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# Molecular analysis of ectomycorrhizal fungal communities

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**Abstract**: Despite advances in mycorrhizal identification, the goal of elucidating the structure and development of mycorrhizal communities remains elusive. Fruit body production can be sporadic, morphological typing of mycorrhizae is subject to variation with environmental conditions or host, and cultural studies are labor intensive and miss fungi that cannot be isolated. Molecular techniques for identification of fungal symbionts can supplement these techniques and offer an approach that is rapid, is independent of environmental variation, and can be applied directly to large numbers of samples. Molecular approaches to mycorrhizal community analysis attempt to distinguish taxonomic groups so they can be monitored and their interactions studied. Initial characterization of community structure involves enzymatic amplification of DNA directly from mycorrhizal roots using fungus-specific primers, followed by restriction endonuclease digestion to produce taxon-specific restriction fragment patterns. Comparison of these patterns with those obtained from fungal fruit bodies or reference cultures facilitates identification of fungal symbionts. Phylogenetic relationships of fungi that cannot be matched to reference isolates can be inferred by sequencing enzymatically amplified DNA. Future directions that will result from molecular approaches include development of sampling strategies, resolution of species complexes, field observations of host specificity, elucidation of the dynamics of replacement processes (succession), and determination of the role of dispersal in community development. As additional techniques are developed for population analysis, resolution of questions related to genetic structure, variation, and gene flow will become feasible.

*Key words*: molecular ecology, fungal community structure, PCR.

**Résumé** : En dépit des progrès sur l'identification des mycorhizes, l'objectif de comprendre la structure et le développement des communautés mycorhiziennes demeure difficile. La production des fructifications peut être sporadique, la caractérisation des mycorhizes est sujette aux variations des conditions du milieu et de l'hôte, et les études culturales exigent beaucoup de travail et négligent les champignons qu'on ne sait pas cultiver. Les techniques moléculaires pour l'identification des symbiotes fongiques peuvent suppléer à ces déficiences, et ouvrent une avenue rapide, indépendante des variations du milieu, qui peut être appliquée directement à un grand nombres d'échantillons. Les méthodes moléculaires appliquées à l'analyse des communautés visent à distinguer les groupes taxonomiques, de façon à ce qu'on puisse les suivre et étudier leurs interactions. La caractérisation initiale de la structure communautaire implique l'amplification enzymatique de l'ADN, directement à partir des racines mycorhizées, en utilisant une amorçe spécifique au champignon, suivi d'une digestion par des endonucléases de restriction pour produire des patrons de restriction spécifiques aux taxons. Une comparaison de ces patrons avec ceux obtenus à partir de fructifications de champignons ou de cultures de référence, permettent l'identification des symbiotes fongiques. Les relations phylogénétiques des champignons qui ne peuvent pas être identifiées avec les isolats de référence peuvent être déduites par le séquençage d'ADN enzymatiquement amplifié. Les nouvelles avenues qui pourront être explorées grâce aux techniques moléculaires, incluent les stratégies d'échantillonage, la résolution de complexes d'espèces, les observations au champ de la spécificité des hôtes, la compréhension des processus dynamiques de remplacement (succession), et la détermination du rôle de la dispersion dans le développement de la communauté. A mesure que de nouvelles techniques se développeront pour l'analyse des populations, il deviendra possible de répondre aux questions portant sur la structure génétique, la variation, et le flux de gènes.

*Mots clés* : écologie moléculaire, structure des communautés fongique, PCR.
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## Introduction

A biological community is defined by its species composition and the interactions among those species. Species composition depends upon rates of loss or gain of species from the community, which in turn depends upon the population dynamics of individual species. Population dynamics are controlled by processes that alter allele frequencies (selection, gene flow, and drift) and stochastic events acting on populations. Thus communities are complex units that are constantly in flux as species interactions ebb and flow and as species are added to and lost from the community.

Plants form several types of mycorrhiza, including vesicular–arbuscular mycorrhizae (VAM), ectomycorrhizae (ECM), ectendomycorrhizae (a variant of ECM), ericoid, and orchidaceous. Therefore, mycorrhizal communities can be composed of one to several types of mycorrhiza, depending upon the specificity of host and fungus. This paper will concentrate on fungi that form ecto- and ectendo-mycorrhizae. I will first discuss some factors that influence mycorrhizal community structure.

### Host specificity

Host specificity plays a major role in dictating mycorrhizal community structure. Molina et al. (41) discuss several components of specificity. Of principle importance is host range of the fungi. Molina et al. (41) grouped ectomycorrhizal symbionts along a host range continuum, from narrow (often genus specific), to intermediate (often family specific), to broad (able to colonize several different orders or classes of host). Host receptivity to mycorrhizal colonization also varies, ranging from nonreceptive (nonmycorrhizal) to receptive. Among receptive hosts, receptivity may be narrow (forms mycorrhizae with a relatively small number of fungi) to broad (forms mycorrhizae with a large number of symbionts) (41).

It is important to acknowledge that specificity can be more than the sum of interactions between host and fungus combinations, there may also be higher order interactions. Massicotte et al. (38) report that some *Rhizopogon* species that were restricted to one host in monoculture were able to extend their host range to a companion plant in dual culture. They conclude that the colonization potential of a fungal mycelium depends upon whether it is linked to a compatible host. Competitive exclusion may also prevent certain host–fungus combinations from occurring in the field.

### Connections between hosts

Read (see Ref. 48) has documented the importance of nutrient flow between plants via their mycorrhizal mycelium in laboratory systems. Observations of this type have led to speculation that mycorrhizal linkages may play a key role in mycorrhizal community structure and dynamics (44). Perry et al. (45) used the term guild to refer to linked networks of ectomycorrhizal hosts. Thus a network of hosts with a common set of mutualists could function as an additional hierarchical layer between populations and communities.

There is some evidence for this hypothesis. Perry et al. (46) found that competition among mixtures of hosts varied depending upon whether they were inoculated with narrow or broad host range symbionts, suggesting that belowground interactions were mediating competition. The concept of guild may also extend across mycorrhizal types. Although the majority of plants maintain fidelity to a single class of mycorrhizae, a substantial number of hosts form more than one type of mycorrhiza (see Ref. 41). Therefore, the opportunity exists for participation of a single host in more than one guild simultaneously.

### Competition

To persist as an active component of the community, mycorrhizal fungi must successfully exploit a continuum of physicochemical environments, from the interior of the host root to the soil environment surrounding the root. Persistence depends upon the success of a symbiont in exploiting these niches and in competing with other fungi for root resources. Competition may be for existing root tips or new, uncolonized root tips. Competition for existing root tips may result in replacement. Competition for new root tips depends upon colonization potential. If the root tip grows slowly, then the resident fungus will usually successfully colonize at root flush (26). However, if roots grow more rapidly than the hyphae, there is an opportunity for colonization by fungi on adjacent roots or in the soil (59).

### Dispersal

If unable to successfully compete, mycorrhizal fungi must disperse in time or space to avoid local extinction. Temporal dispersal involves production of resting stages, such as sclerotia, chlamydospores, or meiospores (ascospores or basidiospores). Resting stages may be long lived. Sclerotia of *Cenococcum geophilum* can survive for several years in clear-cut forests (50) and spores of some ascomycetes may remain dormant for many years between cycles of activity (56).

Symbionts may also form inactive stages in roots, much like the latent pathogens described by Wilcox (60). Formation of sclerotial bodies and sclerotiallike inclusions has been described in dematiaceous root endophytes (61) and in post-fire ascomycetes that colonize roots (17). Whether this is a significant strategy for mycorrhizal fungi is not clear. Symbionts are known to remain alive for months after the above-ground portion of the tree has died (22) and could potentially maintain this condition for years in living roots. Therefore, a mycorrhizal community is also defined by its past history in terms of the inoculum potential of resting structures, as well as the current complement of active mycorrhizae.

Most ectomycorrhizal symbionts appear to be well adapted for spatial dispersal via active or passive discharge of air-borne meiospores from fruit bodies. Hypogeous basidiomycetes and ascomycetes are specialized for dispersal by animals, including mammals, reptiles, and soil invertebrates. Only a few mycorrhizal fungi produce conidia, such as the ascomycetes *Sphaerosporella brunnea* and *Muciturbo* spp. (56), and the dematiaceous hyphomycetes (9). Resting structures, such as sclerotia and chlamydospores, may also act as propagules for spatial dispersal via passive movement of soil by wind or water (41).

### Environmental heterogeneity

Heterogeneity of the environment exists on several scales. Large-scale disturbances, such as fire, alter large tracts of the landscape and repeated cycles of perturbation result in a mosaic of different-aged stands. Within a stand, local disturbance, such as wind throw and recruitment of new hosts, causes a patchy habitat distribution. The soil environment is

also highly heterogeneous and can be partitioned into multiple niches, each with its own unique combination of nutrient source, moisture level, physicochemical properties, and particle size distribution.

Environmental heterogeneity contributes to the diversity of community structure by allowing coexistence of fungi with different ecological specificities. There is evidence that heterogeneous selection in local populations can maintain or increase genetic variability in genes that control ecological traits (39). Environmental heterogeneity would provide considerable opportunities for maintenance of ecological diversity among mycorrhizal fungi.

## Analysis of mycorrhizal communities

The objective of mycorrhizal community analysis is to identify taxa that comprise the community and monitor them and their interactions with other members of the community over time. Traditional approaches to community analysis include collection of fungal fruit bodies, morphological typing of mycorrhizae, and isolation of mycorrhizal fungi from roots.

Studies of fruit bodies have been used to characterize habitat types (6, 8, 43), document successional changes (13, 54), and monitor long-term decline in mycorrhizal communities (4, 31). This approach can be rapidly applied by experienced mycologists, but suffers from the periodicity and seasonality of fruiting and misses altogether those mycorrhizal taxa that do not fruit or form inconspicuous fruit bodies. There is also dispute as to the extent of correlation between aboveground and belowground communities (33).

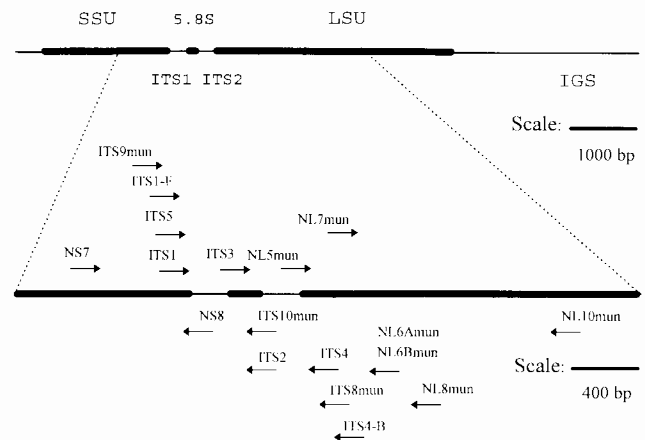
Morphological characterization has long been used to distinguish mycorrhizal roots (see 64). Agerer (1) has recently expanded and refined this approach. This technique has been applied to mycorrhizal communities, including studies of outplanted seedlings (10, 11), the impacts of perturbation on community structure (20), and studies of host specificity (38). An advantage of this approach is that mycorrhizal types can be rapidly identified by an experienced researcher. The major problem is that the extent of intraspecific variation in morphology among taxa and of the same taxon on different hosts or different environments is not well known.

The most labor-intensive technique to differentiate taxa is to isolate the fungi from roots (40). This approach has been used extensively to characterize communities (see Refs. 11 and 30) and allows differentiation of fungi that form superficially similar mycorrhizae. Problems include intraspecific variation in culture morphology (e.g., Refs. 18 and 53), and many mycorrhizal symbionts do not grow in culture.

Molecular approaches alleviate most of these problems because the techniques are largely independent of environmental or host variation. Although serological techniques have been advocated to study communities (2), most community analyses now use the polymerase chain reaction (PCR), a technique for enzymatic amplification of DNA sequences from minute quantities of template DNA (42). Since DNA sequences are amplified directly, PCR can be applied to any tissue, including cultures, fruit bodies, or mycorrhizal roots.

Since a primary objective of community analysis is to distinguish species, the molecular markers used should be universally conserved among the organisms studied and exhibit maximum variability between species and minimal variation within species. The nuclear-encoded ribosomal DNA

**Fig. 1.** Eukaryotic nuclear-encoded ribosomal RNA gene repeat (rDNA). The upper diagram shows the organization of the structural RNA coding genes (SSU, small subunit or 18S gene; LSU, large subunit or 28S gene) and spacer regions (ITS, internal transcribed spacer; IGS, intergenic spacer). The lower diagram expands the part of the rDNA that is commonly used for PCR–RFLP and DNA sequencing analysis. Arrows (not drawn to scale) indicate the position of primer annealing sites and direction of extension. Primers with a "mun" suffix were designed by the author and have the following sequences (written 5′ to 3′): ITS8mun, CTT CAC TCG CCG TTA CTA; ITS9mun, TGT ACA CAC CGC CCG TCG; ITS10mun, GCT GCG TTC TTC ATC GAT; NL5mun, GCA TAT CAA TAA GCG GAG GA; NL6Amun, CAA GTG CTT CCC TTT CAA CA; NL6Bmun, CAA GCG TTT CCC TTT CAA CA; NL7mun, TTG GGA ATG CAG CTC TAA ATG; NL8mun, TTG GTC CGT GTT TCA AGA CG; NL10mun, GGA ACC TTT CCC CAC TTC. ITS1-F and ITS4-B were described by Gardes and Bruns (23). The remaining primers are from White et al. (58).




(rDNA), which encodes the structural RNAs in the ribosome, largely satisfies these criteria. The rDNA (Fig. 1) is tandemly repeated and highly conserved in prokaryotes and eukaryotes. Ribosomal RNAs are also encoded in the mitochondria and have been used for molecular identification (7).

An advantage of the rDNA for species identification is that molecular drive (15) tends to homogenize the rDNA repeats within a species, although intragenomic variation between repeats, particularly in highly variable regions, is known to occur (see Ref. 28). Another advantage of the rDNA is that a range of variation is exhibited by different regions, with the ribosomal RNA coding regions being most conservative, the internal transcribed spacers moderately conserved, and the intergenic spacer most variable (see Ref. 28).

The region of the rDNA most commonly amplified and studied for community analysis is shown in Fig 1. Various primers have been designed to amplify this region. Some are from highly conserved regions and thus will amplify across a wide range of organisms; others have been designed to specifically amplify only fungi or certain groups of fungi.

### PCR–RFLP analysis

The simplest method of assaying amplified DNA segments for sequence variation is by restriction fragment length poly-

morphism (RFLP) analysis. This involves digestion of the amplified fragment with restriction endonucleases followed by gel electrophoresis. Restriction endonucleases recognize a specific sequence (usually 4–6 base pairs) and cleave the DNA where that sequence occurs. We routinely use four enzymes, *Alu*I (AGCT), *Hha*I or its isoschizomer *Cfo*I (GCGC), *Rsa*I (GTAC), and *Hin*fI (GANTC), that cleave frequently and have no overlap in their recognition sequences. This ensures that all mutations detected by restriction digests are independent.

Several studies have amplified the ITS region followed by PCR–RFLP analysis to distinguish isolates. Gardes et al. (25) demonstrated the efficacy of primers ITS1 and ITS4 (58) to distinguish isolates of *Laccaria* spp. and *Thelephora* from pure cultures and from mycorrhizal roots. Although ITS1 and ITS4 are highly conserved across a diverse range of taxa, apparently there were sufficient differences with the plant host DNA that the fungal DNA was amplified preferentially. More recently Gardes and Bruns (23) have described primers ITS1-F and ITS4-B that are fungal specific and that have been optimized to amplify basidiomycete DNA. Henrion et al. (27) used several sets of primers (including ITS1 and ITS4) to amplify the ITS region, the nuclear small subunit gene, and the nuclear large subunit gene from various ectomycorrhizal fungi. Primers were also described to amplify a portion of the IGS region from the 3′ end of the large subunit gene to the 5S gene, which is located in the IGS in some fungi. These same primers were used by Erland et al. (21) to characterize the basidiomycete *Tylospora fibrillosa* and other ectomycorrhizal fungi. Again the ITS region was useful for distinguishing species, but the portion of the IGS amplified exhibited no intraspecific variation among the isolates of *T. fibrillosa* studied. Stoyke et al. (53) used a larger fragment to characterize endophytic fungi from the roots of alpine plants. They used primers A (=ITS9mun) and D (=NL10mun) to amplify a fragment approximately 2.1 kilobases (kb) in length from cultures isolated from roots. Restriction analysis of this fragment identified *Phialocephala fortinii* as the dominant root endophyte on these subalpine sites.

Choice of primers for PCR–RFLP analysis should take into account several factors: (*i*) the taxonomic diversity of the fungal isolates being studied, (*ii*) specificity of the primers, and (*iii*) the presence of introns.

The ITS region is sufficiently variable that species-specific RFLP patterns can be easily distinguished using one or two restriction enzymes. This is partly due to the low selection constraints on this region, but also to the fact that insertion/deletion events are common and easily detectable by gel electrophoresis. The ITS region allows discrimination of closely related species but may not be sufficiently variable to distinguish sibling biological species. Anderson and Stasovski (3) were forced to use the IGS region to distinguish biological species of *Armillaria* after the ITS proved too homogeneous. Species-specific restriction patterns may also be found in divergent regions of the rRNA coding genes, particularly in the eukaryotic D1 and D2 divergent domains near the 5′ end of the large subunit gene (29).

Specificity of primers dictates the type of material that can be used for amplification. Highly conserved primers can only be used to amplify PCR products from pure cultures to avoid spurious amplification of nonfungal sequences. Fungal-specific primers can be used to amplify from fruit bodies or from mycorrhizal root tips without danger of contamination.

To maximize the potential to distinguish species, obtain desirable levels of specificity, and avoid intron-containing regions, we designed the primers NL6Amun and NL6Bmun. These primers are fungal specific and do not amplify plant DNA. The primers differ at just two positions (NL6Amun is matched to the consensus sequence of several Pezizales, while NL6Bmun is matched to several Agaricales). They exhibit specificity among fungi at annealing temperatures above 56°C, although the variable sites are not universally conserved in ascomycetes and basidiomycetes. When paired with the primer ITS5 or ITS1 (58), a region of about 1 kb, extending through the ITS region and the first divergent domain in the large subunit gene, is amplified. ITS5 and ITS1 anneal to sites only two bases apart, but ITS5 occurs immediately upstream of an intron in some ascomycetes (18, 49). Amplification of this intron may confuse RFLP analysis (18). ITS1 avoids the intron but has a tendency to form a primer dimer and to misprime at several sites in the rDNA repeat unless annealed at temperatures above 48°C.

### DNA sequencing

Restriction analysis, while useful for screening isolates, only detects a small amount of the variation present. While this may be sufficient for distinguishing taxa and identifying isolates by comparison to fruit bodies, it is not adequate for determining phylogenetic affinities of unknown isolates. More information can be obtained by DNA sequencing.

An advantage of the ITS5/ITS1–NL6Amun fragment for PCR–RFLP analysis is that we are able to use it as a template to sequence the divergent domain D1 of the large subunit gene using the primer pair NL5mun–NL6Amun. Once this sequence is computer aligned with a diverse taxonomic group of reference isolates, phylogenetic analysis can be undertaken to infer phylogenetic affinities of the unknown symbiont. The NL5mun–NL6Amun region is generally too variable for reliably inferring phylogenetic relationships above the family level. Nonetheless, the analysis allows us to place unknowns into the major fungal groups and determine the closest relatives among reference taxa.

### Genus- and species-specific probes

An alternate approach to identification is to design species- or genus-specific PCR primers or probes. Simon et al. have described family-specific (51) and genus-specific (52) PCR primers for VA mycorrhizal fungi. This approach has also been used to design genus- and species-specific primers for E-strain fungi (K.N. Egger, unpublished data). Such primers are useful for studies of specific taxa or closely related groups of taxa, since they allow amplification and identification of the target fungi directly from roots. A similar approach is to design highly specific oligonucleotide probes that can be hybridized to DNA extracts from roots to detect specific genera or species (7, 25).

## Future directions

Molecular techniques provide methods to supplement or replace conventional methods of assessing mycorrhizal com-

munities. As with any new technology, this opens up new research areas that would otherwise not be feasible to undertake. I will shift my attention to some future directions that will develop as a result of molecular methodologies.

## Sampling

Few studies have attempted to resolve the question of sample sizes required for community analysis. Tews and Koske (55) examined sample size in relation to VAM communities. Estimates of VAM spore density required at least 40 samples and even then the standard error was rarely less than 50%; to achieve a standard error of 95% may require thousands of samples. Estimates of species richness were more feasible, requiring from 9 to 30 samples to recover all the VAM species in the dune ecosystems studied. Equivalent studies need to be done for ectomycorrhizal fungal communities, which are more species rich than VAM communities, to determine numbers of root tips, cores, and plots necessary to accurately assess species richness and population numbers in stands.

## Analysis of community structure

Many research questions relate to species distributions within communities and to the specificities of individual taxa. These studies rely upon the ability to distinguish species.

### *Resolution of species complexes*

One of the major impacts of molecular analysis will continue to be resolution of species complexes. Many highly variable species that appear to be physiologically and ecologically diverse, when studied using molecular methods, turn out to be complexes of biologically distinct species (3, 16, 19, 24, 29, 35). Molecular methods are particularly powerful for resolving species complexes, which is a prerequisite to differentiating taxa for community analysis.

### *The role of host specificity in community development*

We know much less about host specificity in the field than we do in the laboratory. One would expect fungal community composition to change with ingress of hosts that form different types of mycorrhiza or that form mycorrhizae with different fungal taxa within a class of mycorrhiza. These relationships need to be studied and will benefit greatly from molecular approaches applied to field situations. Another important question is the relationship between host diversity and mycorrhizal diversity. Few studies have examined whether mycorrhizal communities are more diverse in pure stands of the host versus mixed stands. To understand the role of host specificity in community development, we need field studies based upon powerful molecular techniques.

### *Dynamics of replacement processes*

Replacement of mycorrhizal types may occur at many scales, from seasonal replacement with spring and fall root flush to long-term replacement corresponding to successional patterns in the host community. Lodge and Wentworth (36) also document negative associations among VA and some ectomycorrhizal fungi that suggest replacement is taking place. The presence of multiple mycorrhizal fungi on single root tips appears to be common (5, 36) and indicate either that replacement is occurring or symbionts are coexisting. Marks and Foster (37) reported that 1–4% of mycorrhizal roots in one study had two symbionts, and there have been reports of up to three symbionts on a single root (65). Molecular approaches are particularly effective at detecting multiple symbionts on single roots (25) and this approach is particularly effective using fungal-specific primers. Further studies to document the dynamics of multiple symbioses will allow us to determine if these observations represent coexistence, perhaps between fungi with different ecological specificities or forming different mycorrhizal types, or a replacement process.

A succession from early- to late-stage symbionts has been proposed based upon a decade-long study of mycorrhizal fruit bodies in a newly planted birch stand (13). On a spatial scale, as the root systems of the trees extended outward so did the occurrence of early-stage fungi, with late-stage fungi fruiting closer to the base of the tree. Temporally, the diversity of species increased as stands aged and early-stage fungi appeared to be replaced in older portions of the root system by late-stage fungi, which often had narrower host ranges.

While these studies propose a successional model for community development, several researchers have noted that some supposedly early-stage fungi remain on roots for extended periods of time and narrow host range fungi can be primary colonizers (12, 41). More important may be the position of the host in the successional sequence. Kropp and Trappe (32) observed that western hemlock was colonized by typically broad host range fungi and hypothesized that host-specific fungi were more common on pioneering plants than on later stage plants such as hemlock. These and other observations led Molina et al. (41) to the conclusion that the successional model is inadequate when applied to highly heterogeneous natural forests, where environmental heterogeneity may allow early to late and narrow to broad host range fungi to persist. Molecular studies of species composition of mycorrhizal communities and determination of the scale of environmental heterogeneity will help to resolve this debate.

### *The role of dispersal in community development*

Further research on the dynamics of mycorrhizal communities should help clarify the role of dispersal in community development. Most mycorrhizal fungi produce airborne dispersive propagules, usually meiospores. However, Deacon and Fleming (13) suggest that there are differences in spore germination between early- and late-stage fungi. Spores of early-stage fungi, which they propose are ruderal species that establish readily from spores, germinate at a higher frequency than spores of late-stage fungi. They propose that late-stage fungi do not readily infect from spores, instead seeming to require the more substantial food base provided by more established trees. As evidence of this they observe that spores of many late-stage fungi require substances produced by an active mycelium to break dormancy (see 13). In contrast, spores of early-stage fungi appear to be more sensitive to stimulation by root exudates than late-stage fungi and may be more likely to colonize as monokaryons (13).

If this hypothesis is true, it suggests that meiospores may play different roles in early- and late-stage fungi. In early-stage fungi meiospores may be dispersive propagules, while

in late-stage fungi they may primarily be agents of gene flow between established mycelia. The ability of monokaryotic mycelia to colonize dikaryotic mycelia via the Buller phenomenon is well established in Basidiomycetes and lends some credence to the hypothesis that basidiospores function as agents of gene flow. Molecular studies of community and population structure and dynamics should help resolve this question.

### Analysis of populations

While the objective of community analysis is to differentiate taxa, the objective of population analysis is to characterize genetic diversity and the distribution of this diversity, within a species (genetic structure), and to estimate the evolutionary forces (such as levels of inbreeding, genetic drift, mating system, gene flow rates, etc.) acting on populations by assessing variation in allele and genotype frequencies.

Unlike sequences used for community analysis, the markers used to study population variation exhibit a high degree of intraspecific variability. Many techniques exist for assessing population variation, including techniques that involve DNA hybridization, such as DNA fingerprinting (see Ref. 14). Microsatellite DNA analysis (47), also known as short sequence repeat (SSR) analysis, is similar to DNA fingerprinting but utilizes the greater utility of PCR. Several approaches are available to detect allelic differences in specific PCR products, including single strand conformation polymorphism (SSCP) analysis, heteroduplex analysis, denaturing gradient gel electrophoresis (DGGE), and a related technique, temperature-gradient gel electrophoresis (TGGE) (see Ref. 34 for a review of these techniques). Finally there are techniques that utilize PCR but with nonspecific primers, such as random amplified polymorphic DNA (RAPD) analysis (62, 63) and arbitrary primer PCR (AP-PCR) (57).

Several of these techniques can only be applied to pure DNA samples from individual fungi, including DNA fingerprinting (depending upon the probe used), RAPD, and AP-PCR analysis. This necessitates collection of large numbers of isolates, a time consuming task for mycorrhizal fungi. The other techniques can be applied to fungal DNA amplified directly from mycorrhizal root tips and these approaches will undoubtedly expand as more taxon-specific primers are developed. Application of these approaches to detect allelic variation in fungal populations will advance our understanding of genetic structure, genetic variability, and gene flow, and how these processes impact upon community dynamics.

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