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Ericoid mycorrhizal fungi: some new perspectives on old acquaintances

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Abstract

Many ericaceous species colonize as pioneer plants substrates ranging from arid sandy soils to moist mor humus, in association with their mycorrhizal fungi. Thanks to the symbiosis with ericoid mycorrhizal fungi, ericaceous plants are also able to grow in highly polluted environments, where metal ions can reach toxic levels in the soil substrate. For a long time this mycorrhizal type has been regarded as an example of a highly specific interaction between plants and fungi. More recent studies have been challenging this view because some ericoid mycorrhizal endophytes seem also able to colonise plants from very distant taxa. A molecular approach has allowed the investigation of genetic diversity and molecular ecology of ericoid mycorrhizal fungi, and has revealed that ericaceous plants can be very promiscuous, with multiple occupancy of their thin roots. The molecular analysis of sterile morphotypes involved in this symbiosis has also led to deeper understanding of the species diversity of ericoid fungi. Genetic polymorphism of ericoid fungi is wider than previously thought, and often increased by the presence of Group I introns in the nuclear small subunit rDNA.

Introduction

Most plants belonging to Ericales are able to associate symbiotically with soil fungi to form a distinctive type of mycorrhiza, termed ericoid mycorrhiza. This association was initially investigated in members of the family Ericaceae, more abundant in the northern hemisphere (Bonfante and Gianinazzi-Pearson, 1979; Pearson and Read, 1973), but a morphologically similar mycorrhizal association was described also in the family Epacridaceae, widely distributed in the southern hemisphere (Ashford et al., 1996; Read, 1996; Reed, 1989). This is not surprising since phylogenetic analyses indicate that Ericaceae and Epacridaceae, traditionally considered as two separate families of the order Ericales, are closely related and epacrids may represent a sub-clade of the Ericaceae (Crayn et al., 1998).

The morphology of ericoid mycorrhiza is highly conserved in different plant species (Figure 1). The epidermal cells of the fine ericaceous hair roots harbour more or less dense coils of fungal mycelium (Bonfante and Gianinazzi-Pearson, 1979; Perotto et al., 1995) which appear to remain enclosed within single root cells (see also Figure 3). As in all endosymbioses, the intracellular fungal symbiont is separated from the plant cytoplasm by a plant-derived membrane, which invaginates to follow fungal growth and coil formation. In the infected cells, the plant nucleus moves to a central position.

Although ericoid mycorrhizal plants are commonly found as understorey vegetation in boreal and mediterranean forests, ericaceous shrubs can become dominant in many natural and semi-natural heathland communities. This happens especially in environmental conditions where a slow decomposition of the plant litter occurs, resulting in acidic soils rich in recalcitrant organic matter but low in available mineral nutrients such as N and P (Cairney and Burke, 1998). The survival of ericaceous plants under such nutrient-stressed conditions is thought to depend on the formation of mycorrhizal symbiosis, and the evolution of the association is regarded as having been driven by the selective advantages conferred by fungal infection (Straker, 1996). The saprotrophic potential of ericoid fungi and their ability to degrade complex and recal-

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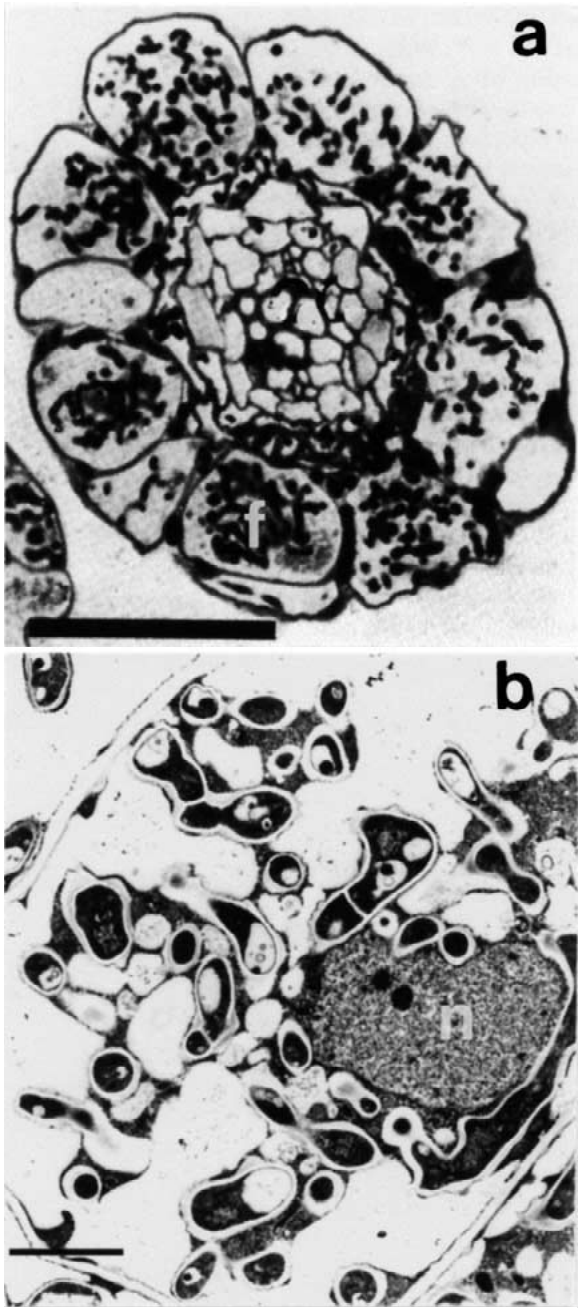

Figure 1. Colonization of *Calluna vulgaris* hair roots by ericoid mycorrhizal fungi. (a.) Transverse section of a hair root to show the general structure of the root. Cells of the outer epidermal layer harbour fungal coils (f) formed by a sterile mycorrhizal morphotype (mycelium G1). The collapsed cortical cells are visible just underneath the epidermis, surrounding the small central cylinder. Bar is 100 μm. (b.) Ultrastructure of an epidermal cell colonised by the sterile mycorrhizal morphotype PSIV. The fungal hyphae form a dense coil that surrounds the plant nucleus (n), which occupies a central position in the cell. Bar is 1 μm.

citrant polymeric substrates have been demonstrated over the years by several authors (e.g., see Bending and Read, 1996, 1997; Cairney and Burke, 1998; Leake and Read, 1991; Perotto et al., 1995; Varma and Bonfante, 1994). Based on these results, it is widely accepted that the major benefit conferred to the ericaceous host plant by mycorrhizal infection is the enzymatic degradation of organic polymers in the soil, and the transfer of some of the resulting products to the root (Smith and Read, 1997). Thanks to their mycorrhizal status, host plants can access otherwise unavailable organic N and P (Nasholm and Persson, 2001).

In addition to their general role in nutrient uptake, it has also been demonstrated that ericoid mycorrhizal fungi confer to their host the ability to compete successfully with other plant species. For example, *Calluna vulgaris* is the most common ericaceous species in the oceanic north west of Europe, where it can form almost pure plant communities (Rodwell, 1991). The superior ability of mycorrhizal *C. vulgaris* to compete over *Nardus stricta* was demonstrated in pot cultures under different nutrient conditions (Genney et al., 2000), and suggested to depend on allelopathy rather than competition for nutrients (see also Pongè, 1998).

Despite the widespread occurrence of ericaceous plants, little progress has been made in the understanding of the molecular bases of plant–fungus interactions during ericoid mycorrhiza formation and functioning. This is in striking contrast with the achievements in the study of arbuscular and ectomycorrhiza, where the use of model systems and the application of functional genomics have led to the discovery of entire sets of genes and proteins regulated during symbiosis (e.g., see Gianinazzi-Pearson, 1996; Van Buuren et al., 1999; Voiblet et al., 2001; and papers in this issue). Delays in this aspect of ericoid mycorrhizal research can be attributed to the difficulty of performing biochemical and molecular studies on this association, due to the minute size of mycorrhizal hair roots, but also to the limited consideration for ericaceous plants in agro-forestry. However, interest in this association has been increasing recently because of the ability of ericoid mycorrhizal plants to grow on polluted sites contaminated by heavy metals, and their potential applications in bioremediation. Some of the tolerance mechanisms, which will be reviewed later, were originally investigated by Bradley et al. (1982), but have been analysed in more detail for both the fungus and

the plant (Martino et al., 2000a,b; Sharples et al., 2000a,b).

The development and use of molecular techniques has contributed greatly to gain insights on fungal diversity as well as the molecular ecology of mycorrhizal fungi. These techniques have been applied extensively to the study of ericoid fungi, and the derived knowledge has modified substantially our view of the ericoid mycorrhizal symbiosis, until recently thought to be an endomycorrhizal symbiosis with a high degree of specificity, restricted to few genera of plants and fungi (Straker, 1996). The aim of this contribution is to review some aspects where progress in the study of ericoid mycorrhizal fungi has allowed a better understanding of their physiology (in particular the mechanisms of metal tolerance), or have opened new perspectives on their ecology.

Ericoid fungi: a good model system to study the molecular bases of fungus–heavy metal interactions

The molecular mechanisms that govern interactions between metals and organisms are extremely complex, and depend on the organism involved as well as on environmental factors. It should be remembered, however, that many metals play essential functions in the organisms, and uptake must therefore involve recognition of metal species. Insufficient levels of essential metals can result in stress responses just as severe as those resulting from excess metals, and therefore cells must have developed mechanisms to avoid both extremes (Tomsett, 1993). Some heavy metals have no known functions and are therefore toxic at all concentrations. Molecular recognition allows organisms to differentiate between essential and non-essential ions and, if necessary, to partition them in different ways.

The mechanisms that allow survival of mycorrhizal fungi under heavy metal pollution are relatively poorly understood. Recent reports indicate that ecto- and endomycorrhizal isolates tolerant to high concentrations of heavy metals are selected in contaminated soils (Colpaert et al., 2000; Weissenhorn et al., 1993). Strains of the ericoid mycorrhizal species *Oidiodendron maius* isolated from Cd/Zn/Al-polluted sites also displayed a better ability to grow *in vitro* on media containing these metals when compared with isolates from non polluted sites (Lacourt et al., 2000; Martino et al., 2000a,b). Similarly, populations of arsenate resistant *Hymenoscyphus ericae*, another well known ericoid mycorrhizal partner, have been isolated from As/Cu-contaminated mine soils (Sharples et al., 2000b,c).

There are two major strategies that fungi can adopt to protect themselves against heavy metal toxicity. Avoidance restricts entry of metal ions into the cytoplasm, and relies on decreased uptake or increased efflux of metal ions, or on their immobilization outside the cell. Sequestration occurs to reduce cytoplasmic concentration of free metal ions, either through the synthesis of chelating compounds or by compartmentalisation into the vacuole (Gadd, 1993; Leyval et al., 1997). These general mechanisms of metal tolerance have also been found in mycorrhizal fungi, but it is still unclear if they also regulate transfer to the plant.

In mycorrhizal fungi, as in other fungi, the cell wall is a major binding site for heavy metals. It has been suggested that adsorption to the fungal wall is an important mechanism that reduces metal supply to the host (see Leyval et al., 1997). In ericoid mycorrhiza, the protective effect of the fungus reported by Bradley et al. (1981) has been ascribed to the fact that *Hymenoscyphus ericae* displays strong affinities for metallic cations. In addition, the excretion of a loosely adhering extrahyphal slime has been shown by Denny and Ridge (1995) to correlate in different fungal strains with the tolerance and the amelioration of zinc toxicity. Strains of the ericoid mycorrhizal species *O. maius* derived from polluted and unpolluted soils were investigated for their ability to bind zinc ions. Significant differences were found in the amount of metal adsorbed to the fungal mycelium, probably due to different binding capacity of the cell wall (Figure 2).

Fungi can also interact with metals in the surrounding environment by releasing extracellular metabolites that can modify heavy metals' bioavailability (Gadd, 1993). Mycorrhizal fungi can modify the mobility of metals in their surrounding environment, and solubilization in particular can be very important as it can contribute to bring into soluble forms essential ions that are normally found as insoluble organic and inorganic compounds (Marschner, 1995; Smith and Read, 1997). The efficiency of mycorrhizal fungi in increasing metal availability to plants would appear to be a potential problem in soils that are naturally enriched in toxic metal species or polluted by anthropic activities. By contrast, it is well documented that the ericoid mycorrhizal symbiosis can reduce metal toxicity to the host, allowing plants to survive in soils with potentially toxic amounts of soluble and insoluble metal species (Bradley et al., 1982; Sharples et al., 2000b,c).

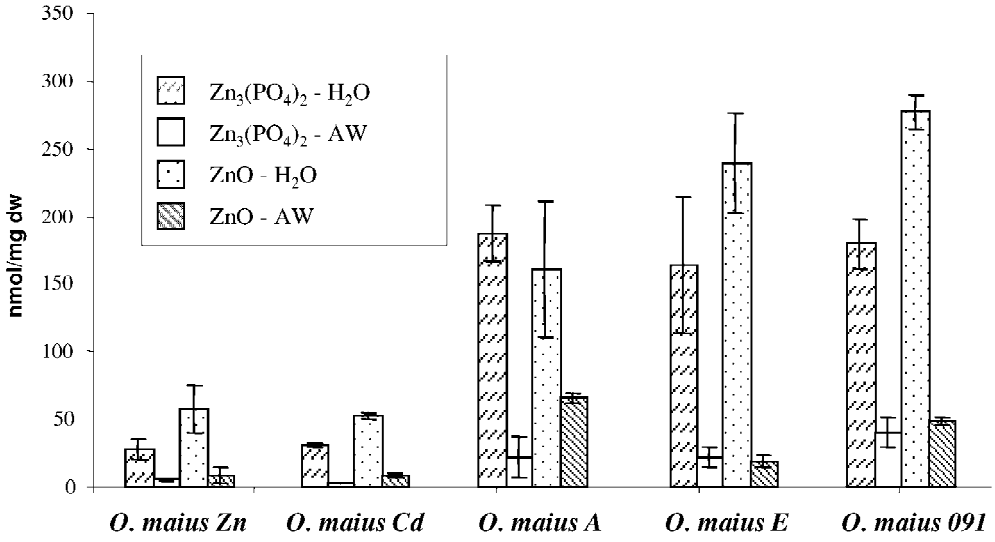

Figure 2. Adsorption of zinc to the mycelium of five different strains of the ericoid mycorrhizal fungus *Oidiodendron maius*. Strains *O. maius* Zn and Cd were isolated from polluted sites near Krakow (Poland), whereas the other strains were derived from unpolluted soils in different geographic locations. Fungi were grown on a solid malt medium added with ZnO or $Zn_3(PO_4)_2$. Significant differences in metal binding properties can be observed. Most of the metal could be released by an acidic wash (AW, HCl 0.5 M). Data are the mean of three independent experiments ± standard deviation.

In these conditions, a possible strategy to reduce metal availability to the plants is suggested by the observation that the ability of ericoid fungi to solubilize insoluble inorganic metal compounds is strongly reduced in fungal strains isolated from polluted soils (Martino et al., unpublished results).

In addition to metabolites that directly interact with metals, fungi can also respond to the presence of metals with the release of specific proteins in the surrounding medium. It was somewhat surprising to observe that the presence of zinc in the culture medium sharply increased the secretion and activity of extracellular enzymes that hydrolyse polymeric substances such as the pectin component of plant cell walls (Martino et al., 2000a). The significance of this increased production is unclear, but oligalacturonans may function as better metal chelators than larger polymers, thus protecting the fungus during saprotrophic growth.

When heavy metals cross the cell wall, they are accumulated in the cell by an active, energy dependent process (Morley et al., 1996). Essential metals are transported across the cell membranes by transport systems specific for these elements (Hughes and Poole, 1989). Non-essential metals can be sometimes transported by the same systems specific for essential metals, or they can use transporters for other nutrients. The mechanism of arsenic tolerance in ericoid mycorrhizal fungi has been investigated by Sharples et al. (2000b,c). This element enters the cell through the phosphate transporter, causing mycorrhizal fungi to enhance both phosphate and arsenate uptake. Sharples et al. (2000c) found that active and specific efflux mechanisms are adopted by ericoid mycorrhizal fungi from polluted sites, so as to decrease cellular concentrations of arsenic while retaining phosphate. These studies are just starting to unravel the cellular and molecular mechanisms of fungal metal tolerance, and they will provide important information to understand and exploit the potential of these fungi in bioremediation.

Biodiversity of ericoid mycorrhizal fungi: what is the true taxonomic range?

Information on the diversity of ericoid mycorrhizal endophytes in the Ericaceae and Epacridaceae has been collected over the years by several authors (e.g., Berch et al., 2001; Bergero et al., 2000; Cairney et al., 2000; Chambers et al., 2000; Hambleton and Currah, 1997; Hutton et al., 1994; Liu et al., 1998; Monreal et al., 1999; Perotto et al., 1996; McLean et al., 1999; Sharples et al., 2000a), and these collective data have contributed to change our view on the specificity of the association with regards to the fungal partner.

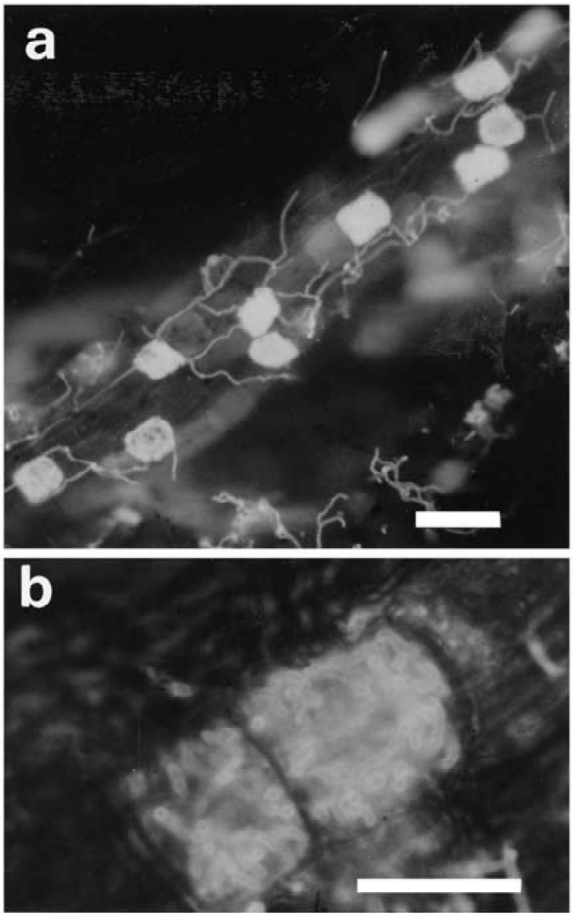

Figure 3. Confocal fluorescence microscopy after staining of a *C. vulgaris* hair root with wheat germ agglutinin-FITC. This lectin binds to the chitin present exclusively in the fungal wall. (a.) Low magnification showing root epidermal cells colonised by fungal coils. The colonised root cells can be well separated from each other and indicate that the fungus does not spread from cell to cell. (b.) The fungal hyphae can be better visualised in two coils shown at higher magnification. Bar is 20 μm.

Fungi recovered from ericoid roots and confirmed to be mycorrhizal in *in vitro* synthesis experiments are mostly sterile when brought into culture. The first two taxa to be recognized as symbionts of ericaceous hosts were both Ascomycetes (see Read, 1996; Smith and Read, 1997). *Hymenoscyphus ericae* (Read) Korf & Kernan is a member of order Helotiales, most frequently found in an anamorphic stage, *Scytalidium vaccinii* Dalpé, Litten & Sigler, proposed by Egger and Sigler (1993). The other known species, *Oidiodendron* spp. (anamorphic fungi), have their teleomorphs mainly in the Onygenales. *H. ericae* and *O. maius* Barron appear to be the dominant fungi in the diverse assemblages of symbionts colonizing the plants investigated to date (Hambleton and Currah, 1997; Johansson, 2001; Monreal et al., 1999; Perotto et al., 1996; Read, 1996; Sharples et al., 2000a; Straker, 1996). It is interesting to note that one or other of the species may be dominant at a particular site. For instance, *C. vulgaris* roots were mostly colonized by *H. ericae* at mine and natural heathland sites in south-west England (Sharples et al., 2000a), whereas *O. maius* was the dominant species in the same plant at a heathland site in northern Italy (Perotto et al., 1996).

Ribosomal DNA sequence comparisons over the past few years are providing new evidence for definition of genetic diversity and taxonomic status of these well-known endophytes. To date, the number of mycorrhizal species within *Oidiodendron* is uncertain, both because of misidentifications of field-collected and historically important strains (Hambleton et al., 1998b) and current re-evaluation of species concepts and boundaries within this genus (Hambleton et al., 1998b; Lacourt et al., 2001). Higher-level taxonomic affiliations and relationships of *Oidiodendron* and related teleomorphs have also been questioned (Hambleton et al., 1998a,b).

Species boundaries are a matter of debate also for *H. ericae*. Vrålstad et al. (2000) found that the ectomycorrhizal symbiont of *Piceirhiza bicolorata* shared approximately 95% ITS1 sequence identity with *H. ericae*, and can be considered part of a single major aggregate with the latter. Several unidentified sterile ericoid symbionts from different ericaceous hosts were also found to segregate with *H. ericae* in sequence based phylogenetic trees (Monreal et al., 1999; Sharples et al., 2000a). In view of the range of genetic diversity observed in such clusters it is presently unclear if a single species is indicated under the name *H. ericae* as currently applied. It is more likely that the *H. ericae* aggregate may correspond to a generic level comprising more than one species showing mycorrhizal behaviour. This generic entity appears to be quite separate from other members of the genus *Hymenoscyphus* (T. Vrålstad, personal communication).

In spite of the likely dominance of a few defined mycobionts, other ascomycetous taxa have been recognized among ericoid symbionts. *Acremonium strictum* W. Gams, an anamorphic fungus with affinities in the Hypocreales (Glenn et al., 1996) formed ericoid mycorrhiza *in vitro* with *Gaultheria shallon* (salal), although association with the host was somewhat atypical (Xiao and Berch, 1996). *Phialocephala fortinii* Wang & Wilcox, another anamorphic

fungus with likely affinities in Helotiales based on the small subunit (SSU) rDNA sequences (LoBuglio et al., 1996), was associated with ericaceous roots. However, its status as an ericoid mycorrhizal symbiont is dubious, as it appeared to form intracellular microsclerotia instead of typical hyphal coils in *in vitro* experiments with *Menziesia ferruginea* (Stoyke et al., 1992).

Molecular data are leading to increasing recognition that ericoid fungi encompass a wider spectrum of taxa than once thought, so that the number of mycorrhizal species is currently uncertain. Different authors (e.g., Hambleton and Currah, 1997; Hutton et al., 1994; Liu et al., 1998; McLean et al., 1999; Monreal et al., 1999; Perotto et al., 1996) have hypothesised that sterile mycelia with ericoid mycorrhizal behaviour represent a heterogeneous group of so far mostly unidentified taxa. Recent comparisons of ITS sequences have indeed indicated that several sterile isolates represent taxa having no close relative among species currently included in GenBank or EMBL databases, as indicated by the low percentage similarity of their closest matches in BLAST or FASTA searches. Isolates obtained from *C. vulgaris* in England were found to have highest similarity (approx. 80%) with *Perrotia* spp. (fam. Hyaloscyphaceae, ord. Helotiales) (Sharples et al., 2000a). An isolate from salal was found to be close to *Phialophora finlandia* Wang & Wilcox (Monreal et al., 1999) an ectendo-mycorrhizal species. Interestingly, *in vitro* inoculation of salal seedlings with this species provided some evidence that this species may form typical ericoid mycorrhizae (Monreal et al., 1999).

Relationships of symbionts of ericaceous and epacridaceous hosts have also been the subject of recent research, as endophytes from Epacridaceae may readily form typical ericoid mycorrhiza in Ericaceae and *vice versa*, suggesting close relationships among the fungi (Liu et al., 1998; McLean et al., 1998; Read, 1996; Steinke et al., 1996). This relatedness can also be found by DNA sequence analysis. For example, Bergero et al. (2000) found that one of the dominant sterile mycorrhizal morphotypes obtained from *Erica arborea* plants in a mediterranean forest in Italy had its closest relative (94% ITS sequence similarity) in an epacrid endophyte obtained from *Astroloma pinifolium* (Epacridaceae) in Australia (McLean et al., 1998).

Despite the relatedness among some sterile morphotypes, the occurrence of *H. ericae* and *Oidiodendron* spp. in the epacrids is so far supported mainly by molecular data. Mycorrhizal isolates from *Epacris impressa* formed either distinct clusters or clustered with *Hymenoscyphus ericae* or *Cistella grevillei* (Hyaloscyphaceae, Helotiales) (McLean et al., 1999), although the low degree of sequence identity and the limited range of taxa included in this study may have led to incorrect interpretations, as discussed by Sharples et al. (2000a). Chambers et al. (2000), based on ITS sequences, found that a fungal isolate from *Woollsia pungens* (Epacridaceae) was to be identified as an *Oidiodendron* species.

Ericoid fungi are also likely to encompass members of basidiomycetes. Early circumstantial evidence of basidiomycete fungi forming ericoid mycorrhiza in roots of *Rhododendron*, *Calluna* and *Vaccinium* (Bonfante-Fasolo, 1980; Englander and Hull, 1980; Peterson et al., 1980) were paralleled by recent observation of hyphae with clamp connections and dolipore septa forming typical mycorrhizal coils in roots of *E. arborea* in Italy (Bergero et al., 2000). These associates, however, could not be isolated and their identity remains to be positively ascertained. In addition, direct microscopic observations of the morphological diversity of ericoid fungi in field collected *E. arborea* roots, together with failure to bring many of them into culture, indicate the occurrence of a variety of unculturable mycobionts (Bergero et al., 2000). These results are in contrast with the general opinion that ericoid symbionts have good saprotrophic abilities and are able to grow on common culture media (Leake and Read, 1991). The use of PCR techniques to amplify fungal DNA directly from mycorrhizal roots may help unravelling their taxonomic nature.

The genetic diversity and the phylogeny of ericoid mycorrhizal fungi are discussed in more detail in another paper of this same issue (Berch et al., 2001), and we will focus therefore on some features of ericoid mycorrhiza that may be of ecological and evolutionary relevance.

Ericoid mycorrhizal roots are composite structures with multiple fungal occupants

The morphology of mycorrhizal roots described earlier indicates that epidermal root cells could potentially function as separate units, challenged and colonized by a variety of fungi resident in the rhizosphere (Figure 3). ITS-RFLP analysis of ericoid fungal isolates from different mycorrhizal plant species has demonstrated that multiple occupancy is a common phenomenon in ericaceous roots. We have observed

that the mycorrhizal roots of *C. vulgaris* from a heathland site in northern Italy harbour quite a diverse community of mycorrhizal fungi that formed typical mycorrhizal structures after resynthesis trials with axenic plants (Perotto et al., 1996). Similar investigations carried out on ericaceous (Bergero et al., 2000; Hambleton and Currah, 1997; Monreal et al., 1999) and epacridaceous (Chambers et al., 2000) plants also indicate the simultaneous presence of fungi with different ITS-RFLP patterns in the same root system.

Intraspecific genetic polymorphism can be revealed by sensitive techniques such as RAPD. When applied to mycological studies, this technique has been successfully used to identify races or even individual genets of filamentous fungi (Smith et al., 1992). RAPD was used to investigate ericoid fungal isolates derived from a single plant of *C. vulgaris*, either identified as *O. maius* or as sterile morphotypes that were distinguishable for their ITS-RFLP profiles (Perotto et al., 1996). RAPD analyses showed a high intraspecific polymorphism in the *O. maius* isolates, and a lower variability within populations of sterile mycelia. Many isolates of *O. maius* showing polymorphic RAPD bands were derived from the same plant of *C. vulgaris*, thus indicating that the roots of ericaceous plants are a complex mosaic where different populations of mycorrhizal fungi coexist, each represented by a variable number of genets. Multiple occupancy seems therefore to be a common phenomenon in ericoid mycorrhiza, and it has also been reported for example in arbuscular (Clapp et al., 1995) and ectomycorrhizal roots (Brand, 1992). This promiscuous situation may have important functional implications for the plant. The simultaneous association with many and diverse symbiotic fungi may represent an important strategy to broaden the array of functions in the colonization of difficult substrates. The hypothesis is more accreditable if such diversity mirrors a functional diversity, and different species of ericoid mycorrhizal fungi are indeed able to perform different physiological functions. For example, they can hydrolyse complex substrates, or they do so to a different extent (e.g., Leake and Read, 1991; Perotto et al., 1997; Varma and Bonfante, 1994). Even individuals within the same species have been shown to perform differently. For example, Cairney et al. (2000) found that isolates of the ericoid fungus *H. ericae* display different abilities to utilize inorganic and organic nitrogen sources. Different abilities of *O. maius* strains to tolerate high concentrations of heavy metals were demonstrated by Martino et al. (2000a,b), even when they were derived from the same site (Lacourt et al., 2000).

Group I introns in ericoid fungi: a fascinating evolutionary puzzle and a potential problem for molecular studies based on PCR-RFLP

Analysis of the nuclear ribosomal genes has revealed an unusual feature for many ericoid fungal isolates. Amplifications using universal primers designed on the SSU rDNA have yielded fragments which were often much larger in size than expected (Egger et al., 1995; Perotto et al., 2000; Vrålstad et al., 2000) due to the insertion of Group I introns. Group I introns form a structural and functional group characterized by four conserved regions which play a role in the formation of secondary structures. They are widespread but irregularly distributed in lower eukaryotes, especially algae and fungi (Gargas et al., 1995; Johansen et al., 1996). Group I introns occur at several locations along the chloroplast and mitochondrial genome, but seem to be restricted to the rDNA genes in the nuclear genome. They have been shown to splice both *in vitro* and *in vivo* due to the autocatalytic properties of the intron RNA, and are able to insert into intronless copies of the same gene with a process called *homing* (Cech, 1990).

In fungi, nuclear Group I introns have been found both in Ascomycetes and Basidiomycetes. Although it has not yet been clarified if they play a specific biological function in their fungal host, they represent a fascinating evolutionary puzzle and a suitable system for studying lateral movement of genetic elements. Horizontal transmission of Group I introns among fungal taxa has been discussed since their discovery on account of their scattered distribution and presence/absence in related taxa (e.g., Hibbett, 1996; Holst-Jensen et al., 1999; Nishida et al., 1998). Phylogenetic analysis has also indicated that horizontal transmission of Group I introns may have occurred between organelles (Turmel et al., 1995) and between fungi and plants during the intimate contacts established during pathogenic or symbiotic interactions (Nishida and Sugiyama, 1995). In ericoid symbiosis, like in all endosymbioses, the fungal and plant cytoplasm are only separated by a thin interface (Perotto et al., 1995), and so a possibility exists that some introns may be transmitted from the fungus to the host, although this aspect has never been investigated.

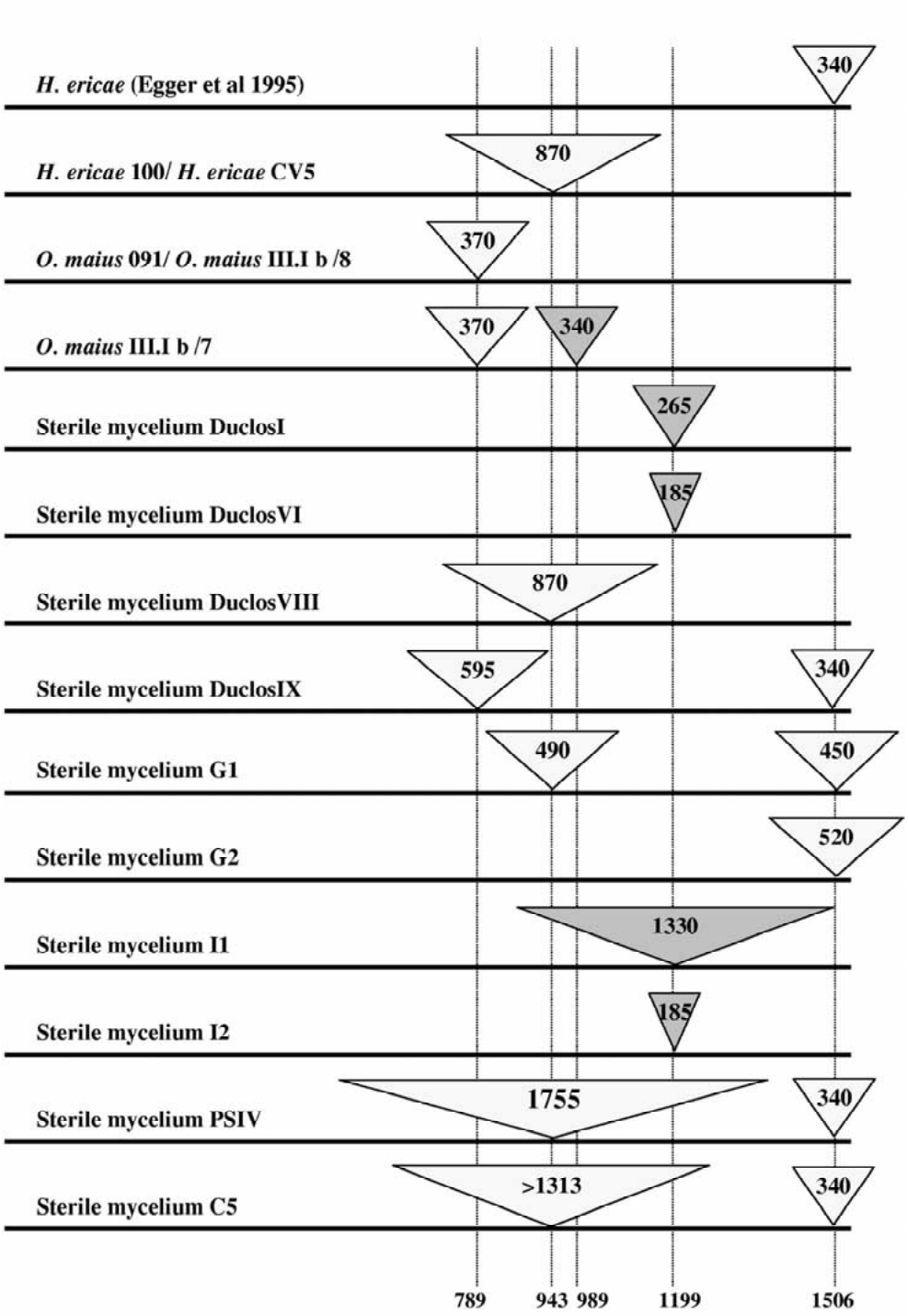

Figure 4. Diagram showing the position of DNA insertions in the SSU rDNA of ericoid isolates. The size of DNA insertions and their exact position (indicated below the diagram and relative to *Escherichia coli* rDNA) were established on the basis of sequence information. White triangles indicate typical Group I introns, whereas dark grey triangles indicate members of a novel class of ribosomal introns. Modified from Perotto et al. (2000).

Following an earlier report of a Group I intron in *H. ericae* (Egger et al., 1995), many additional introns have been found at different locations in the SSU rDNA of a large collection of ericoid fungi (Figure 4) including several isolates of *H. ericae, O. maius* and sterile mycelia (Perotto et al., 2000). The analysis of ericoid mycorrhizal fungi has also allowed the identification of a new class of ribosomal introns that lack the consensus sequences typical of Group I introns. The presence of four distinct and well conserved sequence regions in this new class of introns, together with their occurrence at specific sites different from those colonised by the more typical Group I introns (Figure 4), indicate that they represent a distinct population of insertional elements (Perotto et al., 2000).

Heterogeneity among isolates of the same species, expressed by the presence/absence of specific introns, was found for both *O. maius* and *H. ericae* (Perotto et al., 2000; Vrålstad et al., 2000). The optional presence of Group I introns in the rDNA of ericoid fungi may be a problem in molecular studies that rely on RFLP profiles of either conserved or variable rDNA regions. Since optional introns can be present or absent in the ribosomal genes of strains belonging to the same species, analyses based solely on RFLP profiles may result in misleading information on the diversity of these fungi.

Host range specificity in ericoid mycorrhiza: tight or loose friends?

As already mentioned, ericoid mycorrhiza was until recently considered an example of a highly specific association between few genera of plants and mycorrhizal fungi. The increase in our knowledge on the genetic diversity of ericoid mycorrhizal fungi, described in the previous paragraphs as well as in Berch et al. (2001), has contributed to modify our perception at least with respect to the fungal partner. Experimental evidence is accumulating to suggest that the range of plant species able to associate with ericoid mycorrhizal fungi may be also much wider than previously thought. Duckett and Read have reported in 1995 that *Hymenoscyphus ericae*, the best studied ericoid mycorrhizal symbiont, is also capable of forming *in vitro* intracellular coils in the rhizoids of liverworts. Further support to this observation derives from molecular studies that allowed Chambers et al. (1999) to assign a mycelium isolated in nature from the leafy liverwort *Cephaloziella exiliflora* to the genus *Hymenoscyphus*.

Other recent observations suggest that the host range of ericoid mycorrhizal fungi may be extended also to ectomycorrhizal plants in nature. Ericoid and ectomycorrhizal plants often coexist in natural ecosystems such as boreal or mediterranean forests, where ericaceous plants constitute the understorey vegetation of dominant ectomycorrhizal tree species (Read, 1991). Strong genetic similarities between fungi forming ericoid and ecto-mycorrhiza have been revealed by Vrålstad et al. (2000) in a boreal forest. Following ITS sequence comparison, the ericoid mycorrhizal fungus *H. ericae* was the closest relative of the mycobiont of *Piceirhiza bicolorata*, an ectomycorrhizal morphotype common in post-fire sites. Although the ability of some of these ectomycorrhizal isolates to form ericoid mycorrhiza with an ericaceous host has given so far inconclusive results (Vrålstad, 2001), these data demonstrate that the *H. ericae* aggregate groups together genetically related fungi, able to form distinct mycorrhizal types.

The possible sharing of mycorrhizal fungal isolates among ericoid and ectomycorrhizal plants was also investigated in a mediterranean forest by Bergero et al. (2000). These authors have demonstrated that several fungi (including *Oidiodendron* spp. and sterile morphotypes) were associated with both types of mycorrhizal plants. Fungal associates isolated from ectomycorrhizal *Quercus ilex* root tips formed ericoid mycorrhiza with *Erica arborea in vitro*, and molecular analyses indicate that some of these *Q. ilex* fungal associates were conspecific with mycorrhizal endophytes naturally occurring in *E. arborea* roots (Bergero et al., 2000). The physical and nutritional relationships between these ericoid fungal strains and the ectomycorrhizal plant require further investigation, but it is interesting to note that one ericoid mycorrhizal morphotype could infect *in vitro Q. ilex* seedlings with structures that resembled ectoendomycorrhizal infection (Bergero et al., 2000). The only other fungal species that may be able to form, at least *in vitro*, both ericoid (Monreal et al., 1999) and ecto- or ectoendomycorrhiza (Wilcox and Wang, 1987), depending on the host plant, is *Phialophora finlandia*.

The recent results just described open the exciting possibility that the same ericoid fungus may connect plant species with a different mycorrhizal status. Several studies have shown that the root systems of different plants become interconnected via the hyphae of shared mycorrhizal fungi, by means of which nu-

trients can be translocated (Arnebrant et al., 1993; Bethlenfalvay et al., 1991; Francis and Read 1984; Graves et al., 1997; Newman and Eason 1993; Simard et al., 1997; Whittingham and Read, 1982). Hyphal links have been reported for ectomycorrhizal fungi (Brownlee et al., 1983) and arbuscular fungi (Francis and Read 1984), but they have also been hypothezised in some more complex situations involving plants with different mycorrhizal status (Smith et al., 1995; Taylor and Bruns 1997). In the case of ericoid mycorrhizal fungi, the possibility of physical links between ericaceous and ectomycorrhizal plants (or liverworts) *via* their fungal associates remains an open question, as well as the nature of functional relationships with these hosts (Read, 2000). It also remains to be established whether or not the same fungus can form different types of mycorrhizal symbioses with distinct hosts in natural conditions. The classical bioassay to establish the mycorrhizal nature of a fungal isolate is the inoculation *in vitro* onto axenic seedlings. Although this assay remains an important test to elucidate the mycorrhizal potential of a fungal isolate, the conditions used are very different from those found in nature and the results must be interpreted with caution (Read, 1996).

Although interesting from a biological point of view, due to the more complex molecular mechanisms involved in the establishment of a tight symbiosis, the type of association of ericoid fungi with different host plants may not necessarily be relevant from a physiological point of view. It has been suggested that root–fungus associations might diverge from classical mycorrhizal types and yet function physiologically as a mycorrhiza under natural conditions (Jumpponen and Trappe 1998; Jumpponen et al., 1998). A study by Abuzinadah and Read (1989) showed that *Oidiodendron* enhances the growth of *Betula pendula* on a medium containing proteins as sole N source without producing a classical ectomycorrhizal infection.

The association of ericoid mycorrhizal fungi with ectomycorrhizal plants may be ecologically relevant in some stressed conditions. For example, genetic relatedness between ericoid and ectomycorrizal fungal associates has been revealed in forest environments subjected to fire (Bergero et al., 2000; Vrålstad et al., 2000). It could be hypothesised that one mycorrhizal host could thus provide an efficient source for biotrophic infection of other plant species. A role as a reservoir of ericoid fungi has also been suggested for liverwort rhizoids by Duckett and Read (1995).

In conclusion, recent biochemical, physiological and molecular studies have allowed us to gain better insights on ericoid mycorrhizal fungi. Identification of some mechanisms involved in metal tolerance provides some clues to understand the success observed for *H. ericae* and *O. maius* in colonizing polluted soils. However, the large number of recent publications on the genetic diversity of ericoid mycorrhizal endophytes indicates that the spectrum of fungal taxa has been largely underestimated, and that other fungi with potentially important functions may still to be discovered.

The molecular ecology of ericoid mycorrhizal fungi, and in particular the range of potential interactions with plants of different mycorrhizal status, is a more delicate issue still open to debate, where contrasting scenarios can be envisaged. Most of the results so far obtained indicate the possibility of a continuum from loose non-mycorrhizal to mycorrhizal associations between ericoid fungi and host plants, possibly related to environmental conditions and to the host plant species. This scenario is mainly based on ITS sequence similarities between isolates colonizing different host plants and on *in vitro* cross-inoculation available only for some fungal isolates. However, other data in the literature indicate that an opposite scenario cannot be discounted, where mycorrhizal specificity may be extremely high and even related to species or subspecies levels. Douglas et al (1989) have observed that an isolate of *Oidiodendron maius* from ectomycorrhizal Sitka spruce could not form ericoid mycorrhiza on an ericaceous host, which could normally harbour other fungal isolates of the same species. Similarly, ericoid mycorrhizal isolates belonging to the *H. ericae* aggregate only formed ericoid mycorrhiza on the ericaceous host when inoculated on ericaceous and tree species, whereas only ectomycorrhizal associations with tree species were observed in a similar cross-inoculation trial when ectomycorrhizal field isolates belonging to the same *H. ericae* aggregate were used (Vrålstad, 2001). These contrasting results indicate that although we have made considerable progress on the molecular analysis of ericoid mycorrhizal fungi, there are still exciting and unanswered questions that need to be addressed. In this process, it will be important to evaluate the results of genetic analyses relative to those from *in vitro* studies to understand the relationships of ericoid fungi with potential host plants in nature.

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