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Moving from pattern to process in fungal symbioses: linking functional traits, community ecology and phylogenetics

A growing appreciation of the ubiquity of plant–fungal symbioses and their fundamental importance to plant communities (Smith & Read, 2008; Rodriguez *et al.*, 2009) has led to a recent radiation of research at the ecological intersection of botany and mycology. With new tools helping fungal ecologists frame new questions – and answer long-standing ones with new precision – fungal ecology has entered a transformative phase. As high-throughput and next-generation molecular tools begin to yield unprecedentedly large data sets describing the diversity and composition of fungal communities (e.g. Bidartondo & Gardes, 2005; Jumpponen & Jones, 2009), fungal ecologists are using computational and

analytical innovations (e.g. Taylor *et al.*, 2008) to re-cast questions in terms of process, rather than of pattern alone.

A consensus emerged at the 2009 joint annual conference of the Botanical and Mycological Societies of America (Snowbird, UT, USA; <http://2009.botanyconference.org>) that incorporating functional traits and phylogenetic information into community studies is key to addressing underlying processes – a critical step for moving fungal ecology to a more predictive science. Such a perspective adds to an increasing awareness of the ways that evolution and ecology are linked through functional biology and can be examined at scales ranging from gene expression to broad ecological modes (James *et al.*, 2006; Edwards *et al.*, 2008; Nygren *et al.*, 2008). With a rich history of using molecular approaches for community surveys, an ever-clearer understanding of the fungal tree of life, and a growing wealth of genome sequences, fungal ecologists are poised to examine fungal diversity, functional traits and phylogenetic relationships in novel ways – and to view them through the lens of genomics to characterize, manipulate and conserve fungal ‘community symbiomes’.

Characterizing fungal communities using molecular tools

Because many fungal associates of plants are microscopic and/or unculturable, fungal ecologists long have employed molecular tools to characterize fungi in substrates ranging from leaf litter to flower nectar. Such methods have expanded, especially in the last two decades, with high-throughput 454 and Illumina sequencing platforms providing previously unimaginable sampling depth and breadth (e.g. Buee *et al.*, 2009; Jumpponen & Jones, 2009). Although still limited in sequence length and in the degree to which communities can be accurately described (see Avis *et al.*, 2010), such data sets complement culturing, whole-community fingerprinting (e.g. denaturing gradient gel electrophoresis; Bonito *et al.*, 2010), nonsequence-based molecular approaches (e.g. terminal restriction fragment length polymorphism; Dickie & FitzJohn, 2007) and cloning (e.g. Geml *et al.*, 2009) to illuminate fungal diversity.

Presentations at Snowbird showcased not only these approaches but also the progress in bioinformatics tools needed to analyze such data. For example, József Geml and colleagues (University of Alaska Fairbanks, AK, USA) compared sequence data from curated collections of sporocarps of mycorrhizal *Lactarius* to clone libraries from soil, highlighting unexpected spatial partitioning of these fungi in boreal and tundra ecosystems. Ari Jumpponen and colleagues (Kansas State University, KS, USA) used 454 technology to compare the diversity and composition of phyllosphere fungi between rural and nonrural trees, uncovering a striking effect of urbanization on highly diverse fungi associated with healthy foliage.

Despite an ever-increasing number of studies and advances in molecular tools, discussions at the meeting highlighted challenges that still limit our ability to synthesize large-scale data sets across studies. For example, sampling methods often are optimized for a given system of interest (Peay *et al.*, 2008) and may differ sufficiently among studies to preclude robust comparisons. By contrast, at times standard methods are imperfect despite their apparent generality. For example, many fungal ecologists categorize communities using sequence data from the nuclear ribosomal internal transcribed spacer (nrITS), which are widely represented in GenBank, easily amplified and useful for rapid estimation of taxonomic richness. However, reliance on this locus often precludes phylogenetic analysis of taxonomically diverse survey data, and a growing number of authors have highlighted the many difficulties that limit its utility for taxonomic identification using GenBank (e.g. Vilgalys, 2003; Nilsson *et al.*, 2006). As a result, authors often 'play it safe' by delineating operational taxonomic units (OTU) based on nrITS sequence similarity. However, not only does a general rule for approximating species boundaries remain elusive (Nilsson *et al.*, 2009), but estimates of richness differ markedly when the same data are organized into OTU using different software applications (U'Ren *et al.*, 2009).

The meetings represented an important opportunity to address such issues – not only through interactions among researchers, but also more formally through a two-day statistical workshop on community analysis, which was sponsored by the Fungal Environmental Sampling and Informatics Network (FESIN, a research coordination network supported by the National Science Foundation). The workshop provided hands-on training for > 70 students, postdocs, faculty members, and government scientists in community characterization software and how to draw the strongest insights from survey-based studies. Participants agreed that high-quality analyses and interpretation are key not only for their own sake, but also for understanding the biogeography of fungal–plant associations (Peay *et al.*, 2010; see pp. 878–882 this issue) and the co-evolutionary context they represent (Arnold *et al.*, 2010; see pp. 874–878 of this issue). With the number of community survey studies continuing to rise, and methods for their analysis continuing to improve, participants agreed that the field is ripe for two major steps forward: identifying and measuring functional traits in fungal communities, and interpreting such traits through community phylogenetics.

New perspectives on functional traits

Examining functional traits provides a mechanistic perspective on the abiotic and biotic processes governing community assembly. Because the same traits can be measured on

different species, a functional-trait approach allows communities comprising different taxa to be compared, expanding our ability to generalize or contrast processes structuring communities in very different ecosystems (Diaz & Cabido, 2001; Westoby & Wright, 2006).

The rhizosphere has long been a focus for understanding functional aspects of plant–fungal interactions, with a rich history of studies on arbuscular mycorrhizal and ectomycorrhizal associations. Rhizosphere survey data have been complemented recently by functional studies of nutrient transport and interactions at the cellular and molecular levels (e.g. Jargeat *et al.*, 2003; Govindarajulu *et al.*, 2005). Novel methods for understanding enzyme activity in soil are elucidating functional aspects of nutrient cycling (Sinsabaugh *et al.*, 2008), and new refinements are providing enzyme profiles directly from recently excised mycorrhizal root tips (Courty *et al.*, 2005; Pritsch *et al.*, 2005). Such findings link fungi identified in surveys to their products *in vivo*, facilitating the exploration of mycorrhizal responses to various environmental conditions (Phillips *et al.*, 2008) and linking aspects of ecosystem function to particular species (Dong *et al.*, 2007). Presentations at Snowbird reminded researchers that such studies need not tie function only to chemistry; for example, Kabir Peay (University of California, Berkeley, CA, USA) showed that functional traits such as mushroom height, fruit body production and dispersal vectors are associated with the prevalence of particular fungal species colonizing seedlings planted at varying distances from established mycorrhizae-forming vegetation.

Coupled with estimates of niche occupancy or trait diversity, functional studies provide a context for addressing how fungal communities will respond to environmental change. In addition to examining community responses to factors associated with climate shifts, discussion at Snowbird focused the functional trait lens on issues such as nutrient deposition. In one example, Peter Avis (Indiana University Northwest, Gary, IN, USA) examined the prediction that greater phosphorus uptake and transport abilities should be exhibited by mycorrhizal fungi where nitrogen (N) is less limiting to plants (e.g. N-fixing plants or high N-deposition environments). Observing an ectomycorrhizal community shift to favor species of *Russula*, he found those with cystidia-coated ectomycorrhizas more often in areas of high N (Avis *et al.*, 2003, 2008). Because cystidia produce calcium oxalate crystals that increase soil phosphate availability, he proposed this as a mechanism by which competitive abilities may be enhanced.

An improved understanding of fungal functional traits is promising also from a molecular perspective. Complementing previous phylogenetic analyses (e.g. Hibbett *et al.*, 2000; Arnold *et al.*, 2009), recent genome sequencing has provided insight into the interplay of symbiosis and

saprotrophy (Martin *et al.*, 2008) and highlighted genomic signatures of ecological strategies such as pathogenicity (Soanes *et al.*, 2008) and endophytism (Parrent *et al.*, 2009). Recently, functional genes also have been identified and measured directly in fungal communities (e.g. Blackwood *et al.*, 2007; Nygren *et al.*, 2008).

In almost all cases, a gap remains in linking genome structure to gene expression. In the coming years, transcriptomics will be especially critical; in the meantime, translating the growing genomic database into ecologically meaningful and quantifiable traits is an area where collaboration among bioinformaticians, physiologists, biochemists, mycologists and ecologists is likely to yield especially great rewards. One way to immediately maximize the inferential power of genomics and functional trait studies lies in phylogenetics, especially when applied at a community scale.

Community phylogenetics: linking function to ecology, evolution and genomics

After taking chance into account (see McGill *et al.*, 2006), community assembly at local scales can be conceptualized as the interplay of abiotic filters and biotic interactions such as competition and mutualism – with functional traits determining which organisms successfully pass a given filter and establish. Functional traits often are tied directly to evolutionary history, such that inferences about them are strongest when factors such as biogeographic history (Peay *et al.*, 2010) and phylogenetic relationships are considered.

Dating at least to Darwin, biologists have observed that closely related species are often ecologically similar – implying that they may succeed in similar environments and may compete strongly when they co-occur (Cavender-Bares *et al.*, 2009). Thus, a phylogenetic perspective is useful for interpreting community assembly rules. In Snowbird, a transition from species-level or genotype-level characterization of communities to approaches based on a phylogenetic perspective was showcased by Steve Kembel (University of Oregon, OR, USA) and Elisabeth Costello (Stanford University, CA, USA), who provided hands-on training in Phylocom (Webb *et al.*, 2008) and UniFrac (Lozupone *et al.*, 2006) at the FESIN workshop.

Increasingly, researchers are using such tools to generate hypotheses about connections between taxa and their function (Webb *et al.*, 2002; Cavender-Bares *et al.*, 2009). For example, a community featuring species distributions that are over-dispersed with regard to phylogeny may indicate competition among functionally similar species (Kraft *et al.*, 2007). In contrast, one that features phylogenetically clustered distributions may speak to highly conserved traits that allow related organisms to successfully pass through an environmental filter. Community phylogenetics provides an indirect way to identify key functional traits without

explicitly measuring them, and also can be used to direct efforts to measure such traits.

Several presenters focused on community phylogenetics approaches at Snowbird. For example, Jeri Parrent and colleagues (University of Guelph, ON, Canada) examined the diversity and spatial organization of four functional traits in arbuscular mycorrhizal fungi (AMF): percentage root colonization; extraradical hyphal length; plant biomass; and plant phosphorus content. Evolutionary reconstructions of functional traits across the AMF phylogeny showed that several were highly conserved within lineages (Powell *et al.*, 2009), and that both phylogenetic and trait diversity showed significant evenness in an old-field AMF assemblage. They concluded that phylogenetic structure is an honest signal for functional diversity within AMF communities, and suggested that phylogenetic evenness may represent functional complementarity of community members, which can positively and synergistically affect plants (see also van der Heijden *et al.*, 1998; Maherali & Klironomos, 2007).

An attractive feature of the community phylogenetics approach is its utility at multiple different spatial scales. For example, Kabir Peay characterized ectomycorrhizal community structure across a plant-soil ecotone in tropical rainforests of Borneo. Although little is known about the functionality of such fungi in these forests, phylogenetic clustering and biased representation of several families in particular soils suggested that conserved traits within these lineages may promote their occurrence in particular soil types. Similarly, Ivan Edwards's (University of Michigan, MI, USA) phylogenetic analysis of Agaricomycotina from three forest types demonstrated significant phylogenetic clustering of fungi from sites with similar overstorey composition. By contrast, Sara Branco (University of Chicago, IL, USA) showed that serpentine and nonserpentine soils contained radiations by the same major clades of fungi, suggesting the lack of a physiological barrier for mycorrhizal fungi.

Synthesis

The combination of functional trait analysis and community phylogenetics offers great promise in developing integrated, predictive models for factors shaping the assembly of symbiotic fungal communities on which plants depend. However, a number of challenges – including accurate enumeration and identification of fungal community members, diagnosis of key fungal functional traits, standardizing their measurement, and understanding their evolutionary history – remain before full realization of this approach is possible. Resolving these challenges will help to discern the common processes underlying the dazzling mosaic of diversity that has been uncovered by molecular characterization of plant-symbiotic fungal communities.

Missing pieces for improving our understanding of functional diversity of fungi and implementing a community phylogenetics approach include: (1) exploiting new technologies to obtain phylogenetically informative but species-resolving loci in environmental surveys, and/or applying supertree methods to link nrITS data sets to deeper phylogenetics; (2) careful evaluation of intraspecific variation in ecological modes as a prelude to interpreting the evolutionary history of ecological function; and (3) examination of the conservation or lability of certain functional traits within fungal lineages. The first issue will be addressed through mycologists' ongoing efforts to capture fungal biodiversity, and community-wide efforts to improve curation, informatics tools, metadata, accessibility and phylogenetic analyses associated with such work. The second will be informed by genomics and metagenomics analyses coupled with empirical assessments of function, and enhanced by classically trained mycologists who know these organisms well.

Together, these approaches, coupled with the types of analyses characterized by recent studies examining the evolution of fungal ecological modes (e.g. James *et al.*, 2006; Arnold *et al.*, 2009), will inform the third missing piece – phylogenetic analysis with regard to the origins and evolutionary trajectories of functional traits. Thus, the utility of this emerging approach lies in its multidisciplinary nature and in the ever-greater interaction of diverse researchers interested in an array of levels of biological organization and ecological function.

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The *Sphagnum* air-gun mechanism resurrected

In a recent paper, Duckett *et al.* (2009) present experimental data that they argue reject the air gun mechanism for spore discharge in *Sphagnum*. Since Nawaschin (1897) published the results from physical tests, the air gun mechanism has been widely accepted as the means by which *Sphagnum* spores are propelled into the air (e.g. Ingold, 1965; Maier, 1973; Cronberg, 1992), but no one until Duckett *et al.* has tested it further. According to the air gun notion, air pressure builds up in a cavity in the lower half of the spore capsule when the capsule dries, contracts longitudinally and changes shape from a sphere to a cylinder. The spores are located in a sac in the upper part of the capsule, below the operculum, on top of the air cavity (Nawaschin, 1897) – this may be seen if one holds a fresh, semitransparent capsule towards the light, where the spore mass is darker than the air cavity below. The air cavity constitutes approx. 35% of the external volume of a cylindrical capsule – the rest is the spore sac (50%) and capsule tissue (15%; see illustration in Nawaschin, 1897) – a similar figure is derived by multi-