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REVIEW

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Intraspecific physiological variation: implications for understanding functional diversity in ectomycorrhizal fungi

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Abstract Several thousand fungal species worlwide are thought to form ectomycorrhizas (ECM) with tree hosts and there is currently much interest in determining the functional significance of such diversity in natural and managed ecosystems. While only a few taxa have been investigated in detail, it is clear that ECM fungi display extensive intraspecific variation in a range of physiological and other life-history parameters. Thus, comparative investigations of single (or even a few) isolates of different species are unlikely to provide reliable information on functional capabilities. Extensive screening of taxonomically well-defined isolates is required. This must take into account spatial and temporal variation in gene expression in mycelia growing in axenic culture or in association with a host plant.

Key words Mycelial growth · Metal sensitivity · Enzyme activities · Gene expression

Introduction

On a global scale, several thousand fungi are thought to form ectomycorrhizas (ECM) with tree hosts in natural and managed forest habitats (Molina et al. 1992). At a more local level, below-ground ECM fungal species richness is also high. Recent molecular investigations have shown that communities within single monoculture forest stands comprise diverse arrays of species, many of which remain ill-defined taxonomically (e.g. Gardes and Bruns 1996; Dahlberg et al. 1997). However, scant data are available on the relative abundance of such taxa in and between different habitats. Furthermore, while overall diversity is thought to be important to ecosystem functioning, the functional significance of individual taxa is very poorly understood. As highlighted by Bruns (1995), the factors controlling ECM fungal diversity at global, regional, and even single-root levels remain the subject of debate. Thus, diversity in below-ground ECM fungal communities may be symptomatic of niche differentiation (resource partitioning), competition and/or environmental disturbance. Bruns (1995) cited the major barriers to understanding ECM fungal diversity, along with its functional significance, as our inability to identify below-ground mycelia and a lack of information regarding taxon-specific life histories. Although the growing availability of molecular tools for fungal identification in recent years has led to excellent progress in identification, our appreciation of the below-ground activities of ECM fungi remains very poor and constitutes a major hurdle to understanding the functional importance of diversity.

It is likely that many ECM fungal taxa fulfil broadly similar ecological functions and that a degree of 'functional redundancy' exists in ECM fungal communities (see Allen et al. 1995). Given their taxonomic diversity, however, communities of ECM fungi are still likely to retain a vast amount of functional heterogeneity. Functional redundancy in ECM fungi has been addressed to some extent by partitioning taxa into guilds such as "late-stage", "early-stage" and "multi-stage" (Mason et al. 1982; Danielson 1984) or "protein", "non-protein" and "intermediate" (Abuzinidah and Read 1986). Addressing the difficulties associated with the former classification, Smith and Read (1997) argued that, with our current level of understanding, broader descriptors such as "r-" and "K"-selected are more meaningful. Partitioning into guilds by relative ability to utilise, for example, certain nitrogenous substrates may also be misleading, particularly based on the relative abilities of single, or only a few, isolates (e.g. Abuzinidah and Read 1986). This tendency to generalise ecological functionality from comparative studies of a few isolates reveals little of the intrinsic physiological potential of most ECM fungal taxa.

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A survey of the literature indicates that few comparative studies have been conducted using five or more isolates of a single ECM fungal species (Table 1). In fact, only ca. 20 species from nine genera have been investigated at this level for at least one parameter, meaning that our appreciation of intraspecific diversity in ECM fungi is currently scant indeed. However, as larger populations of these taxa are screened, it is increasingly evident that intraspecific physiological variation in ECM fungi is large, and that this may cloud our understanding of functional diversity. The aim of this review is thus to consider the extent to which intraspecific physiological variation exists in particular ECM fungal taxa and its implications for interpretation of experimental data in the context of functional diversity.

Mycelial growth

Marked intraspecific variation in rates of mycelial growth in axenic culture has been reported for several ECM fungal taxa (e.g. Theodorou and Bowen 1971; Littke et al. 1984; Cline et al. 1987; Ho 1987; Hutchison 1990c; Meyselle et al. 1991; Kieliszewska-Rokicka 1992). A similar level of intraspecific variation may also occur during growth through soil when in symbiosis with a host plant (Colpaert et al. 1992; Lamhamedi et al. 1992a; Thomson et al. 1994; Ek 1997; Timonen et al. 1997). This may influence host carbon allocation and the extent of below-ground fungal respiration (Ek 1997). Extramatrical mycelial growth from the host plant may further be influenced to a variable extent by the nutrient status of soil, as shown for soil nitrogen with *Paxillus involutus* (Batsch: Fr.) Fr. (Arnebrant 1994).

Temperature and/or pH may affect isolates of ECM fungi differentially, with strains varying in temperature optimum for growth (Laiho 1970; Theodorou and Bowen 1971; Samson and Fortin 1986; Cline et al. 1987) and, in some cases, maximal growth temperature (Samson and Fortin 1986). Although in most cases, temperature-related differences in growth rate show no apparent correlation with the geographical origin of strains (Samson and Fortin 1986; Cline et al. 1987; Tibbett et al. 1998b), the optimal growth temperature of 11 USA strains of *Pisolithus tinctorius* varied with the latitude of their origin (Cline et al. 1987). Equally, while marked intraspecific differences in response of *Laccaria laccata* (Scop.: Fr.) Cooke and *Cenococcum geophilum* Fr. to external pH have been observed, there was no apparent relationship with the pH of the soils from which the strains were isolated (Hung and Trappe 1983). It must be noted, however, that most of these studies were conducted on a maximum of only four isolates of each species. More extensive screening is required to infer such relationships with any confidence.

The growth form of mycelia may also vary within a species, with particular isolates producing more-or-less dense mycelia during growth on agar-based media (Littke et al. 1984; Kieliszewska-Rokicka 1992). More importantly, intraspecific variability in the ability to produce rhizomorphs (*sensu* Cairney et al. 1991) may also exist. A very useful approach to the study of intraspecific genetic variation in ECM fungi has been the synthesis of artificial dikaryotic mycelia from monokaryotic mycelia derived from sporocarps. The method has the advantage of defined and closely-related genetic material, commonly involving progeny derived from a single sporocarp, but provides little indication of the variation existing in the field. In a study of 78 dikaryotic mycelia synthesised in this way from single-spore cultures of a South African *Pisolithus* species, Lamhamedi and Fortin (1991) found that 37% of the dikaryons produced no rhizomorphs during growth on agar media. The remaining mycelia produced rhizomorphs under the same conditions, but varied in the number of hyphae making up individual rhizomorphs. Even when dikaryons were mycorrhizal with *Pinus pinaster* in growth pouches, there was variation in the formation of rhizomorphs. Only 37% of isolates produced fine mycelial aggregates, while the remainder produced more robust rhizomorphic structures (Lamhamedi and Fortin 1991). In a subsequent study of 16 dikaryons synthesised from monokaryotic mycelia from a single *Pisolithus tinctorius* (Pers.) Coker & Couch sporocarp, all but one dikaryon produced rhizomorphs during symbiosis with *Pinus elliottii* (Rosado et al. 1994). However, variation in the extent of rhizomorph growth was observed among the dikaryons, stressing that both rhizomorph development and growth can be variable in even closely related *Pisolithus* isolates. The host genotype may also influence the extent of rhizomorph growth (Rosado et al. 1994; Timonen et al. 1997). Since rhizomorphs are believed to be important in bi-directional long-distance solute translocation and to provide hyphae with a degree of protection against edaphic stresses (see Cairney 1992), such variation in rhizomorph production and structure may influence the effectiveness of individual isolates of ECM fungal taxa as mycobionts in the field.

Host-fungus interactions

A number of studies attest to significant variation in the ability of isolates of some fungal species to form ECM with particular host taxa (e.g. Marx 1981; Bougher et al. 1990; Jacobson and Miller 1992; Thomson et al. 1994; Bonfante et al. 1998). The ECM-forming abilities of individual isolates may further be differentially influenced by soil conditions such as nutrient availability, water status and/or temperature (Marx et al. 1970; Bougher et al. 1990; Bougher and Malajczuk 1990; Thomson et al. 1994). Indeed, the influence of different isolates of some ECM species on the growth of host plants may differ with the field conditions, with some isolates performing better than others under particular conditions (Le Tacon et al. 1992).

Table 1 Ectomycorrhizal fungal taxa for which five or more isolates have been screened for variation in various parameters (+++ much variation, ++ some variation, + slight variation, – no significant variation)

Fungus	Number of isolates	Parameter	Variation	Reference
Amanita muscaria (L.: Fr.) Pers.	5	sensitivity to Zn	+++	Brown and Wilkins (1985)
Fuscoboletinus aeruginascens (Secr.) Pom & Smith	14	temperature optimum for growth	++	Samson and Fortin (1986)
Fuscoboletinus paluster (Peck) Pom.	7	temperature optimum for growth	+++	Samson and Fortin (1986)
Fuscoboletinus spectabilis (Peck) Pom. & Smith	7	temperature optimum for growth	–	Samson and Fortin (1986)
Hebeloma cylindrosporum Romagn.	61[a]	glutamate dehydrogenase activity	+++	Wagner et al. (1988)
H. cylindrosporum	61[a]	nitrate reductase activity	+++	Wagner et al. (1989)
H. cylindrosporum	61[a]	acid phosphomonoesterase activity	+++	Meyselle et al. (1991)
H. cylindrosporum	61[a]	IAA production	+++	Gay and Debaud (1987)
H. cylindrosporum	50[b]	nitrogen source utilization	+++	Gay et al. (1993)
Laccaria bicolor (Maire) P.D.Orton	20[b]	solubilisation of inorganic P sources	+++	Nguyen et al. (1992)
L. bicolor	5[b]	ECM formation	+++	Kropp et al. (1987)
L. bicolor	0[b]	ECM formation	+++	Kropp and Fortin (1988)
L. bicolor	67	solubilisation of inorganic P sources	+++	de la Bastide et al. (1995a)
Paxillus involutus (Batsch: Fr.) Fr.	9	pH optimim for growth	+++	Laiho (1970)
P. involutus	9	temperature optimum for growth	++	Laiho (1970)
P. involutus	9	carbon source utilisation	+++	Laiho (1970)
P. involutus	9	nitrogen source utilisation	+++	Laiho (1970)
P. involutus	8	acid phosphomonoesterase activity	+++	Kieliszewska-Rokicka (1992)
P. involutus	18	sensitivity to Al	+++	Rudawska and Leski (1998)
P. involutus	10	sensitivity to Zn	+++	Denny and Wilkins (1987)
P. involutus	5	sensitivity to Cu	+++	Howe et al. (1997)
Pisolithus tinctorius (Pers.) Coker & Couch	11	temperature optimum for growth	+++	Cline et al. (1987)
P. tinctorius	8	nitrate reducatse activity	+++	Ho (1987)
P. tinctorius	8	acid phosphomonoesterase activity	+++	Ho (1987)
P. tinctorius	8	alkaline phosphomonoesterase activity	–	Ho (1987)
P. tinctorius	8	acid phosphodiesterase activity	+	Ho (1987)
P. tinctorius	8	alkaline phosphodiesterase activity	+	Ho (1987)
P. tinctorius	8	cytokinin production	+++	Ho (1987)
P. tinctorius	8	IAA production	+++	Ho (1987)
P. tinctorius	8	gibberellin production	+++	Ho (1987)
P. tinctorius	21	ECM formation	++	Marx (1981)
P. tinctorius	16	ECM formation	++	Rosado et al. (1994)
P. tinctorius	16	rhizomorph production	++	Rosado et al. (1994)
Pisolithus sp.	*78*[b]	*ECM formation*	+++	*Lamhamedi et al. (1990)*
Pisolithus sp.	10[b]	influence on host nutrition and growth	+++	Lamhamedi et al. (1992a)
Pisolithus s.	10[b]	influence on host drought tolerance	+++	Lamhamedi et al. (1992a,b)
Pisolithus sp.	10	sensitivity to Al	+++	Egerton-Warburton and Griffin (1995)
Pisolithus sp.	41[b]	antifungal activity	+++	Kope and Fortin (1991)
Pisolithus sp.	78[b]	rhizomorph production	+++	Lamhamedi and Fortin (1991)
Pisolithus sp. I	30	nitrogen source utilisation	+++	Anderson et al. (1999)
Pisolithus sp. II	6	nitrogen source utilisation	+++	Anderson et al. (1999)
Pisolithus spp.	6	chemotropic effect of host roots	+++	Horan and Chilvers (1990)
Pisolithus spp. (comprising several polypeptide groups)	20	ECM formation/influence on host growth	+++	Burgess et al. (1994a)
Pisolithus spp. (comprising several polypeptide groups)	10	induction of host chitinase/peroxidase	+++	Albrecht et al. (1994)
Scleroderma citrinum Pers.	5	acid phosphomonoesterase activity	+++	Antibus et al. (1992)
Suillus bovinus (L.: Fr.) Rouss. (as *Boletus bovinus* L.: Fr.)	5	carbon source utilisation	+	Ferry and Das (1968)
Suillus cavipes (Opat.) Smith & Thiers	8	temperature optimum for growth	–	Samson and Fortin (1986)
Suillus granulatus (L.: Fr.) O.Kuntze	8	temperature optimum for growth	+++	Cline et al. (1987)
S. granulatus	12	ECM formation	+++	Jacobson and Miller (1992)
Suillus grevillei (Klotzsche) Sing.	29	temperature optimum for growth	+	Samson and Fortin (1986)
Suillus luteus (L.: Fr.) Rouss.	12	sensitivity to Al	+++	Leski et al. (1995)
Suillus placidus (Bon) Sing.	5	nitrogen source utilisation	++	Keller (1996)
Suillus plorans (Roll.) O. Kuntze	6	nitrogen source utilisation	+++	Keller (1996)
Suillus sibiricus (Sing.) Sing.	6	glucose oxidase activity	+++	Iwase (1992)

[a] Mixture of wild-type and synthesised dikaryons
[b] Synthesised dikaryons

As in studies of mycelial development, artificial dikaryons have been used to investigate intraspecific variability in fungus – host interactions. Dikaryons of *Laccaria bicolor* (Maire) Orton derived in this way have, for example, been screened for their ability to form ECM with *Pinus banksiana* (Kropp et al. 1987; Kropp and Fortin 1988; de la Bastide et al. 1995b). Some dikaryons were able to readily form typical mycorrhizal structures under a range of conditions, but others failed to infect the host. The basis for the differential infectivity was suggested to reside in the ability of the dikaryons to produce hormones and enzyme activities and/or differences in the fungus-root recognition process (Kropp and Fortin 1988). The possibility that spontaneous mutation, and so a loss of ECM-forming ability, occurred in some of the constituent monokaryotic mycelia during culture storage (see below) could not, however, be ruled out (Kropp et al. 1987). More detailed investigations of the process of ECM formation indicate that variation exists in *Laccaria bicolor* dikaryons in the extent to which a fungal sheath forms around host roots and the depth to which Hartig net hyphae penetrate (Wong et al. 1989, 1990a,b). Differences in lectin-binding characteristics of dikaryon hyphae that may relate to differential abilities in fungus-host recognition have also been observed (Lei et al. 1991).

Phytohormone production has been shown to vary strongly within ECM fungal species. Ethylene production varies between isolates of *Laccaria bicolor* but data so far obtained, albeit from only four synthesised dikaryons, indicates no correlation with ECM-forming ability (Livingston 1991). Indole-3-acetic acid (IAA) production, suggested to be important in ECM differentiation, is known to vary widely in *Hebeloma*, *Pisolithus* and *Rhizopogon* species (Gay and Debaud 1987; Ho 1987; Ho and Trappe 1987), as does cytokinin production in *Rhizopogon* species (Ho and Trappe 1987). The production of IAA in axenic culture, while influencing ECM-forming ability, is not strictly correlated to the ability of individual isolates (of *Hebeloma cylindrosporum* Romagn. at least) to form ECM (Gay et al. 1994). Thus, production of hormones can, at best, only partially explain the observed intraspecific variability in the potential of ECM fungi to form mycorrhizas.

Intraspecific variation in ECM-forming characteristics has also been studied in some detail for *Pisolithus* species. Marx and colleagues (e.g. Marx 1979) observed variation in infection levels and host growth responses in field-collected North American isolates of *Pisolithus tinctorius*. During screening of 78 dikaryons synthesised by pairings of mycelia derived from germinated basidiospores of a single sporocarp of a South African *Pisolithus* sp., Lamhamedi et al. (1990, 1992a, b) also noted significant intraspecific variation in the ability of dikaryons to form ECM with *Pinus banksiana* and *Pinus pinaster*. Similar variation was reported among 16 synthetic dikaryons derived from a single North American *Pisolithus tinctorius* sporocarp with respect to their ability to infect different families of *Pinus elliottii* (Rosado et al. 1994). Intraspecific differences have also been observed in the extent to which dikaryons stimulate host plant growth, increase nutrient content and produce antifungal compounds (Lamhamedi et al. 1990, 1992a; Kope and Fortin 1991). The variation in host benefit has been suggested to relate either to the percentage infection achieved by the mycelia (Lamhamedi et al. 1990), the extent to which extramatrical mycelia explore soil (Lamhamedi et al. 1992a), or a combination of the two. However, it is interesting to note in the latter context that Ek (1997) found no relationship between the extent of extramatrical mycelial growth and respiration and the amount of nitrogen transferred to the host plant by two *Paxillus involutus* isolates. Intraspecific variation also appears to exist in the extent to which dikaryons can protect the host against water stress. Lamhamedi et al. (1992a, b) measured clear differences in stomatal conductance, transpiration rate and hydraulic conductance in *Pinus pinaster* infected with different *Pisolithus* sp. dikaryons under different soil moisture regimes. Plants infected with dikaryons that produced extensive extramatrical mycelia were less affected and recovered from water stress more readily (Lamhamedi et al. 1992b). The ability to confer protection against water stress was not, however, strictly correlated with the production of large-diameter rhizomorphs (see above), suggesting that other physiological factors influence this response.

While the variation observed by Lamhamedi and colleagues clearly represents intraspecific variation in a single *Pisolithus* species, the picture is less clear for other *Pisolithus* isolates. Recent molecular investigations indicate that the group previously considered as a single taxon (*Pisolithus tinctorius*) actually comprises a number of more-or-less cryptic species (Anderson et al. 1998a; Martin et al. 1998), making difficult the task of assessing intraspecific variation in field-collected isolates of this taxon. It has been recognised for some time that *Pisolithus* isolates collected from pine stands are generally poorer colonisers of *Eucalyptus* spp. than those collected from *Eucalyptus* stands (Malajczuk et al. 1990). Indeed, at the ultrastructural level, differences in chemotropic response, rate of sheath formation and structure of the fungus-root interface formed with eucalypts (namely thickened host epidermal cell walls abutting the fungus and a lack of interfacial acid phosphatase activity) suggest that pine isolates of *Pisolithus* are recognised as incompatible by eucalypts (Horan and Chilvers 1990; Lei et al. 1990). Burgess et al. (1994b) investigated total soluble polypeptide patterns in 100 Australian *Pisolithus* isolates collected from under a range of host trees and found a strong relationship between groups of isolates with similar polypeptide profiles and their respective host tree species. The ability of selected isolates to infect and influence the growth of *Eucalyptus grandis* was subsequently assessed. The results indicate considerable variation (2–45 times greater growth than uninoculated controls) within the sampled population (Burgess et al. 1994a).

Evidence has also been obtained of altered symbiosis-related peptide expression and patterns of host peroxidase and/or chitinase activities thought to be important in the establishment of the symbiosis (Albrecht et al. 1994; Burgess et al. 1995). Moreover, in addition to pine isolates infecting *Eucalyptus grandis* poorly, these authors found that isolates from stands of some Western Australian indigenous *Eucalyptus* species colonised *Eucalyptus grandis* (a native of Queensland) equally poorly, suggesting variation in host preference within a single host genus. The recent comparative rDNA internal transcribed sequence (ITS) comparison of isolates from pine and eucalypt stands supports the existence of separate *Pisolithus* species with preferences for either host (Martin et al. 1998). Similarly, ITS sequence comparisons indicate that multiple *Pisolithus* species exist in native Australian forest stands (probably with *Eucalyptus* spp. as hosts) (Anderson et al. 1998a), raising the possibility of a degree of interspecific host preference within Australian *Pisolithus*. Even within a single host species, *Pisolithus* isolates can show variation in their ability to infect different host genotypes. Tonkin et al. (1989) showed that different clones of *Eucalyptus marginata* are differentially infected by *Pisolithus* isolates from *Eucalyptus marginata* stands depending on the maturity of the plant from which the clones were derived.

A degree of variation in the ability to form ECM with particular hosts has also been noted in *Suillus granulatus* (L.: Fr.) O.Kuntze (Jacobson and Miller 1992). Specifically, isolates of this taxon from North American *Pinus strobus* stands showed greater specificity for their host (as determined by combined data for percentage ECM colonisation, plant growth response and Hartig net development) than isolates from *Pinus densiflora* in Korea and *Pinus wallichiana* in Nepal. Similarly, Bonfante et al. (1998) observed that two isolates of *Suillus collinitus* (Fr.) O. Kuntze from Mediterranean or alpine environments showed differential abilities to successfully infect *Pinus* species from their respective habitats. While these examples may indeed represent intraspecific variation, the geographical separation and/or restricted host availability of the populations and concomitant restricted gene flow may have yielded cryptic biological species of *Suillus granulatus* (Jacobson and Miller 1992). This, however, remains to be resolved.

Nitrogen utilisation

Intraspecific variation in the extent to which various inorganic and organic nitrogen sources are used for growth has been observed in a number of taxa (Laiho 1970; Lundeberg 1970; Finlay et al. 1992; Gay et al. 1993; Keller 1996; Tibbett et al. 1998c; Anderson et al. 1999; Wallander et al. 1999). Physiological studies have also highlighted intraspecific variation in rates of NH_4^+ absorption (Littke et al. 1984) and in the activities of the primary enzymes of inorganic nitrogen assimilation. Although multiple isolates of only a few species have been studied, variation in nitrate reductase activity can be large (Ho 1987, 1989; Wagner et al. 1989). Indeed, Ho and Trappe (1987) reported a 30-fold difference in nitrate reductase activity between two isolates of *Rhizopogon vulgaris* (Vitt.) M. Lange. Wide variation in glutamate dehydrogenase activity has also been reported in a large population of field-collected and synthesised dikaryons of *Hebeloma cylindrosporum* (Wagner et al. 1988). Interestingly, subsequent growth experiments with the same dikaryons, while identifying a small amount of variation in growth on different nitrogen sources, found no correlation between this and the activities of primary enzymes of nitrogen assimilation (Gay et al. 1993). The authors thus suggested that nitrate reductase and glutamate dehydrogenase are not growth-limiting in this fungus. They further stressed the polygenic nature of the process of fungal growth and that activity of a further key enzyme showing little intraspecific variation may limit growth in this fungus. These are important observations and raise questions about the suitability of growth experiments on indicator substrates as presumptive indicators of enzyme activity. Field surveys of $\delta^{15}N$ signatures of ECM fungal sporocarps, thought to be indicative of the relative abilities of the fungi to access organic nitrogen sources in soil, indicate that large intraspecific variation occurs in some taxa (Taylor et al. 1997). The extent to which such variation reflects variation in intracellular nitrogen fractionation, nitrogen source utilisation or availability in soil microsites, however, has yet to be considered in detail.

Enzyme activities

Extracellular and cell-surface-bound phosphatase activities are frequently implicated in the phosphorus nutrition of ECM fungi and their hosts. While most ECM fungi appear to produce these activities to some extent, it is clear that significant intraspecific variation exists. Ho (1987) screened eight USA *Pisolithus tinctorius* isolates for cell-surface-bound phosphomono- and phosphodiesterase activities and found isolate-specific variation, particularly for acid phosphomonoesterase activity. There was no evidence that the differential activities were related to the geographical origins of isolates. Subsequent work in the same laboratory identified considerable variation in cell-surface-bound acid phosphomonoesterase activity in four *Amanita muscaria* (L.: Fr.) Pers. isolates and in alkaline phosphomonoesterase activity in three isolates of *Suillus brevipes* (Peck) Kuntze (Ho 1989). Antibus et al. (1986, 1992), Kieliszewska-Rokicka (1992) and Cao and Crawford (1993) recorded similar variation in cell-surface-bound acid phosphomonoesterase activities in *Cenococcum geophilum* (3 isolates), *Scleroderma citrinum* Pers. (5 isolates) and *Paxillus involutus* (8 isolates) and *Pisolithus tincto-*

rius (4 isolates), respectively. The small number of isolates notwithstanding, activities for isolates from deciduous hosts were twice those of isolates from coniferous hosts. Variation in acid phosphomonoesterase activity can be even greater, with Meyselle et al. (1991) reporting up to 15-fold variation in activity in a population of 61 field-collected isolates and/or artificially synthesised dikaryotic mycelia of *Hebeloma cylindrosporum* Romagn. Recent work also indicates extensive variability in *Hebeloma crustuliniforme* (Bull.: Fr.) Quél, with some isolates producing significantly more extracellular and/or cell-surface-bound acid phosphomonoesterase activities than other isolates (Tibbett et al. 1998a, b).

Tibbett et al. (1998a, b) suggested that the enhanced extracellular acid phosphomonoesterase activity at low temperature in the Alaskan isolate reflects expression of a cold-induced isozyme. There are many qualitative reports of intraspecific variation in phosphomonoesterase isozymes in ECM fungi. Variable phosphatase isozymes have been found within a number of ECM species including *Amanita muscaria* (Ho 1989), *Pisolithus tinctorius* (Ho 1987; Cao and Crawford 1993) and *Rhizopogon vinicolor* Smith (Ho and Trappe 1987), along with several *Suillus* species (Zhu et al. 1988; Ho 1989; Sen 1990; Keller 1992; El Karkouri et al. 1996). These authors have also identified considerable intraspecific variation in isozymes of a range of intracellular enzymes of *Suillus* species, indicating considerable physiological diversity. For some species, at least, variation in isozyme pattern appears to be greater between isolates from different forest regions than those collected in single forest stands (Zhu et al. 1988; Sen 1990; El Karkouri et al. 1996), suggesting that habitat variation and/or host tree selection (see above) are important determinants of variation. Interpretation of these data are, however, limited by the relatively small numbers of isolates so far investigated from individual sites (<15). Lapeyrie et al. (1991) screened two isolates each of *Hebeloma crustuliniforme*, *Paxillus involutus* and *Pisolithus* sp. for their relative abilities to solubilise phosphorus-containing mineral complexes. Even within such a narrow population of isolates, intraspecific variation was observed. For example, one *Pisolithus* sp. isolate showed an apparent inability to solubilise the substrates and one *Paxillus involutus* solubilised Ca-phytate but not inorganic phosphate complexes; the other isolates of both taxa solubilised at least some organic and inorganic complexes relatively freely. Similarly, considerable variation was observed in 67 field-collected isolates of *Laccaria bicolor*, and within a population of 20 synthesised dikaryotic mycelia derived from a single sporocarp of the same taxon (Nguyen et al. 1992; de la Bastide et al. 1995a). In the latter case, the phosphorus-solubilising ability of the dikaryons was distributed around that of the parent mycelium but did not appear to be heritable. The physiological mechanisms underlying phosphorus solubilisation include proton extrusion, organic acid production and/or phosphatase activities. Organic acid production can vary strongly at the intraspecific level (Iwase 1992); however, each of these attributes seems likely to be polygenically controlled and understanding the genetical basis of variation is difficult (Nguyen et al. 1992; de la Bastide et al. 1995a).

Although there has been little study of plant cell-wall-degrading enzymes in multiple isolates of ECM fungal taxa, variation here also seems likely to be considerable. Giltrap (1982) conducted a series of presumptive tests for polyphenol oxidase activity. While little activity was observed in 1–4 isolates of some species, others such as *Amanita rubescens* (Pers.: Fr.) S.F.Gray, *Boletus subtomentosus* L.: Fr. [= *Xerocomus subtomentosus* (L.: Fr.) Quél.], *Leccinum scabrum* (L.: Fr.) S.F.Gray and *Suillus luteus* (L.: Fr.) S.F.Gray demonstrated considerable intraspecific variation, with individual isolates displaying very strong phenol oxidising activities. Similar intraspecific variation has been observed in a range of other ECM taxa and is further complicated by the fact that intraspecific variation exists also in the production of such activities on different growth media in some taxa (Hutchison 1990b). Variation in activity has also been observed in four *Pisolithus tinctorius* isolates for components of the cellulase complex. Cao and Crawford (1993) reported that all four isolates produced both α-galactosidase and β-glucosidase, but only a single isolate produced β-galactosidase activity. In addition, isoforms of α-galactosidase varied with the isolate, with one isolate producing three isoforms of β-glucosidase not evident in the other strains. Intracellular glucose oxidase activities, assayed in seven Japanese isolates of *Tricholoma robustum* (Alb. & Schw.: Fr.) Ricken s. Imazeki, varied by almost fourfold (Iwase 1992). Two isolates of each of a number of other *Tricholoma* species also showed variability (<10 times) in this respect. Although not directly involved in degradation of components of the plant cell wall, glucose oxidase, along with other carbohydrate oxidases, has been indirectly implicated in partial lignin degradation by ECM fungi. These enzymes produce H_2O_2, which, in the presence of Fe^{2+}, produces hydroxyl radicals that may contribute to lignin fragmentation (see Burke and Cairney 1998). The data of Iwase (1992) suggest that the ability of ECM fungi to modify lignin in this way varies greatly, even within a species.

Metal sensitivity

A relatively large number of studies have addressed the existence of intraspecific variation in the sensitivity of ECM fungi to toxic metals. Even where relatively few (<5) isolates have been used, it is clear that a large degree of variation exists within some species in this respect (Thompson and Medve 1984; Brown and Wilkins 1985; Colpaert and Van Assche 1987, 1992; Howe et al. 1997; Vodnik et al. 1998). Furthermore, the physiological basis for insensitivity may vary within a species. For

example, Howe et al. (1997) showed that two Cu-insensitive isolates of *Paxillus involutus* accumulate Cu-binding proteins when exposed to the metal. A third, equally insensitive isolate did not accumulate such proteins, suggesting that insensitivity in this isolate is mediated by another mechanism. There may also be intraspecific variation in the extent to which isolates mediate metal insensitivity in their hosts. Aggangan et al. (1998) found differences in the extent to which three isolates of *Pisolithus* protected *Eucalyptus urophylla* against Ni. While possibly reflecting intraspecific differences, these data, however, are equally likely to represent intraspecific differences recently observed in this taxon (see above).

It has been suggested by some authors that such variation is related to the metal status of the soils from which particular strains were isolated, and that some ECM fungal taxa thus demonstrate adaptive tolerance to metals in the environment (e.g. Colpaert and Van Assche 1987). In the case of Al sensitivity, broader-scale screening experiments support this hypothesis for some taxa, with Egerton-Warburton and Griffin (1995) and Leski et al. (1995) demonstrating a strong correlation between Al insensitivity and the concentration of the metal in the environments from which isolates of a *Pisolithus* sp. and *Suillus luteus* were obtained. Variation in sensitivity to Al in 19 isolates of *Paxillus involutus*, however, showed no relationship with the contamination status of their soils of origin (Rudawska and Leski 1998), suggesting constitutive sensitivity in this species.

Although intraspecific variation exists in ECM fungi with respect to sensitivity to other metals, evidence for adaptive tolerance to these is less compelling, and the data are somewhat contradictory. Thus, although variation in Zn sensitivity in five isolates of *Amanita muscaria* was not related to Zn in their native soils (Brown and Wilkins 1985), Colpaert and Van Assche (1987) noted a much greater insensitivity in single isolates of *Aamanita muscaria*, *Suillus luteus*, *Suillus bovinus* (L.: Fr.) Rouss. and *Thelephora terrestris* Ehrh.: Fr. from a severely Zn-contaminated site. Similarly, while recording intraspecific variation, Denny and Wilkins (1987) found no evidence for adaptive Zn insensitivity in 10 isolates of *Paxillus involutus*. Colpaert and Van Assche (1992), meanwhile, reported that single isolates of *Amanita muscaria*, *Suillus luteus* and *Suillus bovinus* from heavily Cd-contaminated sites were significantly less sensitive to Cd that those from unpolluted environments. The small sample size of the latter notwithstanding, the apparent discrepancies may reflect the fact that the Zn-contaminated environments from which Wilkins and his colleagues collected their isolates were in fact less contaminated by the metals than the sites from which the insensitive isolates used by Colpaert and Van Assche were collected (Colpaert and Van Assche 1987). Clearly more extensive collection and intraspecific screening of strains from a range of environments will be required to resolve this conflict.

Other considerations

Intrinsic variation may be confounded further by changes that appear to occur in individual mycelia following isolation. There are several reports suggesting that isolates of some fungal taxa demonstrate a decreased ability to form ECM with their hosts during prolonged maintenance in axenic culture (Laiho 1970; Marx and Daniel 1976; Marx 1981; Thomson et al. 1993). Inoculation of a host plant and subsequent re-isolation of the fungus can often increase the mycorrhiza-forming efficacy of susceptible isolates (Marx 1981; Thomson et al. 1993), although the ability to form ECM may decline again in these isolates after only a few months in axenic culture (Thomson et al. 1993). Such a response is not, however, ubiquitous. Three isolates of *Laccaria bicolor* showed no decrease in ECM-forming ability during 18 years of continued subculture under axenic conditions, nor did their ability to form ECM increase following passage through a host plant (Di Battista et al. 1996).

The *Laccaria bicolor* isolates studied by Di Battista et al. (1996) did, however, display reduced mycelial growth rates and decreased ability to produce sporocarps during long-term maintenance in culture. A reduction in the ability to initiate sporocarp formation has also been reported for other ECM taxa during >1 year in axenic culture (Giltrap 1981). Similarly, mycelial growth rates of a variety of taxa were shown to decline during axenic culturing over only a few months, although infection of a host plant and re-isolation of mycelia could mediate short-term enhancement of growth rate (Thomson et al. 1993). Particular isolates, at least of *Pisolithus* spp., may further display changes in the growth form of mycelia, with variation in the extent of rhizomorph production having been noted in replicate cultures of some isolates (Lamhamedi and Fortin 1991), while a temporal switch from dense to diffuse growth has been observed in another isolate (Anderson et al 1999).

Physiological variation has also been recorded in replicate cultures of individual isolates of various taxa. Hutchison (1990a), for example, observed variation in utilisation of various carbon and/or nitrogen substrates, including urea, pectin, casamino acids and lipids. In most cases, this variation was confined to the extent of utilisation (presumed to reflect variation in production of the necessary degradative enzymes), but in some instances some replicates of particular isolates produced no growth on a substrate while others did. It is not clear whether these observations reflect temporal changes due to storage in culture or some other source of variation. Scheromm et al. (1990), however, noted a decline in the ability of an isolate of *Hebeloma cylindrosporum* to utilise NH_4^+ as sole nitrogen source over a period of only a few weeks. Furthermore, Anderson et al. (1999) reported that, two isolates of a *Pisolithus* sp. maintained in culture for >10 years utilised protein in the form of bovine serum albumin (BSA) relatively freely

while recent (<1-year-old) isolates were limited in the extent to which they utilised this substrate. Although further work is required here, data to date suggest a temporal change in the ability of this *Pisolithus* species to utilise BSA.

Clearly, temporal variation in the growth, ECM-forming abilitiy and physiology of ECM fungi can arise during storage in axenic culture, even where continued subculturing in the absence of a suitable host plant is employed. Di Battista et al. (1996) suggested that such variation represents phenotypic differences within isolates, arising from modification (e.g. via DNA methylation) of genes involved in growth or mycelial physiology. Alternatively, it may reflect chromosome polymorphisms arising from rearrangements of gene organisation during mitoses or via the action of transposable elements (Di Battista et al. 1996). Where variation within individual isolates cannot be related to storage in culture, it may indicate differential expression of genes at points from which subcultures of mycelia from single Petri dish cultures were initiated. As argued elsewhere, expression of many genes will not be uniform even within individual axenic mycelia of ECM fungi (see Cairney and Burke 1996). Subculturing from different regions (even within the growing front) of individual mycelia may thus influence the outcome of short-term experiments as hyphae in mycelial plugs initiate growth and acclimate to the new growing medium. Such acclimation may be expressed in the form of different lag phase lengths, which may influence growth and substrate utilisation experiments (Finlay et al. 1992).

Conclusions

There is no doubt that interspecific differences exist in the functional roles played by ECM fungi in forest ecosystems; however, we currently have scant appreciation of the extent of such diversity. This may be partly explained by the tremendous taxonomic diversity of mycobionts believed to prevail globally and by the fact that, to date, only a handful of taxa have been investigated in any detail. Our understanding of functional diversity is further hampered by the degree of intraspecific variation identified in the few ECM taxa so far studied in detail. It seems likely that some of the observed intraspecific variation represents ecotypic adaptation according to environmental pressures in different geographical regions or, on a smaller scale, to localised heterogeneity in edaphic conditions. With the exception perhaps of differential sensitivity to Al, however, this question has not been addressed in sufficient detail. While a few studies have aspired to such comparisons, the numbers of isolates screened from different environments have been small, precluding meaningful data analysis. It is only through extensive multi-isolate screening that the extent of ecotypic adaptation can be ascertained.

Some apparent intraspecific variation may reflect misidentification of isolates, such as the apparently facultatively saprophytic strains of *Bjerkandera subtomentosus* reported by Lundeberg (1970) which were later identified as isolates of the wood-rotting *Bjerkandera adusta* (Willd.: Fr.) Karst (see Hutchison 1990a). Equally, as appears to be the case with *Pisolithus* species for example, variation may reflect the existence of separate biological species within what has been previously regarded as a single species grouping. Thus isolates separated by differential host species availability or simply by geographic isolation may, through reduced gene flow or other mechanism, lose mating compatibility (see Molina and Trappe 1986; Jacobson and Miller 1992).

There is a need also to critically assess axenic culture-based work in the light of changes in gene expression that have been shown to occur during maintenance under standard laboratory conditions. From the data summarised in this review, it is clear that a comparative study between, for example, a single newly obtained isolate of one taxon and a single isolate of another taxon maintained in axenic culture for a number of years is likely to provide no useful information regarding their comparative ecological functioning. Yet experiments of this nature continue to be conducted and conclusions regarding ecological functioning inferred from the published data.

Changes in gene expression in ECM fungi have been shown to occur as a result of interaction with a host plant in symbiosis (Tagu and Martin 1996; Tagu et al. 1996). It can thus be argued that physiological and/or morphological traits expressed in axenic culture do not necessarily reflect those expressed by the fungi under the influence of a host plant in symbiosis. This clearly increases the difficulties in extrapolating data from such experiments to ecological functioning in the field. While this is undoubtedly so, we must be mindful that several of the caveats outlined above for axenic culture studies also apply to work with intact fungus – plant systems. In particular, considerable intraspecific variation has been demonstrated in the extent and morphological development of extramatrical mycelial systems, meaning that, even *in planta*, extensive screening of studies using multiple isolates are required to give a reasonable estimate of mycelial characteristics. We must assume the same to be true also for physiological characteristics of extramatrical mycelial systems. Although only studied so far using isolates of *Paxillus involutus*, it is clear that intraspecific variation exists in terms of the cost-benefit of association with a single host species. Isolates of *Paxillus involutus* are known to vary in the extent to which they transfer absorbed NH_4^+ to the host (Wallander et al. 1999). Ek (1997) has also shown intraspecific variability in the ratio of host-derived carbon allocated to fungal biomass and respiration: nitrogen transfer to the host plant. Furthermore, in a heterogeneous substrate such as soil, individual ECM mycelia display considerable spatio-temporal heterogeneity in physiological and morphological characters (Ek et al. 1994; Cairney and Burke 1996; Timonen

and Sen 1998) that will serve to confound simplistic interpretation of the functional contributions of individual taxa. Phenotypic variation of this nature, arising from epigenetic events and/or altered gene expression has, for example, been documented in mycelia derived from different regions of individual *Laccaria bicolor* genets (de la Bastide et al. 1995a). Consequently, although we might intuitively predict that populations of ECM fungal species comprising a few large mycelial genets (e.g. Dahlberg and Stenlid 1994; Anderson et al. 1998b; Bonello et al. 1998) will exhibit less functional variation than a localised population of many short-lived genets (e.g. Gryta et al. 1997), this may not necessarily be the case. Spatio-temporal variation in activities within a single large mycelium may be great and, if not considered, has the potential to further cloud our vision of the functional significance of ECM fungal taxa in forest ecosystems.

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