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Key words: *Arabidopsis,* ethylene, *Piriformospora indica,* plant defences, plant–fungus interaction, signalling, symbiosis.

Advancing our current understanding of plant–fungal symbioses: bridging scales from local to global

The recent joint meeting of the Botanical and Mycological Societies of America (July 25–29, 2009; Snowbird, Utah, USA) was an apt venue for a symposium examining the ecology and evolution of the symbiotic interactions that occur between plants and fungi. These highly diverse and cryptic symbioses, studied for many decades, are ancient and ubiquitous. Recently, the field has undergone rapid expansion, drawing from molecular systematics and ecological genomics to develop new DNA-based and RNA-based tools and associated bioinformatics. At a session on the phylogenetic and functional patterns of host plants and their associated fungi, speakers described research programs that combine cutting-edge and traditional methods to amass data about the nature and distribution of these interactions across scales, from local to global, in a co-evolutionary context. Several threads united the research programs presented, collectively pointing to an alignment of questions investigated at different scales to reveal a comprehensive picture of processes that shape symbiotic plant–fungal communities. This emergent view shows that the fields of botany and mycology are maturing in an understanding of plant–fungal interactions, founded on a rich collection of observations and burgeoning theories, now coupled with fresh perspectives garnered by using new tools. This Commentary captures these developments and provides an introductory overview to a cluster of Letters published in this issue of *New Phytologist.* Each letter focuses on one of three important areas within plant–fungal symbiosis that emerged from the meeting and will help to set the way forward: plant–fungal symbiotic co-evolution (Arnold *et al.*, pp. 874–878); biogeography of these symbiotic partners (Peay *et al.*, pp. 878–882); and phylogenetic community ecology of plants and their symbiotic partners (Parrent *et al.*, pp. 882–886). Full details relating to presentation titles, author affiliations and related information about the 2009 Annual Botany & Mycology Conference can be found online at http://2009.botanyconference.org.

> *'With symbiotic interactions between plants and fungi spanning a complex continuum from mutualism to parasitism …, an ongoing conversation between mycologists and botanists is critical.'*

Innovative research that has steadily accrued in areas spanning micro-evolutionary processes, patterns of specificity, biogeography, and community phylogenetics of plants and fungi was highlighted at this year's meeting showcasing this emergent multidimensional view of plant–fungal symbioses. As it becomes easier to characterize inter-specific and intra-specific genetic variation of both plants and fungi, we are poised to test theories about community assembly and co-evolution on landscape and global scales that previously were prohibitive. Speakers highlighted advancements in the understanding of interactions at the genotype level that affect the next generation, providing new evidence for local adaptation (presented by Louis J. Lamit), and how narrow receptivity of plant species leads to low ecological redundancy of mycorrhizal fungi, so that keystone fungal taxa can determine plant establishment, survival and diversification (presented by Martin Bidartondo). Such processes at local scales are critical for understanding the mechanisms of co-evolution with direct implications for macro co-evolution. Additionally, it is critical to identify ecologically relevant models of symbioses for the broad context of research from genomics to climate change. Symposium speakers Elizabeth Arnold and Kabir Peay, as well as the MSA Presidential Address by Roy Halling highlighted progress in understanding fungal biogeography and dispersal, previewing the value of such data for fueling ecologically relevant hypothesis-driven research about current and future distributions of fungi and their plant hosts. Additionally, Peay, among others, emphasized the exquisite sensitivity of symbiotic fungi to the spatio-temporal scale and ecological context of sampling, illustrated by his finding of a large difference in biodiversity patterns of plant hosts and fungal symbionts in different ecosystems. Extrapolations from Peay's data suggest that tropical ecosystems rich in plant biodiversity can have 10–40 times the number of plant hosts to fungal symbionts of temperate ecosystems, which points to a potential disconnect between biodiversity patterns of host species and symbionts. The greater diversity of symbionts in temperate systems relative to host plants indicates that there may be a different pattern for symbiont diversity on a global basis, begging for follow-up studies and meta-analyses, and indicating the importance of examining biodiversity in areas previously thought to be low on biodiversity based solely on plant studies. Furthermore, Parrent presented data indicating that shifts in richness of constructed field communities of fungal symbionts were connected to their phylogenetic diversity. This finding suggests that competition among mycorrhizal fungi in the field, presumed to be stronger among closely related fungi, trumps plant–fungal interactions. Finally, Avis's presentation of community shifts of fungal symbionts in woodlands experiencing elevated rates of nitrogen deposition illustrate the new quest to identify the tissue-level traits and their phylogenetic dispersal that may mediate community-level shifts occurring belowground, and recast traditional thinking about drivers of community assembly in changing environments.

With symbiotic interactions between plants and fungi spanning a complex continuum from mutualism to parasitism that is influenced by both plant and fungal partners (Jones & Smith, 2004; Karst *et al.*, 2008; Rodriguez & Redman, 2008), an ongoing conversation between mycologists and botanists is critical. Scientists in both disciplines are poised to profit from new theory in co-evolution that is rapidly being generated and tested by investigators examining other interacting organisms, both symbiotic and free-living, including plant-pollinator systems and even symbioses in the sea (Bascompte *et al.*, 2006; Ollerton *et al.*, 2007), and take into consideration that species (and genotypes) interact within a geographic mosaic (Thompson, 2005). Coupling micro-perspectives and macro-perspectives on symbiotic interactions between plants and fungi with the latest genomic tools is likely to take us in exciting new directions (see Arnold *et al.*, 2010; Peay *et al.*, 2010 – both in this issue). Such tools provide new ways to characterize diversity from local to global scales, and place that diversity into an evolutionary framework spanning from events that have occurred quite recently to more ancient diversification events (Arnold *et al.*, 2009). Moreover, by placing phylogenetically informed interactions in a geographic framework, these tools can help to address broad questions about the ecological processes shaping biogeographical patterns.

As we gain increasingly detailed information about fungal communities, a specific goal mentioned by several speakers is to use phylogenetic analyses to address the conservatism of functional traits and their ecological implications (Cavender-Bares *et al.*, 2009). This potentially powerful approach can proceed by relying on the use of phylogenetic dispersal of a community as a proxy for functional diversity, as highlighted in symposium presentations by Arnold and by Parrent and in the accompanying Letter by Parrent *et al.* This approach also allows broad questions about ecosystem function to be complemented with narrower, more detailed, studies that address function in taxon-specific organismal terms. As discussed by many speakers, and during a discussion following the symposium, additional progress will require remedying the current scarcity of information about plant and fungal functional traits related to these symbioses. Given that fungi, belowground plant organs and mycorrhizal symbioses are diverse and cryptic, this is a vast challenge, but headway has been made with further progress forthcoming. In some instances, it may be possible to mine functional trait data from the literature. For example, it may be possible to infer fungal dispersal syndromes from measures of spore size, spore longevity, or fruiting body height (presented by Kabir Peay). While it has proven relatively straightforward to gather data for aboveground functional traits of diverse plant species (e.g. Grime, 2001; Reich *et al.*, 2003), a few studies have shown that it is possible to gain comparable functional trait data from belowground tissues such as fine roots (e.g. Guo *et al.*, 2008; Comas & Eissenstat, 2009) and fungal hyphae (presented by Jeri Parrent).

Moving forward, it will be useful to identify key functional differences among symbionts, such as hyphal exploration types (e.g. Agerer, 2001) and extend them to arbuscular mycorrhizal fungi (AMF) (presented by Jeri Parrent). Finally, there is an ongoing hunt for relevant and reliable biochemical and molecular traits that can be easily scored using high-throughput methods (Bidartondo & Gardes, 2005).

Overall, as detailed in the accompanying Letters, speakers presenting research in Utah synthesized our current understanding of plant–fungal symbioses and indicated future directions that would be fruitful in advancing this field. These presentations illustrated how research on plant–fungal interactions can profit from new theoretical frameworks such as co-evolutionary theory (Thompson, 2005), conservation-oriented biogeography (Cadotte *et al.*, 2006; Lockwood *et al.*, 2007) and the merger of phylogenetic community ecology with functional trait approaches (Cavender-Bares *et al.*, 2009). In the face of global change – waves of species extinctions and invasions, anthropogenic changes to Earth's soils and atmosphere, damage to ecosystem services – it is critical to advance a comprehensive understanding of the symbiotic interactions between plants and fungi occurring at multiple scales.

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Key words: biogeography, BSA, co-evolution, mycorrhizas, MSA, phylogenetic community ecology, symbioses.

Letters

Interwoven branches of the plant and fungal trees of life

Just as the processes that drive natural selection are now recognized to manifest at levels of biological organization above and below those of the individual – with trait variation, differential rates of birth and death, and heredity pervasive from nucleic acids to cells to populations (Williams, 1966; Dawkins, 1976; Sober, 1984; Keller, 1999; Okasha, 2008) – so too is co-evolution now viewed not just at the level of species, but also at phylogenetic scales ranging from genotypes to major clades (Thompson, 2005). Although strict-sense co-cladogenesis remains a holy grail among some biologists seeking to document reciprocal evolutionary change, researchers have begun to recognize that phylogenetic trees need not branch in parallel to indicate co-evolutionary history. A broader view encompassing ecology brings to light the ways in which co-evolutionary processes can be subtle – yet pervasive, rapid, and interlaced inexorably with geography, chance and the ecological community in which organisms are embedded.

Such a theme runs through the interface of mycology and botany, as showcased at the 2009 meetings of the Mycological Society of America and the Botanical Society of America in Snowbird, Utah. Speakers highlighted the ways in which the plant and fungal trees of life reflect co-evolutionary processes at multiple scales – from roots and leaves to grasslands and forests – often in ways more akin to interwoven branches than to parallel bifurcations. Not only were fungal associates of plants shown to respond to evolutionary change in their hosts, but responses of plants to fungi were a significant theme. (Presentation titles, author affiliations and related information can be found online at http://2009.botanyconference.org.)

Co-evolution of plants and fungi – ancient in nature and ongoing today – has shaped every plant, and by extension every terrestrial ecosystem, in ways we are only beginning to appreciate. By integrating from genotypes to the broadest phylogenetic scales, mycologists and botanists are examining recent and historical traces of important symbioses in ways that will inform a modern co-evolutionary synthesis.

Micro-evolutionary perspectives on plant–fungal co-evolution

Darwin argued that evolution by natural selection has three requisites: trait variation among individuals within a species, heritability of traits, and differential survival and reproduction in favor of individuals with the most beneficial traits (Darwin, 1859). Investigators are beginning to shed light on how these requisites, from the perspective of plants and fungi, influence an organism's partners in symbiosis. For example, new evidence suggests that the composition of plant-associated fungal communities, including fungi as diverse as endophytes, ectomycorrhizal fungi and epiphytic lichens, can differ among genotypes of the trees with which they associate (e.g. Lamit, 2008; Sthultz *et al.*, 2009a). Moreover, studies show considerable functional variation among genotypes of fungal symbionts, frequently with strong effects on host fitness (e.g. Koch *et al.*, 2006; Ji, 2007). Unquestionably, genetically based trait variation has important ecological implications for plant–fungal interactions, consistent with Darwin's first requisite for evolution by natural selection.

In testing Darwin's second and third requisites, studies are moving beyond documenting genotype differences to investigate their heritability and evolutionary significance. Presentations by Jamie Lamit and Chris Sthultz, representing researchers at Northern Arizona University (NAU), showed that even long-lived trees such as pinyon pine (*Pinus edulis*) can be used to study co-evolution between plants and fungi. Strikingly, evidence is mounting that the propensity to harbor a certain composition of fungal symbionts can be passed from one generation to the next (Elamo *et al.*, 1999; Sthultz, 2008). In northern Arizona, USA, *P. edulis* exhibits a genetically based polymorphism in resistance and susceptibility to chronic herbivory by a stem-boring moth (Whitham & Mopper, 1985; Mopper *et al.*, 1991). Ectomycorrhizal communities differ dramatically between resistant and susceptible phenotypes, and long-term removal of moths from susceptible trees does not shift fungal composition to resemble resistant trees (Fig. 1a; Sthultz *et al.*, 2009a). Ectomycorrhizal communities of seedlings grown in soil cores collected under resistant and susceptible trees group by the phenotypic class of their maternal parent, regardless of the source of their soil core, and contain fungal communities resembling those of adult trees of their phenotypic class (Sthultz, 2008).

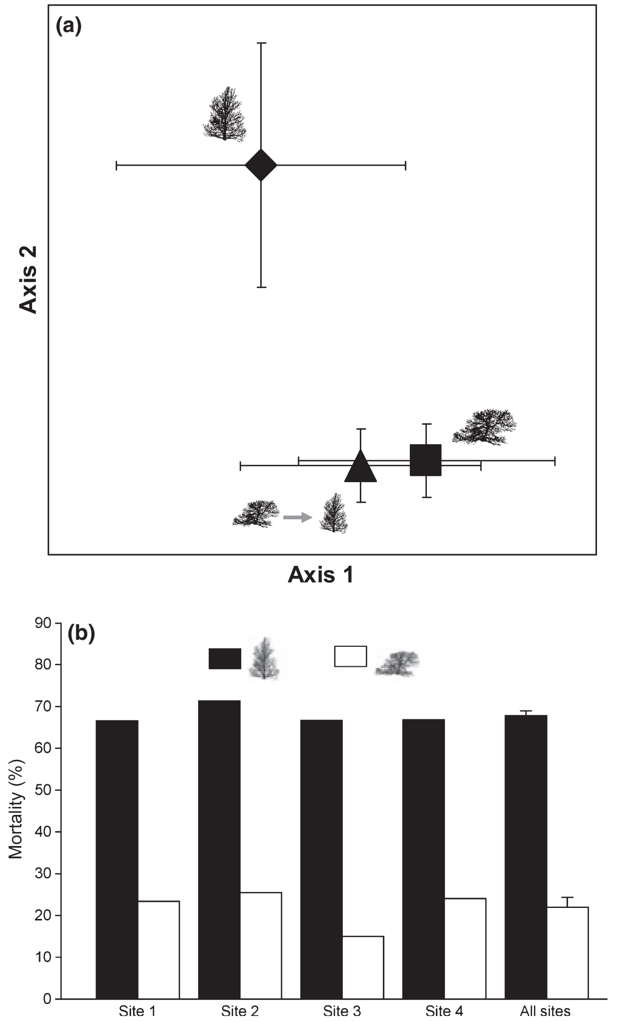

Fig. 1 (a) Nonmetric multidimensional scaling ordination of ectomycorrhizal communities of adult *Pinus edulis* trees that are moth-resistant (diamond), moth-susceptible (square), or from which moths have been removed (triangle). Each symbol represents the mean plus ± 1 SE of ectomycorrhizal community ordination scores of trees for each group. (b) Trees with the moth-resistant phenotype have three times greater mortality than moth-susceptible phenotypes at four different sites and on average. Bar, ± 1 SE. Adapted from Sthultz *et al.* (2009a,b).

A necessary consequence of the genetic basis to plant–fungal community interactions is that selection on one set of partners in symbiosis influences the other. In a recent drought, moth-resistant *P. edulis* suffered threefold higher mortality than moth-susceptible trees (Fig. 1b; Sthultz *et al.*, 2009b). Given the tight connection between tree phenotypes and the fungal community, this suggests that selection on *P. edulis* will influence fungal communities associated with the two phenotypic classes of trees – or that selection is operating on the unique combinations of tree genotype and fungal community in concert. Both possibilities are tantalizing. Ongoing work will aim to uncover whether these patterns are simply the product of directional selection, or are true fingerprints of co-evolutionary phenomena.

At a larger scale, such strong selection may lead to local adaptation and speciation, as evidenced by Nancy Johnson and colleagues' demonstration of local adaptation between the grass *Andropogon gerardii* and its arbuscular mycorrhizal fungi (AMF) (Johnson *et al.*, 2010). In this case, the symbiosis is more efficient from the perspective of both plants and fungi when symbionts share an evolutionary history than when they do not. Complementing such findings was Suzanne Joneson's study of gene regulation during the early stages of the establishment of lichen symbioses, and Tami MacDonald's work on the acquisition of genes that influence the ability of mycobionts to lichenize. These studies brought a 'real time' ecological and genetic perspective to the meetings, linking genotypes to signatures of co-evolution at the species level and above.

Species-level co-evolution in plant–fungal interactions

Many foundational studies in co-evolution focus on highly specialized associations that display strong specificity (e.g. Darwin, 1862; Futuyma & Slatkin, 1983; Jordano, 1987). However, recognizing variation in specificity is fundamental to understanding how diversity is organized spatially, and maintained over ecological and evolutionary time (Thompson, 2005).

Mycologists have long been aware that fungal symbionts of plants range from facultative to obligate, and from cosmopolitan to highly specialized (e.g. Molina *et al.*, 1992). Now, investigators are beginning to focus on the ecological and evolutionary consequences of such variation. Not only are some plant-associated fungal communities variable in their specificity across space – such as endophytes, which exhibit higher specificity at higher latitudes than in the tropics (Arnold & Lutzoni, 2007) – but they also differ in the outcomes of their functions over time and space. Particular species may be mutualists under some environmental conditions, but saprotrophs or parasites under others (e.g. Johnson *et al.*, 1997), and over evolutionary time, changes among ecological states can be unexpectedly frequent (Arnold *et al.*, 2009). The complex interplay that must occur when hosts and/or symbionts shift along the mutualism-to-parasitism continuum is an exciting but under-explored area of research.

To date, our best understanding of variation in such interactions comes from the illuminating case of mycoheterotrophy. Mycoheterotrophic plants, which receive some or all of their organic nutrition from fungal symbionts, have long been key pieces of the co-evolutionary puzzle. These 'saprophytic plants' gained recognition even in early botany textbooks (e.g. Skene, 1924):

> *'It is not certain that any [saprophytic plants] really draw organic food directly from the soil. The fungus may in all cases act as an intermediary. …These plants would properly be regarded as the end of a series, exhibiting the extreme results of the mycorrhizal habit. … There are likely many cases of partial saprophytism which have not been recognized.'*

Early in the molecular revolution, mycoheterotrophic associations were recognized as highly specific, propelling investigations of specificity in other plant–fungal systems. Presentations at Snowbird extended this momentum, documenting specificity among Basidiomycota and liverworts (Bidartondo & Duckett, 2009), AMF and nonphotosynthetic plants (Merckx *et al.*, 2009), ectomycorrhizal fungi and heathland seedlings (Collier & Bidartondo, 2009), ectomycorrhizal fungi and nonphotosynthetic plants (Marc-André Selosse, presentation; Hynson & Bruns, 2009; see also Selosse, 2010), foliar endophytes and their hosts (A. Elizabeth Arnold, Mariana del Olmo, Romina Gazis, Jose Herrera, Demetra Kandalepas, Kali Lader, Michael Weiß, Jana U'Ren, presentations), Dikarya and orchids (Martin Bidartondo, presentation), and even fungi and their own endosymbionts (Michele Hoffman, presentation). Many of these talks provided examples in which fungal phylogenies did not reflect co-cladogenesis with hosts at the species level, yet still provided strong signals of co-evolution.

Notably, these presentations not only provided a perspective on how specificity shapes particular symbioses, but also addressed how specificity can translate to function. In particular, speakers showed that narrow plant receptivity implies low ecological redundancy in fungi, whereby a particular fungus can determine the establishment and survival of a plant, and thus – over the long term – the persistence and subsequent diversification of the lineage that plant represents. Although recognized for plant pathogens, as showcased in a new light at Snowbird by Michelle Hersh, this novel realization for nonpathogenic associations turns the tables on plant conservation by spotlighting conservation of species or groups of fungi, and highlights the sometimes overlooked reciprocity with which plants respond to their fungal inhabitants over evolutionary time. It also echoes our growing understanding of the crucial role of fungi in the colonization of land by plants (Heckman *et al.*, 2001; François Lutzoni, presentation), a critical step in the diversification of the green tree of life.

Co-evolution in the broad sense

No plant in a natural setting exists in the absence of fungi. Spores and hyphae on exterior surfaces, endophytes within leaves and stems, and root-associated fungi ranging from mycorrhizal fungi to dark-septate endophytes comprise a living context for plant ecology in every terrestrial ecosystem (Blackwell, 2000; Rodriguez *et al.*, 2009). Accordingly, most plants live in close association with members of multiple phyla of fungi, each distinctive in its evolutionary history and genomic architecture. Researchers studying broad-scale patterns in co-diversification must often peer through thousands of twigs of the fungal tree of life in the hope of finding a signal of the major branches that lie beneath.

Seeming at times a Quixotic task – and at other times a Herculean one – recent efforts are being informed by the rapid accumulation of fungal genome sequences (e.g. Martin *et al.*, 2008; Eva Stukenbrock, presentation), advances in understanding evolutionary changes among major lineages (e.g. James *et al.*, 2006), integration of phylogenetics with rules of community assembly (Jeri Parrent, presentation) and linking of survey data – including culture-based and environmental sampling – with large-scale phylogenetic inferences (e.g. Arnold *et al.*, 2009). Talks at Snowbird described exciting new perspectives on the timing of major diversification events in fungal–plant associations (F. Lutzoni), the dominance of endophyte communities by different major clades of Ascomycota in plants representing different major lineages (U'Ren) and the insights at multiple levels emerging from studies of the 'microbiomes' of lichens and plants (Lutzoni).

Synthesis

Botanists and mycologists are entering a new decade with a shared perspective that plants co-evolve with the fungi on and in their tissues – even when strict-sense co-cladogenesis is not evident. Convincing data indicate that many plants depend on fungi, particularly in stressful environments, and that fungal symbionts range from mutualism to parasitism and from specificity to generalism, encompassing the ability to change over host ranges, short or long timescales, or as a function of environmental conditions (Rodriguez *et al.*, 2009). Studies described at Snowbird provided evidence of plant–fungal co-evolution using approaches as diverse as micro-evolutionary experiments in the laboratory and field to measuring the congruence of fungal and plant phylogenies from the narrowest to the broadest levels.

Symposium participants agreed that future work will benefit from an interdisciplinary approach fusing traditional evolutionary studies with cutting-edge methods. Identifying traits that vary among plant or fungal genotypes, and how they lead to differences in community structure and function, is critical – and can be achieved through methods ranging from the identification of quantitative traits to detailed studies of gene expression. Speakers also agreed that the traditional approach of studying pairwise interactions is limiting: new research should consider not only diverse fungal symbionts of a given guild, but the simultaneous interactions of symbionts from roots and shoots, and their multipartite associations. This challenging prospect becomes ever more feasible through metagenomics and may be enhanced by ecological network analyses and structural equation modeling (Agrawal *et al.*, 2007; Vacher *et al.*, 2008; Mary Jane Epps, presentation). In part because most fungal communities remain largely understudied, investigators suggested that processes such as local adaptation need more attention: such processes appear frequently in the evolutionary history of plants and fungi, but rarely have studies encompassed sufficient scales – and precise enough tools – to diagnose them. Linking surveys of fungal communities to function (Parrent *et al.*, this issue of *New Phytologist*, pp. 882–886) and geographic distributions (Peay *et al.*, this issue of *New Phytologist*, pp. 878–882) represent key steps forward.

In this context, biologists from many backgrounds are connected by studying plant–fungal interactions. Together they are poised to provide new perspectives on co-evolution, and to move towards a science of 'applied co-evolutionary biology' (Thompson, 2005) to predict outcomes that may ensue as humans spread invasive plant and fungal species, eliminate native biodiversity and otherwise reshape the myriad species interactions that underpin terrestrial plant communities and the ecosystems they support. Understanding how plants and fungi are interwoven at multiple levels of biological organization promises to be key to both foundational and predictive science in the years to come.

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Not every fungus is everywhere: scaling to the biogeography of fungal–plant interactions across roots, shoots and ecosystems

Early natural historians viewed the distributions of fungi as independent of ecology, and instead akin to spontaneous generation: if conditions are right, the appropriate fungi will appear (de Candolle, 1820). Accordingly, Miles Joseph Berkeley (1863), the founder of British mycology, writes to Darwin, 'Indeed were not Fungi so much the creatures of peculiar atmospheric conditions, there would seem to be no limit to the diffusion of their species.' Such a perspective captures a view that characterized the early literature in mycology: fungi may appear to have limited geographical distributions, but dispersal *per se* plays no role in determining such distributions. Nearly a century later, Bisby (1943) recognized endemism in fungi but remained convinced that, 'distribution of hosts and substrata primarily controls distribution of fungi'. Whereas appreciation of spatial and historical patterns of biodiversity led Darwin and Wallace to the theory of evolution by natural selection, the perception that fungi are relatively free from dispersal barriers remained influential well into the 20th century (e.g. Bisby, 1943; Raper *et al.*, 1958).

This assumption has been challenged by recent molecular studies of historical biogeography, ecology and population genetics of fungi (Taylor *et al.*, 2006; Lumbsch *et al.*, 2008). Such studies show that although some fungi are capable of long-distance dispersal (Moncalvo & Buchanan, 2008), the distributions of most reflect the same major dispersal barriers (e.g. oceans and mountains) that drive vicariance events in other organisms (James *et al.*, 1999; Matheny *et al.*, 2009). At first glance the dispersal and distribution of fungi may seem like a topic of interest only in an academic sense. However, broad-scale distributions of fungal pathogens, saprotrophs and mutualists influence key ecosystem properties (Fig. 1), which are currently under pressure from anthropogenic change.

The ecological and historical determinants of fungal distributions – particularly those of symbiotic fungi – were a topic of discussion at a special symposium on the phylogenetic and functional patterns of host plants and their associated fungi, as well as several other sessions, at the Botanical and Mycological Societies of America meeting at Snowbird, Utah, in July 2009. Speakers addressed patterns of fungal distributions at scales ranging from experimental gardens to continents, and at levels of biological organization from genotypes to phyla.

Two talks provided ecological evidence that dispersal limitation should be prevalent among fungi: T. E. Galante (SUNY College of Environmental Science and Forestry, USA) and J. L. Stolze-Rybczynski (Miami University, FL, USA) presented statistical and biomechanical models, respectively, based on direct measurements of basidiospore dispersal from fungal reproductive structures, highlighting how structural differences, such as mushroom height, spore shape and size of Buller's drop, determine dispersal distances. These talks also showed that most spores travel only very short distances from their point of origin – for exam-

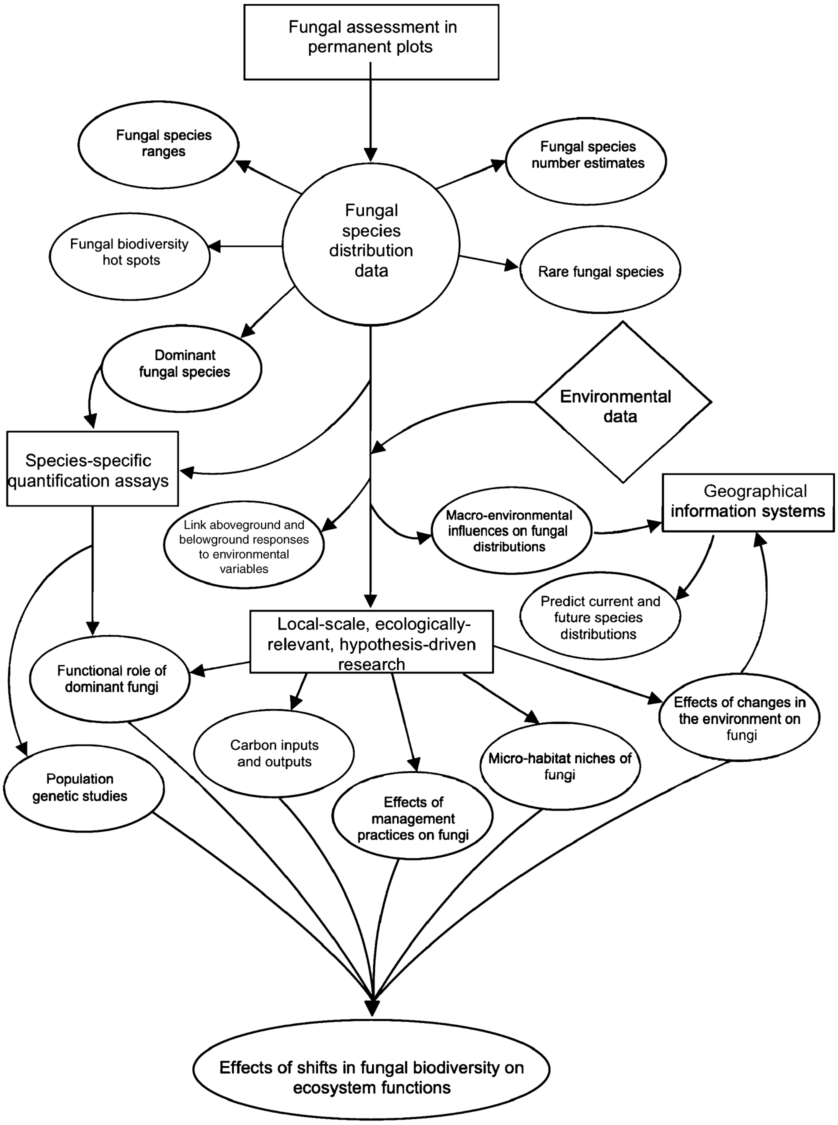

Fig. 1 The potential research outputs of global fungal biodiversity assessments. Rectangles, a research effort; ovals, a research output; diamonds, an instance when data gathered from permanent plots would enhance research efforts.

ple, Galante found that 95% of the spores observed fell within 45 cm of the mushroom from which they originated – and suggest that dispersal limitation may occur even at small to moderate spatial scales. At the community level, differences between species in dispersal strategies can explain patterns of fungal community assembly at landscape scales (Nara, 2009), and isolation and dispersal limitation can lead to significant changes in the species richness and colonization intensity experienced by mycorrhizal host plants (Dickie & Reich, 2005).

At larger spatial and temporal scales, the interplay among dispersal limitation, biogeographical history and adaptive evolution have generated an array of unique fungal assemblages, many of which are just beginning to be characterized by morphological or molecular means. Talks by T. D. Fulgenizi (Humboldt State University, CA, USA) and K. G. Peay (University of California, Berkeley, USA) both described unique ectomycorrhizal communities from the major tropical rainforests of the Amazon and Borneo, respectively. Strong latitudinal changes in fungal community structure were demonstrated for foliar endophytes by A. E. Arnold (University of Arizona, USA), who highlighted the interplay of species diversity and phylogenetic diversity from tropical to arctic environments. A. S. Amend (University of California, Berkeley, USA), presenting a 454 pyrosequencing characterization of indoor environments from every continent, found greater phylogenetic similarity of fungal communities sampled from similar latitudes.

Such latitudinal and biome-level differences in the abundance of particular species, lineages and functional groups are probably linked with ecosystem processes and plant community structure at large spatial scales. For example, the increasing prevalence of ectomycorrhizal symbioses vs arbuscular mycorrhizal symbioses from low to high latitudes and (within tropical forests) from the Amazon to southeast

Asia (Read, 1991), probably affects regional rates of carbon and nitrogen cycling. Still, relatively little is known about how mycorrhizal type and diversity interact with large-scale soil processes in most of the world.

Understanding determinants of fungal community structure across multiple spatial and temporal scales is particularly important given that fungal communities in a variety of ecosystems have been altered markedly by human activities (e.g. Arnolds, 1991; Lilleskov *et al.*, 2002; Mummey & Rillig, 2006). Since the 1980s, compelling evidence has emerged of a decline in fruiting of forest fungi in northern and central European countries (Arnolds, 1991) and modelling of bioclimatic envelopes predicts changing distributions and possible extinction for some British lichen (Ellis *et al.*, 2007). Some pathogenic and mutualistic fungi are expanding their geographical ranges (James *et al.*, 2009; Pringle *et al.*, 2009), and the phenology of fungi in some forests has changed markedly over the last 50 years, in many cases yielding not one annual fruiting season, but two (Gange *et al.*, 2007; Kauserud *et al.*, 2008). Despite the steady increase in mycological studies from tropical regions, many tropical fungal communities remain unstudied, and the continuing decline in forested areas may lead to a large loss of still uncharacterized biodiversity (Arnold & Lutzoni, 2007).

Moreover, evidence is accumulating that fungal responses to anthropogenic change may have far-reaching consequences. For example, complex changes in rates of fungal decomposition of organic matter have been observed in the context of climate alteration (Lensing & Wise, 2006; Allison & Treseder, 2008). A number of studies indicate that fungal species composition, root and/or shoot biomass, rates of herbivory and susceptibility to pathogens, and rates of nitrogen acquisition and cycling efficiency, respond to environmental changes such as elevated CO_2 (Hunt *et al.*, 2005; Chen *et al.*, 2007; Cudlin *et al.*, 2007; Clark *et al.*, 2009). In turn, these processes will shape large-scale distributions of plants and animals. For example, high specificity has been demonstrated for a number of mycoheterotrophic plants (Bidartondo & Bruns, 2002; Bidartondo, 2005), and experimental tests have shown that the distributions of these plants (many of which are rare or endangered) are constrained by distributions of one or a few species of ectomycorrhizal fungi (Bidartondo & Bruns, 2005; Bidartondo & Read, 2008). Thus, the migration of these plants and others in response to climate change may be constrained by the distribution or co-migration of fungal symbionts. Given that many fungi, as well as plants, differ in their dispersal abilities, it is likely that individual species will differ in the rate of migration in response to global change, which will inevitably lead to the creation of novel communities and interactions (Davis, 1986; Keith *et al.*, 2009). These may lead to temporary disequilibria (i.e. where species are not present in otherwise suitable environments) or to the formation of stable communities of plants and fungi much different from those we see today.

Despite compelling evidence that fungal communities are changing, and that these changes have potential ramifications for key ecosystem properties, we still have little ability to predict or generalize at the spatial and temporal scales necessary to inform sound experimental design for ecology and ecosystem science. This is primarily because we have accurate distributional data for only a small fraction of fungal species and lack the ability to extrapolate functional studies from the laboratory to the ecosystem and from single species to communities. Fortunately, our ability to map large-scale distributions is greater than ever before. From a methodological standpoint, fungal community ecologists have harnessed the power of molecular ecology to permit the following: more holistic and quantitative measures of community structure that take into account uncultured fungi and fungi that fruit infrequently; and rapid analyses at levels of biological organization ranging from genotype diversity to phylogenetic structure (Arnold *et al.*, 2007; Peay *et al.*, 2008). Concurrently, communities of researchers are rallying to enhance the quality and content of databases to accommodate and curate such data (Bruns *et al.*, 2008), and ecologists are calling for use of the baseline distribution data for mycorrhizal fungi, ranging from regional to continental scales and encompassing entire ecosystems (Lilleskov & Parrent, 2007). These efforts will use increasingly powerful next-generation sequencing methods to open up the 'black box' of fungal ecology and to identify and focus on species, lineages or functional groups that are key to providing ecosystem services.

Such a change in perspective will also require scaling from the traits of individual fungi and their individual plant partners, across multiple scales, as well as a clear research framework that identifies links between research efforts and gaps in our knowledge (Fig. 1). With this framework in mind, we propose a series of fundamental questions that we hope will motivate and guide a global fungal biodiversity assessment.

(1) What are the large-scale spatial distributional ranges for fungal species and to what extent are these determined by abiotic and biotic environmental variables vs historical patterns of dispersal and migration?

(2) Can changes in fungal distributions driven by environmental change (i.e. climate shifts, habitat loss and changing host/substrate distribution) be predicted for groups with distinct geographical distributions, and how will this affect the future distribution of symbiotic plants or animals?

(3) Are there ecologically dominant fungi in particular ecosystems? What criteria should we use to identify them? How do they contribute directly to ecosystem processes (such as carbon sequestration) and how much do they indirectly affect ecosystem processes (such as net primary productivity)?

(4) If there are widespread, dominant fungal species or lineages across biomes and environmental gradients, to what extent are they functionally and genetically homogeneous?
(5) Can data from traditional, small-scale studies be extrapolated directly to entire ecosystems, or are large-scale pilot studies required to account for interactions and non-additive effects in the scaling-up process?
(6) At what spatial scale can we detect key changes in fungal community structure that are related to essential ecosystem functions or responses to perturbations such as climate change? In other words – which scale is appropriate for detecting community responses to disturbance and at which scale do these changes in the fungal community structure translate to changes in ecosystem processes or services?
(7) Where are the geographical hot spots of fungal biodiversity and why?

The increasing interest by the broader ecological community in fungi, the existence of long-term plot networks and the increasing availability of next-generation sequencing technology make a global assessment of fungal diversity a realistically achievable goal now more than ever. We hope that these questions will help to motivate and guide such an effort and believe that the data generated will answer fundamental questions about the distribution and drivers of fungal diversity, provide baseline data for the incorporation of fungi into other ecological study programmes and help to meet the future challenges of global change.

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Key words: biogeography, dispersal, diversity, mycorrhiza, scaling, symbiosis.

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-