ELSEVIER

Fungal Ecology

Commentary

The shape of fungal ecology: does spore morphology give clues to a species' niche?

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Pick up any botany textbook, and you can read about the morphologies of flowers: "most bird flowers are colorful, with red and yellow ones being the most common… bat flowers are typically dully colored, and many of them open only at night" (Raven et al., 1992 pp. 424—425). The color, smell, and shape of a flower (as well as quality of nectar and phenology) are used to predict the kind of pollinator associated with a species. For example, red flowers that do not smell but produce high quality nectar are associated with hummingbirds, which is why hummingbird feeders are often brightly colored and filled with strong sugar water. Morphology can be manipulated to attract specific pollinators, and pollination syndromes are commonly discussed in the literature (Rosas-Guerrero et al., 2014). Seeds are also a clue to ecology, for example, plants making copious numbers of very small, light, seeds, are likely to use wind as a dispersal vector; seeds with elaiosomes are likely to be dispersed by ants (Raven et al., 1992).

What about a spore that is ornamented? Can a mycologist know anything about ecology from looking at the shape (Roper et al., 2008) or size of a spore, or the size or phenology of a sporocarp? Obviously, morphology is a clue to taxonomy; ascospores look different from basidiospores. A spore of a *Cordyceps* looks like nothing else.

But perhaps morphology can offer more. The effective dispersal of spores plays an essential role in the ability of fungi to reach and colonize new substrata. Many spore features are likely to be under adaptive selective pressures related to the particular ecological niche of a species. In fact, the morphological diversity of spores and the sporocarps holding them is astonishing (Pringle, 2013), and begs for explanation. In some cases, morphological diversity is easily explained, for example, the characteristic forms of hypogeous truffles are the result of natural selection for animal dispersal, and so hypogeous basidiomycete truffles lack functional sterigmata. Understanding these morphologies provides clues in less obvious cases, as when a newly discovered epigeous species also lacks sterigmata capable of ballistospore discharge; animal dispersal becomes an obvious hypothesis (Desjardin et al., 2011). But for the majority of spore and sporocarp morphologies, adaptive significance is unknown. Moreover, we do not know how spore features may or may not relate to a plethora of other ecological strategies – including, for example, does

the spore of a saprotrophic *Amanita* look somehow consistently different from the spore of a mycorrhizal *Amanita*?

While many modern fungal ecologists may eschew the study of spores and sporocarps as throwbacks to a pre-molecular era, Peay (2014), Halbwachs et al. (2015) and Bässler et al. (2014) ask whether there are predictable differences between the spores and sporocarps of saprotrophic and ectomycorrhizal fungi. Among other results, Bässler et al. (2014) found that saprotrophic fungi produce smaller sporocarps than ectomycorrhizal species, and Halbwachs et al. (2015) found that spores of ectomycorrhizal species are more likely to be ornamented. There are a number of challenges to these kinds of analyses – including, trophic status and spore morphology are influenced by phylogeny, and estimating independent changes in either character requires phylogenetically explicit statistical models. Meerts (1999) used phylogenetically independent contrasts to demonstrate lineage specific differences in correlations of spore and sporocarp sizes. In some cases correlations were positive (for example, in *Agaricus* and *Coprinus*) while in other cases, correlations were negative (*Psathyrella*). While Halbwachs et al. (2015) and Bässler et al. (2014) attempt to control for phylogenetic interdependence, there are reasons to query their assumptions and methods. For example, the species included in analyses do not form a monophyletic group, although they are treated as a single group, and ancestral state analyses of nutritional mode and spore surface characters are not provided. In addition, the species are taken from a single geographic region (Northern Europe), and may be dominated by particular subsets of fungal diversity, including ectomycorrhizal *Cortinarius* species with ornamented spores. Nonetheless, both articles ask questions worth thinking about, and provide a valuable path forward.

If these findings hold, what can they teach us about the selective pressures experienced by fungi in different niches? What is the relationship between spore morphology (specifically, ornamentation), dispersal, and symbiosis, and why does sporocarp size give a clue to nutritional mode? The ectomycorrhizal symbiosis has evolved at least 66 times (Tedersoo et al., 2010; Tedersoo and Smith, 2013), likely from decomposer ancestors in each case. Understanding these transitions is a major focus of mycology (Hibbett et al., 2000; Bruns and Shefferson, 2004; Wolfe et al., 2012; Kohler et al., 2015). To

date research has emphasized the genetics underpinning the evolution to symbiosis, and the evolved interaction at the interface of a fungus and plant (Plett and Martin, 2011; Martin and Kohler, 2014; Liao et al., 2014). But the emergence of an ectomycorrhizal symbiosis may well involve correlated changes in spore or sporocarp morphology, for example, perhaps a transition to ornamented spores (Halbwachs et al., 2015).

However, to our knowledge there is no research on the origins of ornamentation, nor do we know if a reversal from ornamented to smooth spores has happened in evolutionary history. The critical question is whether or not there is a fundamental difference in the habitats of saprotrophic and ectomycorrhizal fungi, so that each group requires a fundamentally different dispersal syndrome. Halbwachs et al. (2015) suggest that the predominance of ornamented spores amongst ectomycorrhizal fungi is related to the fact that their primary resource (plant roots) is buried within the soil matrix, rather than exposed as are most resources of saprotrophic fungi. Ornamentation, they hypothesize, may facilitate zoochory by soil invertebrates that would carry the spores closer to their destination. Lilleskov and Bruns (2005) showed that the knobby spores of the ectomycorrhizal fungus *Tomentella sublilacina* were effectively dispersed on the body of mites, so this is a plausible contention. But it is a single example, and we do not know the answers to questions including, is *T. sublilacina* representative of all ectomycorrhizal species, and do unornamented spores NOT cling to invertebrates? Do these mites really carry the spores from the surface to deeper layers of soil? However, Halbwachs et al.'s ideas can be tested with experiments, for example to see whether smooth spores are less effectively dispersed through soil by animals, and whether animal dispersal does indeed help move ectomycorrhizal spores deeper into the soil column or closer to host roots.

The hypothesis linking ectomycorrhizal spore ornamentation to animal dispersal is just one of many plausible conjectures, and for example, the authors might as easily have thought about water. We do know that water can move spores: one particularly elegant experiment involved *Lycoperdon perlatum*, *Fuligo septica*, and yeast. Spores of all species were moved from water in a Petri dish up an inclined glass plane to heights of more than 20 cm (Bandoni and Koske, 1974); spores moved up the plane in response to a slow trickle of water (analogous to rain) that reached and perturbed the air–water interface in the dish. There is also no doubt that water moves species differently, for example, when water is poured over soil *Penicillium* spores do not move well while spores of *Zygorhynchus vuillemini* (now *Mucor moelleri*) move easily (Burges, 1950). But as with insects, we have a limited understanding of how effective water is as a vector through soil, nor do we really understand potential interactions between water and ornamentation.

Perhaps ornamentation facilitates movement by animals, but inhibits dispersal by water, and perhaps ECM of shallow soils benefit from large, ornamented spores, while ECM associated with deep roots benefit from possession of small, smooth spores. This kind of mechanism would help explain or reinforce the strong patterns of vertical zonation seen in many soil fungal communities (Dickie et al., 2002; Taylor et al., 2014). Perhaps in some cases the depth of a species' habitat is more of an influence on morphology than trophic status.

In some cases, literature outside of mycology proves useful. For example, the dispersal of bacteria through soil has also been an active area of research. Cell size clearly plays a role in bacterial dispersal by water; smaller bacteria move more easily with water (Gannon et al., 1991) but soil properties, specifically the size of the spaces or pores within soil, also influence migration (Huysman and Verstraete, 1993). Hydrophobic bacteria move two to three times more slowly than hydrophilic strains, because hydrophobic strains stick to soil particles (Huysman and Verstraete, 1993). Perhaps hydrophilic fungal spores will also move more easily than hydrophobic fungal spores and, because fungal spores are often coated with numerous hydrophobic proteins, termed hydrophobins (Whiteford and Spanu, 2002), the potential for species to
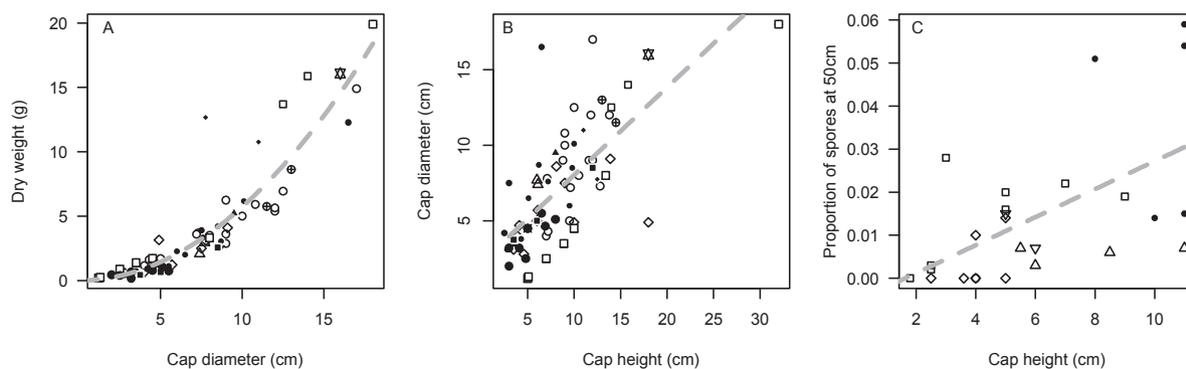
Fig 1 – Allometric relationships between sporocarp characteristics of ectomycorrhizal fungi. Data are measurements made from 69 sporocarps of 15 species collected at Point Reyes National Seashore by KGP during 2008–2009. Each species is represented by a unique symbol. (A) Relationship between cap diameter and total dry weight. Exponential relationship was fit using the NLS2 package in R. (B) Relationship between cap height (measured from the base of the stipe to the surface of the hymenium) and cap diameter, with best fit linear regression shown. (C) Relationship between height of the hymenium and spore dispersal. Spore dispersal was estimated by counting spore deposits on Vaseline coated glass slides placed overnight at different distances away from marked sporocarps. The figure shows the proportion of observed spores falling in the longest distance class (50 cm) and suggests cap height has a positive effect on mean spore dispersal distances.

evolve differences in hydrophobicity is enormous. However, at least one study suggests something quite different: hydrophobins are required for efficient water mediated dispersal of *Cladosporium fulvum* (Whiteford and Spanu, 2001).

Complex questions and arguments also surround the link between sporocarp size and nutritional mode (Bässler et al., 2014). Large sporocarps convey obvious ecological advantages. Larger caps provide more surface area for spore production, and taller mushrooms enable a higher release point for spores, making it more likely that spores will travel long distances (Galante et al., 2011, Fig 1). But these advantages come at a cost, as increasing cap size requires non-linear increases in total biomass (Fig 1). Perhaps ectomycorrhizal fungi can afford larger sporocarps because they access a more predictable source of carbon (Bässler et al., 2014), thereby generating the correlation between niche and sporocarp size. Or perhaps saprotrophic fungi require smaller sporocarps because species often occupy ephemeral patches of habitat, needing to move among habitats annually, and so fruit even when resources are scarce or the environment is suboptimal. Smaller sporocarps may enable consistent reproduction, year after year. Or perhaps there are other explanations. How fungi store and mobilize the carbon and nitrogen needed to produce sporocarps remains an open question (Taylor and Alexander, 2005), and one novel tool to explore the question would involve tracing the carbon and nitrogen sources for saprotrophic fungi that form large sporocarps, for example *Agaricus*, *Macrocybe*, *Leucopaxillus*, and *Macrolepiota*.

While scientists have thought about the shapes of flowers and seeds for hundreds of years, relatively less thought has been directed at sporocarp or spore morphology, except in contexts of taxonomy. Notable exceptions include early work by A. H. R. Buller, E. Parmasto, C.T. Ingold, and H. Clémençon, and more recent research describing relationships between ecological strategies, and sporocarp and spore size, among polypores and autumnal basidiomycetes (Kauserud et al., 2008, 2011). The papers by Halbwachs et al. (2015) and Bässler et al. (2014) highlight our fundamental ignorance about basic aspects of natural history, ideally, they will inspire a new wave of critically needed studies.

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