

Meetings

Bridging mycorrhizal genomics, metagenomics and forest ecology

6th New Phytologist Workshop, in Nancy, France, November 15–16, 2012

After an initial lag phase dedicated to the sequencing of easily cultured saprotrophic fungi (among the first three published genomes were the models *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe* and *Neurospora crassa*) and species of biomedical, agricultural or biotechnological interest, genomics is now poised to rapidly permeate the fields of fungal ecology and evolution. As regards mycorrhizal associations, the story begins in 2008 with the publication of the genome of the agaric *Laccaria bicolor* (Martin *et al.*, 2008), followed 2 yr later by publication of the Black Truffle of Périgord *Tuber melanosporum* genome (Martin *et al.*, 2010). Within the last 2 yr, the Joint Genome Institute (JGI; <http://jgi.doe.gov/>) of the US Department of Energy has made available to the scientific community the genomes of at least a dozen additional mycorrhizal species and many more are expected to be released in the near future. Mycorrhizal fungal genome projects are part of an initiative for systematic sequencing of species representative of ecologically important functional groups, including plant pathogens and wood rotters and will cover all major fungal phyla.

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This wealth of sequence data is an unprecedented resource and is beginning to change research programs and experimental design. A key to this change is that fungal genomics is not a tool restricted to expert geneticists; instead it is serving as a source of inspiration for all scientists engaged in the various aspects of interactions between fungi and plants, or in questions grounded in fungal ecology or evolutionary biology.

In this context, the 6th New Phytologist Workshop, ‘Bridging Mycorrhizal Genomics, Metagenomics and Forest Ecology’, was held at the INRA center in Nancy (France) on November 15–16, 2012. The workshop was organized by Francis Martin (INRA) and

Igor Grigoriev (JGI) with additional financial support provided by the Laboratory of Excellence for Advanced Research on the Biology of Forest Ecosystems (ARBRE) (<http://mycor.nancy.inra.fr/ARBRE/>). Scientists at the meeting sought to discuss the present and future contributions of fungal genomics not only to our understanding of symbiotic associations, but also more generally to forest ecosystem functioning. Many of those present at the workshop had also attended the 2nd Mycorrhizal Genomics Initiative (MGI) Workshop coordinated by Francis Martin and Igor Grigoriev and held just before the New Phytologist Workshop. The MGI (<http://mycor.nancy.inra.fr/IMGC/Mycogenomes/>) is an ongoing joint JGI–INRA program focused on sequencing mycorrhizal fungal species (Martin *et al.*, 2011).

‘Honour to whom honour is due’, the first speaker at the New Phytologist Workshop was Alan Kuo from the JGI, which has enabled roughly half of the fungal genomes sequenced to date through its Community Sequencing Program (CSP), dedicated to plant biomass degrading species, mycorrhizal ones or to the coverage of the entire kingdom Fungi (the ‘1000 fungal genomes’ project, see <http://1000.fungalgenomes.org/home/>). The JGI is also the sequencing center which is actively opening the field of fungal genomics to other disciplines, including ecology and evolutionary biology. Alan showed us how in addition to generating genome sequences which are assembled, annotated and made available on individual genome browsers, the JGI is investing in integrating the data to facilitate cross-species comparisons. For example, one of the most prominent online tools is MycoCosm (<http://genome.jgi.doe.gov/programs/fungi/index.jsf>), which allows easy comparisons of gene functional categories between species, or the fast retrieval of all members of a specific gene family from the different genomes (Grigoriev *et al.*, 2012).

Fungal genomes as resources

Meeting sessions were organized so as to illustrate the different uses of fungal genomics, and the first set of sessions illustrated the value of individual genomes. Several talks highlighted the fact that an individual genome is for many research teams an invaluable resource to address ‘classic questions’ at a genome scale, by giving biologists access to the complete set of genes controlling specific processes. The point was illustrated by both Jan Colpaert (Hasselt University, Belgium) who investigates metal resistance in *Suillus luteus*, and by Uwe Nehls (Bremen University, Germany) who studies transmembrane sugar transporters enabling carbohydrate exchange between the partners of a mycorrhizal symbiosis. However, it was also clear that a huge gap exists between an observed phenotype (e.g. metal resistance) and a list of genes. One strategy to overcome the difficulty in connecting phenotypes to genotypes is to use comparative transcriptomics or genomics with groups of related fungal strains possessing different phenotypes, in

order to more accurately associate the target phenotype to either variability in gene transcription patterns and/or changes in genetic architectures, for example gene family amplifications or contractions. On many occasions the discussion focused on the need for additional sequencing to validate hypotheses generated from genome analyses. This sequencing will be expensive and time-consuming, but the data will greatly improve our understanding of fungal biology.

Genome signatures of fungal trophic strategies

One of the most hotly debated questions in fungal evolution is whether or not transitions between major trophic modes (mutualism, parasitism and saprotrophism), which have taken place independently many times in diverse fungal lineages, are reversible. A barrier to answering this question is a still incomplete knowledge of the natural history of many species. Fungal genomics may allow a 'reverse ecology' approach, and enable the autecology of a fungal species to be predicted from its genetic repertoire. Simply speaking, if a species lacks the genes to decompose wood, it cannot be a wood decomposer, and if mycorrhizal symbiosis specific genes are identified, then the species can be labeled as mycorrhizal. Several talks illustrated the promises, but also the difficulties, of this approach, as each trophic strategy turns out to be more complex and diverse than any of us has suspected. For example, Dan Cullen (USDA, Wisconsin, USA) pointed out that many aspects of plant biomass degradation are still obscure. In brown rot fungi, the contraction of cellulose gene families provides additional evidence that decomposition may involve Fenton chemistry and not classical enzymatic cellulolysis; it seems clear that different fungi use different tools to function as saprotrophs. Dan Cullen and Dimitri Floudas (Clark University, Worcester, MA, USA) also showed that species with different trophic strategies do not always differ with respect to the presence or absence of the key gene families involved in plant biomass degradation, for example, laccases are commonly found in mycorrhizal genomes. Any protein may have multiple functions, and this highlights the need to clarify the role of enzymes in other processes, including development or metabolism. Throughout the meeting, transposable elements and their mobility, especially during meiosis, were discussed as potential drivers of evolutionary change, particularly by Claude Murat (INRA, Nancy, France) during his analysis of the black truffle genome.

One obvious limitation in much of the work published to date is the inclusion of species belonging to distantly related lineages in a single analysis. The approach makes it difficult to identify genetic changes caused by any single evolutionary force. There is an obvious need for sequencing the fungal tree of life at a finer taxonomic grain, to gather genomic data for phylogenetically related but ecologically divergent species. Examples of large-scale sequencing efforts at the class or family levels have been published for pathogenic and saprotrophic ascomycetes (Ohm *et al.*, 2012), and mycorrhizal and decomposer *Amanita* (Wolfe *et al.*, 2012), and at this meeting were illustrated for *Colletotrichum* spp. by Marc-Henri Lebrun (INRA, Grignon, France). Mycologists are increasingly aware that some fungal species have dual or multiple ecological abilities, and a potentially exciting avenue for future

research would target the sequencing of species with this kind of trophic complexity, for example, the endophytic and saprotrophic Xylariaceae (Davis *et al.*, 2003), the endophytic and mycorrhizal Sebaciales (Zuccaro *et al.*, 2011), or the saprotrophic and mycorrhizal fungi colonizing Ericaceae and Orchids (Martos *et al.*, 2009). One intriguing issue involves rare species, which can dominate many fungal guilds, for example in endophytic or ectomycorrhizal communities. The idea that rare species occupy differentiated, original niches recently received some support in a study of plant communities (Mi *et al.*, 2012), and future choices about what to sequence should perhaps take these ideas into account.

The fungal individual

Another fundamental question in mycology concerns the cohesiveness of the fungal mycelium, and the related concept of the fungal individual. As early as 1931, Buller (1931) discussed the 'social organization' of groups of mycelia, and he may not have thought of any single mycelium as an individual. Later, Rayner (1991) argued for the integrity of the different mycelia of a single species, and did not hesitate to consider each as a distinct entity. What are fungal individuals? Are the enormous genets of species of, for example, *Armillaria* described by Smith *et al.* (1992) functioning as a physiological whole, and what role do hyphal breaks or fusions play in delineating distinct ramets? With genomes as a resource, novel experiments are possible. Transcriptomes grounded in a well annotated genome and taken from different parts of an individual could be used as a proxy to infer either physiological specialization or uniformity. This aspect of the meeting's discussion was illustrated by Anders Tunlid (University of Lund, Sweden), who presented as a model the mycelium of the ectomycorrhizal fungus *Paxillus involutus* proliferating in nutrient rich patches of soil. These kinds of transcriptomic experiments, currently restricted to laboratory microcosms, should be transposed in the near future to more natural settings, including perhaps the edges and center of a network growing on a forest floor.

Beyond fungal genomes: environmental genomics

Besides the genomics of single species, metagenomics or 'community genomics' emerged as an active research field. In the case of bacteria, metagenomics has benefited from the large number of available genomes for precise taxonomic annotation of anonymous environmental sequences, as illustrated at this meeting by Matthias Hess's (Washington State University, Pullman, WA, USA) talk on the cow rumen metagenome. In the field of fungal ecology, metagenomics is being developed to dissect the key ecosystem processes primarily serviced by the fungi, including plant biomass degradation in forests. In this context, Petr Baldrian (Institute of Microbiology, Prague, Czech Republic), using a combination of amplicon sequencing (ITS and cellobiohydrolase amplified from either soil DNA or RNA), DNA SIP using ¹³C-labelled cellulose, and soil proteomics dissected the taxonomic and functional complexity of distinct fungal communities specializing on contiguous horizons of a forest soil profile (Baldrian *et al.*, 2012). As

pointed out by Roland Marmeisse (CNRS/University Lyon 1, France) the functional diversity of fungal communities may be better understood using metatranscriptomics, which makes use of soil-extracted polyadenylated mRNA converted into cDNA before sequencing. This approach may occasionally seem frustrating, because most sequences cannot be affiliated to any taxon, but it provides functional data unavailable through the barcoding of soil communities. Moreover, barcoding often targets a specific group, for example, fungi or animals, leaving key soil taxa unstudied, for example the diverse soil Foraminifera (Lejzerowicz *et al.*, 2010). Even within fungi, the current taxonomic distribution of sequenced species is a severe limitation to the precise taxonomic identification of soil fungal sequences. For this reason, ongoing sequencing efforts, such as the CSP 'Metatranscriptomics of Forest Soil Ecosystems' (http://mycor.nancy.inra.fr/blogGenomes/?page_id=3262) are targeting ecologically relevant 'keystone' fungal species found in soil or other substrates where fungi play critical roles.

Questions in fungal ecology: now answered with more power and precision?

Major technological advances in terms of the availability and applicability of molecular methods have brought along massive increases in the quantity of data about fungal individuals, populations, and communities in the environment. Perhaps not surprisingly, the core questions remain: who is doing what and where? Obviously, our early understanding of these questions was not precise enough, and our knowledge of fungal assemblages and their functions in the environment has changed considerably during the last decade. This was illustrated in a talk by Åke Olson (Uppsala BioCenter, Swedish University of Agricultural Sciences, Uppsala, Sweden) on the fungal communities inhabiting logs; their assembly, priority effects, and activity, addressed by both traditional culture dependent and by DNA and RNA based approaches. Methodological issues were also discussed: what is the appropriate sample size or sampling strategy for fungal community description in the environment, which marker(s) should be used (the ITS2 region may be superior to the ITS1 because the variability in its length is less, and so there is less amplification bias, but other markers could be used, too), and how to elucidate the taxonomy of environmental DNA/RNA sequences? Furthermore, as pointed out by Petr Baldrian, ribosomal genes allow description of a pool of sequences, which is not equal to the pool of organisms in a community because of the unknown and potentially variable copy number of ribosomal genes. Clearly, methodological aspects need more rigorous study, in order to ensure that the important ecological questions can be answered with necessary detail and precision.

Other biological interactions also shape genomes?

As stated by Arthur G. Tansley, *New Phytologist's* founder, 'though the organisms may claim our prime interest, when we are trying to think fundamentally, we cannot separate them from their special environments, with which they form one physical system' (Tansley,

1935, p. 299). Elaborating from this point, it is striking that most analyses of genomic data focus on fungal trophic strategies, symbioses with plants, and the ecological roles of fungi in nutrient cycling. It seems the ecology of fungal species is primarily discussed as autecology, while the synecological dimension remains poorly explored. Yet, as is true for plants and animals, fungi have competitors, predators and parasites in the surrounding biotic community and these likely exert strong evolutionary pressures (Rohifs *et al.*, 2007). The interactions may have shaped portions of each fungal genome, as pointed out by Marc-André Seloisse (Université de Montpellier, France). We often forget that some fungi are the basis of entire food webs involving mycophagous invertebrates and protozoans (Crotty *et al.*, 2012). Genetic traits, including those resulting in secondary metabolite profiles, likely evolved to face such aggressors, with features like smells and pigments contributing to defense (for example by mimetism or aposematism; Sherratt *et al.*, 2005). Francis Martin reported that the ectomycorrhizal symbiont *Laccaria bicolor* accumulates transcripts coding for clitocypins and macrocypins in the external layers of the mycorrhizal mantle. These protease inhibitors may protect the fungal hyphae from grazing by predators. As time goes by, further evidence for evolved defenses and currently undescribed arms races may be found in fungal genomes.

Conclusions: not only a matter of DNA sequencing

If one of the major conclusions of this workshop was that fungal genome sequencing efforts must intensify, several presentations also showed us that fungal genomics will benefit from emerging technologies not directly related to DNA sequencing, and involving the phenotype. For example, environmental proteomics may enable the identification of proteins directly extracted from soil samples (Petr Baldrian; Schneider *et al.*, 2012), and powerful, novel analytical systems may enable single cell metabolic profiling (Scott Baker, Pacific Northwest National Laboratory, Richland, WA, USA) or nondestructive measures of plant biomass modification in response to fungal colonization (Anders Tunlid). It is clearly an exciting time to be involved in the science of interactions.

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