps corresponding mostly to centromeres. The genetic map combined with the high-quality sequence enabled mapping of nine quantitative loci for several traits, including cultivar and host specificity. This pathogen carries a smaller arsenal of cell wall-degrading enzymes compared with species of *Aspergillus* and *Botrytis*, suggesting that it might behave more like a biotroph than a necrotroph, especially during the early stages of pathogenesis. Functional analyses have been initiated, for example, the *MgHog1* gene is required for filamentous growth on plant surfaces, and a knockout hampers penetration of the host (Mehrabi *et al.*, 2006). Kema also reported on the 7.1× draft genomic sequence of *M. fijiensis*, which has a genome that is *c.* 80% larger and more complex compared with *M. graminicola*, indicated by a double peak of GC content and a higher frequency and diversity of transposable elements.

Mycotoxins: aflatoxin and fumonisin

Continuing with the ascomycetes, Gary Payne (North Carolina State University, Raleigh, NC, USA) focused his talk on comparative genomics of two closely related aspergilli that exhibit distinct ecologies (Payne *et al.*, 2006). *Aspergillus flavus* is a soil saprophyte that can be an opportunistic pathogen in humans and plants such as maize. This pathogen is economically important because it produces the carcinogenic toxin aflatoxin. *Aspergillus oryzae* is a domesticated fungus that is also economically important because of its widespread use, particularly in Japan, in the preparation of popular food products such as soy sauce and sake. Obviously, *A. oryzae* is safe for food consumption; it is not known to be pathogenic, nor does it produce aflatoxin. Interestingly, the two genomes turned out to be very similar, lending credence to the long-held view that *A. oryzae* is a domesticated strain derived from *A. flavus*. Payne's initial comparative analyses focused on genes for secondary metabolism, one of the most astonishing features of fungi (Kroken *et al.*, 2003). Remarkably, *A. oryzae* has retained an inactive aflatoxin biosynthesis cluster orthologous to that of *A. flavus* at the distal end of chromosome 3. Both *A. oryzae* and *A. flavus* have larger numbers of polyketide synthases (*pks*) and nonribosomal peptide synthetases (*nrps*) relative to other aspergilli. Approximately two-thirds of the *pks* genes have the same splice site; the remaining third have differing gene structure and, interestingly, one *pks* gene in *A. flavus* appears to be deleted in *A. oryzae*. The next question that Payne and his collaborators are tackling focuses on whether the observed differences are species- or strain-dependent. This will be performed using a combination of PCR analysis, oligonucleotide arrays, and Affymetrix GeneChip analyses.

Another fungus which produces mycotoxins and is also a pathogen of maize is *Fusarium verticillioides* (*Gibberella moniliformis*). Fumonisins (FB1) are a group of mycotoxins that are renowned for causing a debilitating form of brain cancer in horses and esophageal cancer in humans. Because these mycotoxins are so harmful to human and animal health, they are highly regulated such that contaminated grain cannot be marketed. In addition to *F. verticillioides*, genomes of three other *Fusarium* species (*F. graminearum*, *F. oxysporum* and *F. solani*) will be publicly available. Assembly of the *F. verticillioides* genomic sequence and automated annotation were completed recently and 87 000 ESTs have also been sequenced. Won-Bo Shim (Texas A & M University, College Station, TX, USA) presented research using the genome to study secondary metabolism in *Fusarium* species. Analyses of the sequences have revealed that the fumonisin (*FUM*) gene cluster is a 15-gene regulon with the PKS encoded by *FUM1*. In total, there are 15 gene clusters that harbor PKS genes in *F. verticillioides*, two of which, *PGL1* (pigment production in perithecia) and *GzFUS1* (common mycotoxin), are

highly conserved with *F. graminearum*. Five clusters, including *FUM*, are unique to *F. verticillioides* among the sequenced *Fusarium* spp. Shim also reported that the regulation of FB1 production occurs coordinately with conidiation, and, using *fcc1*, a mutant in a C-type cyclin gene that is affected in FB1 production, has identified genes that are coregulated with the *FUM* genes. Gene knockouts have revealed both positive and negative regulatory genes. The positive regulators include *GBB1*, a heterotrimeric G protein beta subunit, a C2H2 transcription factor, and a hypothetical cell membrane named *CMG1*. The negative regulators are a monomeric G protein, *GBP1* (Sagaram *et al.*, 2006), and *CPP1*, a protein phosphatase 2A catalytic subunit.

Sclerotinia

As with the *Fusarium* genome sequence, a joint community annotation effort has been under way to map the genome of the necrotrophic and broad-host-range pathogen *Sclerotinia sclerotiorum*. However, this is a homothallic fungus, and therefore a genetic map could not be generated to guide the genome assembly. Instead, an optical map was used to validate and improve the assembly of the 9.9× draft genomic sequence. The mapping of *S. sclerotiorum* together with *Botrytis cinerea*, a closely related pathogen for which the genomes of two strains have been sequenced, has more than 40 annotators. Jeff Rollins (University of Florida, Gainesville, FL, USA) reported on how he is using the genome to empower his own research on the regulation of multicellular patterns and cell fate in fungal fruiting bodies (apothecia). For instance, the genome sequence is facilitating the genetic analysis of the effect of light on apothecial development. Genes for putative photoreceptors, such as the *white collar* ortholog and a cryptochrome-encoding gene (*cry1*), are being functionally characterized through gene-replacement experiments. The automated gene-call prediction identified 14 522 genes in the *S. sclerotiorum* genome. This autocalled gene set has been used as the foundation for producing Agilent microarrays. Initial hybridizations with these arrays will focus on validating probe design and investigating the regulation of apothecial development.

Genome annotation

The final presentation of the workshop was given by Igor Grigoriev (Joint Genome Institute (JGI), Walnut Creek, CA, USA), who has overseen the annotation of many fungal and oomycete genomes, most recently the zygomycete *Phycomyces blakesleeanus*. The JGI annotation pipeline involves automated gene calls followed by manual curation by community members. So far, more than 15 000 fungal genes have been curated manually by over 150 annotators. JGI typically sequences ESTs to aid the annotation process and is constantly looking for additional data to enhance genome annotation. ESTs, proteomics, and microarrays not only help to validate predicted genes but also enable various community-driven activities. Grigoriev showed a number of examples, including preliminary results on metabolic reconstruction for *Pichia stipitis*, microarray analysis for *Laccaria bicolor*, and proteomics studies in *Phanerochaete chrysosporium*. Comparative genomics can also be used to explore genome organization, as was done for *Nectria haematococca*, and study synteny, as in *P. sojae* and *P. ramorum* (Tyler *et al.*, 2006). He also reported on the pioneering approach of combining traditional Sanger dideoxy sequencing with novel methods such as 454 sequencing, as currently performed for the *Phytophthora capsici* project. A future emphasis at JGI will include developing a critical mass of data and tools for comparative genomics. Finally, Grigoriev reiterated his invitation to all interested scientists to join in web-based community annotations (http://genome.jgi-psf.org/euk_cur1.html).

Perspectives

In retrospect, mycologists should be grateful for the visionary decision by the Broad Institute (at that time, Whitehead Institute) to launch the Fungal Genomics Initiative in 2000 (http://www.broad.mit.edu/annotation/fungi/fgi/index.html) and to the American Phytopathological Society (APS) for its decision to follow up with a plant-associated microbial sequencing initiative during 2003 (Leach *et al.*, 2003). These initiatives had a catalytic effect on the research community's interest in genomics, encouraged funding agencies to invest in fungi, and undoubtedly raised interest among other genome centers, such as JGI and Washington University Genome Center, to take up fungal genome projects. Among the 10 fungi or oomycetes on the APS high-priority sequencing list from 2003 (http://www.apsnet.org/media/ps/MicrobialGenomicsSeqFinal03.pdf), nine are now sequenced or in the pipeline. A more recent boost to the sequencing of fungal genomes was provided by the Community Sequencing Program (http://www.jgi.doe.gov/CSP/index.html) of the JGI that began during 2005. This program provides free sequencing of organisms suggested by the scientific community.

Sequencing fungal genomes has had a notable positive influence on genome science, enabling genomicists to hone their techniques with smaller eukaryotic genomes before tackling larger projects. The time has now come when discoveries from these fungal and oomycete genomes are improving our understanding of these fascinating organisms, which reinforces a sense of excitement about the years to come.

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Polyploidy: genome obesity and its consequences

Polyploidy workshop: Plant and Animal Genome XV Conference, San Diego, CA, USA, January 2007

Polyploidy is a major evolutionary feature of many plants and some animals (Grant, 1981; Otto & Whitton, 2000). Allopolyploids (e.g. wheat, cotton, and canola) were formed by combination of two or more distinct genomes, whereas autopolyploids (e.g. potato, sugarcane, and banana) resulted from duplication of a single genome. Both allopolyploids and autopolyploids are prevalent in nature (Tate *et al.*, 2004). Recent research has shown that polyploid genomes may undergo rapid changes in genome structure and function via genetic and epigenetic changes (Fig. 1) (Levy & Feldman, 2002; Osborn *et al.*, 2003; Chen, 2007). The former include chromosomal rearrangements (e.g. translocation, deletion, and transposition) and DNA sequence elimination and mutations, whereas epigenetic modifications (chromatin and RNA-mediated pathways) give rise to gene expression changes that are not associated with changes in DNA sequence. Over time, polyploids may become 'diploidized' so that they behave like diploids cytogenetically and genetically. Comparative and genome sequence analyses indicate that many plant species, including maize, rice, poplar, and *Arabidopsis*, are recent or ancient diploidized (paleo-) polyploids.

The consequences of polyploidy have been of long-standing interest in genetics, evolution, and systematics (Wendel, 2000; Soltis *et al.*, 2003). Research interest in polyploids has been renewed in the past decade following the discovery of multiple origins and patterns of polyploid formation (Soltis *et al.*, 2003) and rapid genetic changes in resynthesized allotetraploids in *Brassica* (Song *et al.*, 1995) and wheat (Feldman *et al.*, 1997). Rapid technological advances have also facilitated genomic-scale investigation of polyploids and hybrids (Wang *et al.*, 2006). Many ongoing studies are focused on investigation of: (i) the evolutionary consequence of gene and genome duplications in polyploids; (ii) genomic and gene expression changes in resynthesized allotetraploids; (iii) genetic and gene expression variation in natural populations of polyploids; and (iv) comparison of genetic and gene expression changes in resynthesized and natural polyploids (Wendel, 2000; Osborn *et al.*, 2003; Soltis *et al.*, 2003; Comai, 2005; Chen, 2007). The presentations given at the Polyploidy workshop, Plant and Animal Genome XV Conference (http://www.intl-pag.org/), reflected these current research themes, reporting on ancient polyploidy events in *Glycine*, expression evolution of duplicate genes in *Arabidopsis*, gene expression changes in resynthesized *Brassica* and wheat allopolyploids, hybridization barriers in *Arabidopsis*, and tissue-specific and stress-induced expression patterns of duplicate genes in cotton and hybrid *Populus*.

'... expression of duplicate genes in response to developmental programs is more strongly correlated than that of duplicate genes in response to environmental stresses, suggesting rapid evolution of duplicate genes in response to external factors'

