Molecular windows into the below-ground interactions of ectomycorrhizal fungi

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Over the past decade the use of molecular techniques has provided new ways to study old questions about how ectomycorrhizal fungi interact with each other and their environment. In their simplest form, these methods enable researchers to identify vegetative stages of fungi that would be difficult, slow, or impossible to identify by morphological or culture-based methods. This has resulted in quantitative views of complex fungal communities, which in turn have revealed new and unexpected patterns in community structure. In addition, molecular methods have been used to identify individual genotypes of fungi. This information has provided insights into the way that particular species of ectomycorrhizal fungi spread in nature. In this article we will mention the basic techniques and briefly discuss some of the findings that have resulted. Advances in this field have recently been comprehensively reviewed for scientific audiences, and we refer readers that have a more technical interest or a need to access the current literature to these reviews (Dahlberg, 2001; Horton & Bruns, 2001).

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Molecular systematics branches into molecular ecology

For the past decade mycology has been in the golden age of molecular systematics. Gene sequences, particularly of the ribosomal RNA genes and spacers, have been determined from large numbers of fungi, compared, and used to test evolutionary hypotheses and ultimately to rework taxonomy and classification. The impact has been huge, and many of the basic techniques have now become part of the mainstream of fungal systematics. These developments in molecular systematics have also directly facilitated the development of what is sometimes referred to as “molecular ecology”. This latter term may incorrectly conjure up an image of studying interactions among molecules in an environmental context, but it is really the study of ecology facilitated by molecular techniques.

In an ecological context molecular techniques are typically used for identification of individual genotypes, species, and higher taxa, and are just beginning to be used to identify and quantify where and when specific genes are expressed in the environment. The molecular systematic work facilitated these developments in two ways: i) many of the techniques employed in systematics were directly transferable to ecological applications, and ii) the sequence data generated from systematic studies provided the basis for identifications in ecological settings.

The basic methods

Most of the molecular techniques employed use the polymerase chain reaction (PCR) as a starting point. This method, which is mentioned repeatedly in other papers in this issue, and illustrated in an earlier review by Mitchell et al. (1995), is particularly useful in ecological studies because of its sensitivity. It enables one to pick out specific fragments of DNA from minute samples and amplify them to useful quantities. The amplification can be taxon-specific. It can be designed to selectively amplify for example only fungal, only basidionycetaceae, or only plant sequences. This is important because environmental samples (e.g., roots or soil) frequently have DNA of many organisms present.

Once DNA fragments are amplified they can then be sequenced directly, and the sequences can then be used to identify the organism from which the molecules were derived (see Fig 1). Fragments can also be cut with restriction enzymes, and the resulting fragment patterns can be compared to those derived from identified fungi – this is the restriction fragment length polymorphism (RFLP) method. The beauty of this approach is that it is fast, and relatively cheap.

When the main purpose of the technique is identifi-
Fig 1 The snow plant, *Sarcode sanguinea* (Ericaceae, Monotropoideae), grows in conifer forests of Oregon, California, and Baja California. It is among the largest myco-heterotrophic plants: a non-photosynthetic plant that obtains all of its nutrition from green plants via shared mycorrhizal fungi. The bright red inflorescences of the snow plant emerge from a coralloid mass of mycorrhizal roots (A, B, C, D). In order to examine plant-fungal specificity, fungal genes (the ITS region in this example) can be amplified from individual root tips via the polymerase chain reaction. These genes can then be sequenced and used to accurately identify the mycorrhizal fungi involved in these disparate symbioses. In the example shown, these results and others demonstrated that throughout most of its geographic range the snow plant only associated with fungi in the *Rhizopogon ellenae* species group, while the related plant, *Pterospora andromedea*, associated with two different related groups of *Rhizopogon*.
cation of unknown fungi, the sequences that are targeted for amplification have almost always been the ribosomal RNA genes and spacers. The advantages of these regions for evolutionary studies have been discussed elsewhere (White et al., 1990; Mitchell et al., 1995). There are three basic reasons why they are also popular for ecological studies: 1) they are universally present—thus, no additional prior sequence knowledge is required to target them; 2) they are present in high copy number—this means that the sensitivity of the assay is high, since a few viable cells provide hundreds of target copies; 3) a huge amount of data is available—this often makes it possible to identify unknown sequences. The internal transcribed spacer region, or ITS, is the most commonly targeted region for identification (38,089 accessions in the database). This region exhibits all three of the features listed above, but it also has one additional advantage that accounts for its popularity: it is often highly variable between species. The reason for this variability is that at least two thirds of the region does not code for any gene. As a result, it is not very constrained by selective pressures and is free to accumulate mutations over time.

Who is there?
One of the most basic questions in ecology is “who is there?”. If all fungi fruited each year in a predictable time and in proportion to their presence at a site, this would be an easy question to address. But as readers of this journal are well aware, fungi are not that cooperative. When they are present only as mycelium either in the soil or associated with roots, they are often very difficult to find and identify. This is a setting where molecular approaches have proven to be very useful. For example, in ectomycorrhizal communities it has been relatively easy to visually sort colonized root types and then identify the fungi on them by using the techniques discussed above (Fig. 1). The results from these studies have revealed complex species-rich communities that vary at fine spatial scales. This is not too surprising as studies based on fruiting also show this pattern (Struhtsma et al., 2001). What was unexpected was that the most abundantly fruiting species were often rare on the roots, and conversely the most abundant species on roots were often poorly represented in the fruiting records (Dahlberg, 2001: Horton & Bruns, 2001). This pattern is due in part to the high abundance of resupinate basidiomycetes such as Tylospora, Piloderma, and Tomentella, but the reason for the apparent ‘missing’ root tips for the abundant fruiters is not yet fully understood. A second surprising finding is that non-photosynthetic plants, such as Menotrpora or Condolworthia, that tap into mycorrhizal fungi to make their living, have very specific fungal associations (Bilateral & Bruns, 2001). Previously, it had been hypothesized that they were generalists with many fungal associates, much as is the case with green plants. This idea made sense because being a generalist would seem to increase a plant’s chances for finding a compatible fungus. One might expect plants that are completely dependent on mycorrhizal fungi such as non-photosynthetic plants, to be the least specific. The fact that the reverse is true forces us to rethink our ideas about the advantages of specialization in mycorrhizal systems.

What happens when systems are disturbed?
A common question in community ecology is what are the effects of disturbance. Molecular techniques have now been applied to this question in mycorrhizal communities for disturbances such as fire, logging, and nitrogen enrichment. These disturbances are common in the forested settings in which ectomycorrhizal fungi are found. Each of these factors had been previously investigated either by fruiting records or by crude morphotyping of mycorrhizal roots, but molecular approaches have now provided a much higher resolution view of the community-level response. In the case of fire, it is now clear that the dominant species change when canopy trees are killed and replaced by seedlings. At least in some settings, many of the species that colonize after fire were already present as resistant propagules (a “spore bank”) prior to the fire (Baar et al., 1999). In less severe fires the effects on the fungal community may be less extreme, with no obvious difference between burned and unburned forest after several years (Jonsson et al., 1999). Nitrogen deposition had been previously shown to suppress fruiting, but the below-ground response was unknown. Recently, several molecular studies have shown that nitrogen enrichments do cause a shift in species below ground, but interestingly the fruiting response is more rapid and obvious than the root-level changes (Peter et al., 2001).

How do they spread?
A second set of fundamental ecological questions is how does a particular species establish and spread, and does it persist over time. For fungi the two basic ways that they can do this are by spore or by vegetative growth. These two alternatives have different genetic outcomes, and so molecular methods can be used to differentiate them. For example, in most basidiomycetes, where the spores are products of meiosis, establishment by spore results in new, unique genotypes, while vegetative spread results in identical genotypes. Several
PCR-based methods have been used to test for genetic identity and to map the spatial extent of individual genotypes.

From earlier work it was thought that vegetative growth probably accounted for much of the colonization in undisturbed forests, and multiple studies with *Stiluit* species and a few other taxa seemed to support this view. Recently, however, several studies have shown that many species frequently establish by spore in undisturbed forest settings. These include *Laccaria amethystina*, *Russula cremoris*, *Lactarius xanthogalactus*, and *Amanita francheti*. This is interesting for at least two reasons. First, some of these species are common below-ground dominants; a distinction that one might expect to be achieved by vegetative growth. Second, the spores of most of these taxa are virtually impossible to germinate under laboratory or greenhouse conditions; thus some additional conditions must occur in nature that facilitate germination and establishment. Clearly we have much more to learn even about even the dominant fungi in forest systems.

Where is this going next?

We now have two conflicting views of mycorrhizal communities: the fruiting pattern and the root-colonizing pattern. Each gives a different picture of which fungi are most important. Other views are also possible and will likely be informative. The mycelial view—which species have the most mycelium in the soil—is an obvious one. At least three independent groups of researchers reported the first mycelial views at the recent International Congress of Mycorrhizae in Adelaide, Australia [<http://www.waite.adelaide.edu.au/SolWatre/3icom.htm>]. Perhaps the most interesting result reported was that mycelia of ectomycorrhizal fungi were at least as common and perhaps much more common than those of saprobiic fungi in forest organic layers. Although quantification issues remain to be worked out, it is clear that the mycelial view will be a very important one.

So far, molecular techniques have been used primarily as markers for species or individuals, but the real potential may lie in an expression view; quantifying the gene expression for key enzymes. In mycorrhizal systems genes related to phosphorus and nitrogen acquisition as well as cellulose and lignin degradation would be obvious targets. To achieve such a goal, detailed knowledge of the genomes of individual species will be required. This may sound like it is a long way off, but such work is well underway in several laboratories (Martin, 2001). The future of molecular ecology is a bright one, and we are now only a few years into it. The real beauty of it is that it provides us with a new set of tools to answer many basic questions about how fungi make their living and compete in complex communities. Although molecular ecology may seem like an exotic road to travel, it is really leading back to a familiar goal: a better understanding of the natural history of the fungal world.

References


