15 Mycorrhizal Specificity and Function in Myco-heterotrophic Plants

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Contents

15.1 Summary

We present an analysis of fungal specificity in myco-heterotrophic orchids and monotropes. We argue that specificity represents a continuum and can only be properly assessed using phylogenetic data. Several green orchids associate with wide phylogenetic arrays of *Rhizoctonia* species, and hence show little specificity, while other green orchids, and all studied achlorophyl-

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lous orchids and monotropes, associate with narrow phylogenetic groups of fungi, and hence show significant specificity. In several species, this tight specificity has been shown to apply from seed germination through adulthood under natural conditions, though not necessarily under in vitro conditions. Patterns of specificity have been correlated with patterns of fungal distribution and habitat variation in several myco-heterotrophs. However, studies of other myco-heterotrophs have shown that tight specificity is expressed even when diverse fungi co-exist with the plant. Moreover, in one case, genetic influences of the host plant have been shown to outweigh environmental influences over the patterns of specificity. Major host jumps and intraspecific host-race formation have contributed to the evolution of specialisation in several myco-heterotrophs. Some achlorophyllous orchids associate with wood-decay or parasitic fungi, but many recent studies have revealed associations with ecto-mycorrhizal fungi in orchids, monotropes, and a liverwort. Tracer studies show that autotrophic ecto-mycorrhizal host plants can provide the fixed carbon to nourish myco-heterotrophs linked by a shared fungal partner. Important outstanding questions concern recognition phenomena, the origins and evolution of specificity, the physiology and ecology of carbon exchange, and whether myco-heterotrophs interact with fungi in fundamentally different ways than do autotrophs.

15.2 Introduction

Leake (1994) defined plants that depend upon fungi for the supply of essential carbon sources, and in which the "normal" polarity of sugar movement from plant to fungus is reversed, as "myco-heterotrophs". Two classes of myco-heterotrophic plant (MHP) were recognised, one in which the ability to fix carbon has been completely lost (the "fully" myco-heterotrophic plants), and one in which, at least in later stages of the life cycle, some autotrophic capability is retained (the so-called "partial" myco-heterotrophs).

Dependence on fungal-derived energy sources has arisen independently on multiple occasions through the evolution of land plants. Full myco-heterotrophy occurs in roughly 400 species distributed through the Ericaceae (Monotropoideae), Polygalaceae, and Gentianaceae of the Dicotyledonae and the Burmanniaceae, Corsiaceae, Lacandoniaceae, Orchidaceae, Petrosaviaceae and Triuridaceae of the Monocotyledonae (Leake 1994). The achlorophyllous, gametophytic stages of some leptosporangiate ferns and lycopods as well as the sporophyte and gametophyte of the hepatic *Cryptothallus mirabilis*, are also myco-heterotrophic (Read et al. 2000). This assemblage of unrelated plants includes many taxa that have been shown to associate with ecto-mycorrhizal (EM) Basidiomycete fungi, and a number of taxa whose mycorrhizal organs contain vesicles and/or arbuscles, indicating associations with arbus-

cular mycorrhizal (AM) glomalean fungi. The repeated evolution of this habit across the two major mycorrhizal categories, and in most major lineages of vascular plants, argues against the view that myco-heterotrophy is a rare and anomalous strategy. Partial myco-heterotrophy, which encompasses all green orchids, appears to be even more widespread.

Seen from this perspective, full MHPs represent one end of an evolutionary continuum across which dependence upon fungi for supply of carbon moves from absolute to partial to none. This perception is contrary to that of Robinson and Fitter (1999) who consider full MHPs to be entirely distinct from all other mycorrhizal plants. Specificity toward particular fungi, a striking feature of at least some full MHPs, can also be viewed as a continuum, with possible ties to the autotroph–myco-heterotroph continuum. Here, we analyze environmental, genetic and evolutionary influences on specificity in the best-studied MHPs of the Orchidaceae and Monotropoideae from a continuum perspective. Specificity phenomena between plants and arbuscular mycorrhizal fungi or ecto-mycorrhizal fungi are reviewed respectively by Sanders (Chap. 16, this Vol.) and Molina et al. (1992).

Knowledge of the fine structure and cellular biology of the mycorrhizal interactions in several partial MHPs has progressed significantly in recent years (Peterson et al. 1998; Schmid and Oberwinkler 1993, 1994, 1995, 1996; Schmid et al. 1995; Uetake et al. 1992, 1997; Uetake and Ishizaka 1996; Uetake and Peterson 1997, 1998), but will not be covered in this review. Instead, we will focus on major gaps in the understanding of myco-heterotrophy that were pointed out in the conclusions of Leake (1994), namely, those concerning the identities and trophic niches of the fungal associates, the ecology of seed germination under natural conditions, and the dynamics of carbon transfer from fungus to plant.

15.3 Evidence for Specificity in Myco-heterotrophs

The fact that most plants display little evidence of specificity in their relationships with mycorrhizal fungi (Molina et al. 1992; also see Sanders, Chap. 16, this Vol.) make it all the more important to determine, in those cases where specificity is seen, the nature and impact of the specific associations. Both the evolution and ecology of specificity in symbioses are important areas of basic inquiry (Bernays 1988; Jaenike 1990; Berenbaum 1996), but to date, few model systems involving specificity *toward*, rather than *by,* fungi have been identified. From an applied standpoint, recognition of a requirement for a specific symbiont could prove critical to the conservation of MHPs.

There is a long and lively history of debate concerning the specificity of orchids toward their fungal symbionts (see e.g. Hadley 1970 versus Clements 1988). Some of the controversy over specificity arises simply from differences

in the often unstated definitions used by different workers. A second major source of confusion has been the problematic identification and taxonomy of some of the fungal symbionts, especially the '*Rhizoctonia*' species that frequently colonise orchids (Ramsay et al. 1986; Clements 1988). A third source of confusion has likely been the wide variation in fungal isolation procedures, and difficulties in distinguishing isolates that are mycorrhizal from those that colonise orchids as non-mycorrhizal endophytes or parasites (Andersen and Rasmussen 1996). New phylogenetic data and methods offer hope for clarifying these points of confusion.

Molecular-phylogenetic and ultrastructural methods are helping to resolve both *Rhizoctonia* systematics, and the problems of discriminating mycorrhizal from non-mycorrhizal isolates. DNA sequence data from ribosomal genes and spacer regions are revolutionising our understandings of fungal systematics and mycorrhizal ecology (Bruns et al. 1992; Swann and Taylor 1993; Berbee 1996; Gardes and Bruns 1996; Hibbett et al. 1997; Karen and Nylund 1997; O'Donnell et al. 1997). These methods have allowed the rapid identification of fungi that are difficult or impossible to isolate in pure culture, thus, circumventing the biases particular to fungal isolation procedures (Taylor and Bruns 1997; Taylor and Bruns 1999b). However, the polymerase chain reaction (PCR) method can introduce its own set of biases, so a combination of techniques is preferable (Taylor and Bruns 1999b), and fungal isolation is obviously a necessary step prior to conducting most experiments.

Problems associated with varying definitions of specificity can be overcome by adapting the modern, consensus definition of specificity from the general evolutionary and ecological literature (Thompson 1994). Specificity, as we define it, is not a binary categorical descriptor, but instead, represents a continuous axis, where the position of an organism on the axis is defined by "*the phylogenetic breadth of the mycorrhizal associations of that particular plant or fungus*." We note that specificity in the fungal partner need not bear any particular relation to specificity in the plant; degree of specificity is a unique attribute of each partner. The description of an organism as 'more' or 'less' specific is best made by comparison with other organisms that are involved in similar interactions. Studies that have investigated specificity in myco-heterotrophic plants are discussed below and summarised in Table 15.1.

15.3.1 Overview of Specificity in Orchids

Most orchids display an unusual life-history strategy in which minute "dust seeds" that lack substantial energy reserves are produced in great number and are typically highly adapted for wind dispersal (Ramsbottom 1922). This strategy is also typical of many MHPs outside the Orchidaceae (Leake 1994). Immediately following seed germination, in the pre-photosynthetic phase

during which most plants utilise their seed reserves, orchids form mycorrhizal associations and extract energy-containing compounds needed for growth from their fungi. While most orchids eventually develop green, photosynthetic organs, fully myco-heterotrophic orchids have given up photosynthesis entirely, and rely upon fungal derived energy sources throughout their life cycle. Of the roughly 400 fully myco-heterotrophic angiosperms, approximately 35 % occur in the Orchidaceae (Leake 1994). These species are distributed across several tribes and many genera that are not sister taxa, showing that the transition to complete myco-heterotrophy has occurred independently on numerous occasions within this family (Dressler 1993).

Orchidaceous mycorrhizae are distinct from other major mycorrhizal categories both anatomically and in the taxonomy of the fungal symbionts. Hyphae proliferate abundantly within certain cortical cells, forming coils, known as pelotons that are reminiscent of Paris-type arbuscular (AM) or ericoid mycorrhizae (Burgeff 1959; Smith and Read 1997), but do not form any "mantle" outside the plant. However, AM vesicles and arbuscles are absent and all reliably described orchid fungi belong to the Basidiomycetes.

15.3.1.1 Rhizoctonia Systematics

Fungi isolated from mycorrhizal organs of adult orchids frequently belong to the anamorphic form-genus *Rhizoctonia* (Burgeff 1959; Currah et al. 1997; Hadley 1982). Higher fungi are often given two names which are based upon two different sets of characters: anamorphic names are based upon vegetative characteristics such as hyphal morphology and asexual spores, while teleomorphic names are based upon features of the sexual reproductive structures, i.e., macro and microscopic features of sporocarps. Sexual structures generally provide more informative characters for taxonomy and systematics than do vegetative structures. However, fungi placed in the form-genus *Rhizoctonia* seldom reveal their basidiocarps, and, hence, are often referred to and identified by their anamorphs. Production of chains of swollen, monilioid cells (asexual resistant propagules) is a uniting anamorphic feature among *Rhizoctonia* fungi. The *Rhizoctonia* species from orchids have often been treated as though they belong to a coherent taxonomic entity, perhaps due to their anamorphic similarities and ubiquity in soil, despite the fact that the teleomorphs differ sufficiently to suggest distant phylogenetic relationships.

Members of the following *Rhizoctonia* anamorph/teleomorph pairs, all of which belong to the Hymenomycetes, have been isolated from orchids: *Opadorhiza/Sebacina, Epulorhiza/Tulasnella, Ceratorhiza/Ceratobasidium* and *Rhizoctonia* DC/*Thanatephorus* (Moore 1987, 1996; Andersen and Rasmussen 1996). While they have not been isolated from orchids, there are a number of additional teleomorphs that have, at times, been linked to *Rhizoctonia*, including *Athelia, Botryobasidium, Cejpomyces*, *Helicobasidium (Ure-*

Table 15.1. Selected studies dealing with specificity in myco-heterotrophic plants

Taxon	Samples	Place	Fungal identification methods[a]
Green orchids			
Amerorchis rotundifolia	19 Adults	Field	*In planta* + isolation: vegetative morphology
Acianthus reniformis	26 Adults	Field	Isolation: morphology of vegetative and sexual stages
Acianthus caudatus	12 Adults	Field	Isolation: morphology of vegetative and sexual stages
Acianthus exsertus	12 Adults	Field	Isolation: morphology of vegetative and sexual stages
20 *Caladenia* spp.	98 Adults	Field	Isolation: morphology of vegetative and sexual stages
Calypso bulbosa	? Adults	Field	*In planta* + isolation: morphology of vegetative and sexual stages
Cypripedium candidum	? Adults	Field	Isolation: vegetative morphology
Cypripedium parviflorum	? Adults	Field	Isolation: vegetative morphology
Dactylorhiza purpurella	21 Adults	Field	Isolation: vegetative morphology
Dactylorhiza purpurella	Seedlings	Lab	In vitro germination tests
5 *Diuris* spp.	28 Adults	Field	Isolation: morphology of vegetative and sexual stages
Goodyera repens	Seedlings	Lab	In vitro germination tests
Goodyera oblongifolia	8 Adults	Field	*In planta* + isolation: vegetative morphology.
Microtis parviflora	18 Adults + 72 seedlings	Field	Isolation: vegetative morphology
Microtis parviflora	Seedlings	Lab	In vitro germination tests
Platanthera hyperborea	13 Adults	Field	*In planta* + isolation: vegetative morphology
Platanthera hyperborea	15 Seedling packets	Field	*In planta* + isolation: vegetative morphology
Platanthera leucophaea	? Adults	Field	Isolation: vegetative morphology
Platanthera obtusata	14 Adults	Field	Isolation: vegetative morphology
Pogonia ophioglossoides	? Adults	Field	Isolation: vegetative morphology
Pterostylis barbata	6 Adults	Field	Isolation: vegetative morphology, anastomosis grouping
Pterostylis nana	8 Adults	Field	Isolation: vegetative morphology, anastomosis grouping
Pterostylis aff. rufa	7 Adults	Field	Isolation: vegetative morphology, anastomosis grouping
Spiranthes sinensis	37 Adults	Field	Isolation: vegetative morphology
Spiranthes sinensis	Seedling	Lab	In vitro germination tests
Spiranthes sinensis	18 Adults + 27 seedlings	Field	Isolation: vegetative morphology, anastomosis grouping

Reference	Identified fungi[b]	Trophic group[c]
Zelmer et al. (1996)	*Epulorhiza* (7), *Moniliopsis* (3)	Unknown
Warcup (1981)	25 of 26 isolates were *Sebacina vermifera*	Possibly ecto-mycorrhizal
Warcup (1981)	*Sebacina vermifer*a (2), *Tulasnella cruciata* (10)	Possibly ecto-mycor rhizal, unknown
Warcup (1981)	*Tulasnella calospora*	Unknown
Warcup (1971)	108 of 110 isolates were *Sebacina vermifera*	Possibly ecto-mycorrhizal
Currah et al. (1988)	*Rhizoctonia* spp., *Rhizoctonia anaticula, Thanatephorus pennatus*, unidentified clamped fungi	Unknown
Curtis (1939)	*Rhizoctonia subtilis*[d]	Saprotroph
Curtis (1939)	*Rhizoctonia subtilis*[d]	Saprotroph
Harvais and Hadley (1967)	A variety of unidentified *Rhizocto-nia* spp., *R. repens, R. solani,* and other fungi	Unknown
Harvais and Hadley (1967)	Unidentified *Rhizoctonia* spp., *R. repens*, and *R. solani* (including pathogenic strains)	Unknown
Warcup (1971)	*Tulasnella calospora*	Unknown
Hadley (1970)	*Ceratobasidium cornigerum* (1/3), *Ceratobasidium* sp. (1/2), *Thanatephorus cucumeris* (3/9), *Rhizoctonia* sp. (2/4)	N/A
Zelmer et al. (1996)	*Epulorhiza* (1), *Ceratorhiza* (34), *Moniliopsis* (3)	Unknown
Perkins et al. (1995)	2 *Epulorhiza* spp. were isolated from s eedlings and adults	Unknown
Perkins et al. (1995)	*Epulorhiza repens, Epulorhiza* sp., 3 *Ceratorhiza* spp.	N/A
Zelmer et al., (1996)	*Epulorhiza* (7), *Ceratorhiza* (7), *Moniliopsis* (5)	Unknown
Zelmer et al. (1996)	*Epulorhiza* (3), *Ceratorhiza* (1), unknown clamped fungus (4), unidentified (1)	Unknown
Curtis (1939)	*Rhizoctonia robusta, R. sclerotica, R. Stahlii, R. subtilis*[d]	Saprotrophs
Currah et al. (1990)	*Epulorhiza anaticula, Ceratorhiza goodyerae-repentis, Sistotrema* sp.	Unknown, Saprotrophs
Curtis (1939)	*Rhizoctonia monilioides, R. repens*[d]	Saprotrophs
Ramsay et al. (1987)	Binucleate *Rhizoctonia* spp., mostly anastomosis group P3	Unknown
Ramsay et al. (1987)	Binucleate *Rhizoctonia* spp., anastomosis groups P1 and P4	Unknown
Ramsay et al. (1987)	Binucleate *Rhizoctonia* spp., anastomosis group P2	Unknown
Terashita (1982)	*Rhizoctonia repens* (32), *Rhizoctonia solani* (16)	Unknown
Masuhara et al. (1993)	22 out of 23 *Rhizoctonia* tester strains, including binucleate and multinucleate isolates	N/A
Masuhara and Katsuya (1994)	All plants contained *Rhizoctonia repens*, 2 also had *R. solani*	Unknown

Table 15.1 (*Continued*)

Taxon	Samples	Place	Fungal identification methods[a]
Achlorophyllous orchids			
Cephalanthera austinae	26 Adults	Field	*In planta* + isolation: molecular (ITS RFLPs, ITS sequences, ML5–6 sequences)
Corallorhiza maculata	9 Adults	Field	Isolation: vegetative morphology
Corallorhiza maculata	? Adults	Field	*In planta* + *ex planta*: vegetative morphology and hyphal tracing
Corallorhiza maculata	104 Adults	Field	*In planta*: molecular (ITS RFLPs, ML5–6 sequences)
Corallorhiza mertensiana	27 Adults	Field	*In planta*: molecular (ITS RFLPs, ML5–6 sequences)
Corallorhiza striata	? Adults	Field	*In planta* + *ex planta*: vegetative morphology and hyphal tracing
Corallorhiza striata	8 Adults	Field	*In planta* + isolation: molecular (ITS RFLPs, ML5–6 sequencing)
Corallorhiza trifida	? Adults	Field	*In planta* + *ex planta*: vegetative morphology and hyphal tracing
Corallorhiza trifida	18+ Adults	Field	*In planta* + isolation: vegetative morphology
Corallorhiza trifida	4 Adults + 24 seedlings	Field	*In planta* + isolation: molecular (ITS RFLPs and ITS sequences)
Galeola altissima	Seedling	Lab	In vitro germination tests
Galeola septentrionalis	? Adults	Field	*In planta* + isolation: vegetative morphology
Gastrodia cunninghamii	? Adults	Field	*In planta* + *ex planta*: vegetative morphology and hyphal tracing
Gastodia minor	? Adults	Field	*In planta* + *ex planta*: vegetative morphology and hyphal tracing
Gastrodia sesamoides	? Adults	Field	*In planta* + *ex planta*: vegetative morphology and hyphal tracing
Neottia nidus-avis	8 Adults + 7 seedlings	Field	*In planta*: molecular (ITS RFLPs, ITS sequences, 28S sequences)
Rhizanthella gardneri	1 Adult	Field	Isolation: morphology of vegetative and sexual stages
Yoania	? Adults	Field	*In planta* + *ex planta* + isolation: vegetative morphology and hyphal tracing
Achlorophyllous monotropes			
Allotropa virgata	? Adults	Field	*Ex planta*: mycorrhiza morphology
Allotropa virgata	37 Soil cores near adults	Field	*In planta* + *ex planta*: mycorrhiza morphology + molecular (ITS RFLPs)
Hemitomes congestum	? Adults	Field	*Ex planta*: mycorrhiza morphology and Trappe (1985)
Monotropa uniflora	30 Adults	Field	*In planta* + *ex planta*: vegetative morphology and hyphal tracing

Reference	Identified fungi[b]	Trophic group[c]
Taylor and Bruns (1997)	14 Species spanning the *Thelephora-Tomentella* group	Ecto-mycorrhizal
Zelmer et al. (1996)	7 *Moniliopsis* isolates	Parasitic
Campbell (1970b)	Mainly *Armillaria melea*, also two unknown fungi	Parasitic
Taylor and Bruns (1997); Taylor and Bruns (1999b)	20 Species spanning much of the Russulaceae	Ecto-mycorrhizal
Taylor and Bruns (1999b)	3 Closely related species in the Russulaceae	Ecto-mycorrhizal
Campbell (1970b)	Unknown brown fungus, unknown white fungus	Ecto-mycorrhizal
Taylor (1997)	A narrow clade within the *Thelephora-Tomentella* group	Ecto-mycorrhizal
Campbell (1970b)	*Mycena thuja*, unknown fungus	Unknown
Zelmer and Currah (1995)	Unknown yellow, clamped Basidiomycete	Ecto-mycorrhizal
McKendrick et al. (2000b)	7 ITS RFLP types, all in the *Thelephora-Tomentella* group	Ecto-mycorrhizal
Umata (1995)	*Erythromyces crocicreas, Ganoderma australe, Loweporus tephroporus, Microporus affinus, Phellinus* sp.	Wood decay saprotrophs
Hamada (1939)	*Armillaria mellea*	Parasitic + saptrotrophic
Campbell (1962)	*Armillaria mellea*	Parasitic + saprotrophic
Campbell (1963)	Unknown, brown, clamped Basidiomycete	Ecto-mycorrhizal
Campbell (1964)	Possibly *Fomes mastoporus*	Wood decay saprotroph (white rot)
S.L. McKendrick, J.R. Leake, D.L. Taylor and D.J. Read (in prep.)	*Sebacina vermifera*-like fungi	Unknown
Warcup (1985, 1991)	The single isolate obtained was named *Thanatephorus gardneri*	Ecto-mycorrhizal
Campbell (1970a)	*Lycoperdon perlatum*	Saprotroph + ecto-mycorrhizal + parasitic
Castellano and Trappe (1985)	*Rhizopogon vinicolor*	Ecto-mycorrhizal
Lefevre et al. (1998)	*Tricholoma magnivelare*	Ecto-mycorrhizal
Castellano	*Rhizopogon vinicolor, Cenococcoum geophilum*	Ecto-mycorrhizal
Campbell (1971)	*Armillaria mellea*	Parasitic

Table 15.1 (*Continued*)

Taxon	Samples	Place	Fungal identification methods[a]
Monotropa uniflora	23 Adults	Field[e]	*In planta*: morphology
Monotropa uniflora	6 Adults	Field	*In planta*: molecular (ML5–6 sequences)
Monotropa hypopitys	10 Adults	Field	*In planta* + *ex planta*: vegetative morphology and hyphal tracing
Monotropa hypopitys	11 Adults	Field	*In planta* + culture
Monotropa hypopitys	? Adults	Field	*Ex planta*: mycorrhiza-fruitbody connections, mycorrhiza morphology
Monotropa hypopitys	9 Adults	Field	*In planta*: molecular (TSOP, ML5–6 sequences)
Monotropastrum sp. ???	? Adults	Field	*In planta* + *ex planta*: vegetative morphology and hyphal connections
Pleuricospora fimbriolata	? Adults	Field	*Ex planta*: mycorrhiza-fruitbody connections, mycorrhiza morphology
Pterospora andromedea	? Adults	Field	*Ex planta* + isolation: mycorrhiza morphology
Pterospora andromedea	31 Adults	Field	*In planta*: molecular (ITS RFLPs, TSOP, ML5–6 sequences)
Pterospora andromedea	Seedlings	Lab	In vitro germination tests
Sarcodes sanguinea	Seedlings	Lab	In vitro germination tests
Sarcodes sanguinea	12 Adults	Field	*In planta*: molecular (TSOP, ML5–6 sequences)
Sarcodes sanguinea	57 Adults	Field	*In planta* + isolation: molecular (ITS RFLPs, ITS sequences)

[a] Isolation refers to the culturing of fungi from pelotons or from whole tissue sections. *In planta* refers to the identification of fungi by direct methods that do not require fungal isolation. *Ex planta* refers to direct observations of fungal morphology or hyphal connections from plants to identifiable fungal structures (fruit-bodies or ecto-mycorrhizal roots). For the molecular methods, ML5–6 refers to fungal mitochondrial large subunit ribosomal gene sequences in the region described by Bruns et al. (1998). TSOP refers to "taxon-specific oligonucleotide probe" and ITS-RFLP refers to restriction digests of the fungal nuclear internal transcribed spacer region.

[b] Fungal taxa are listed exactly as given in the cited publication; note that different authors have used different nomenclatures. For *Rhizoctonia* fungi, various names may refer to the same taxon (e.g. *Rhizoctonia repens*, *Epulorhiza repens*, and *Tulasnella calospora*). In the case of laboratory seed germination tests, only fungi that produced compatible interactions are listed. Fractions in parentheses show the number of compatible strains over the total number of strains tested for a given fungal species.

[c] The trophic niches are listed whether or not the fungi are currently accepted as the legitimate mycorrhizal symbionts. In some cases we infer the trophic category of a fungus from other sources. However, if the authors have made a definitive statement concerning the trophic niche, their conclusion is given preference.

[d] It is not clear how the *Rhizoctonia* epithets used or coined by Curtis fit into modern *Rhizoctonia* taxonomy.

[e] Based on study of herbarium specimens.

Reference	Identified fungi[b]	Trophic group[c]
Martin (1986)	*Russula* species, and unidentified Russulaceae	Ecto-mycorrhizal
Cullings et al. (1996)	Russulaceae	Ecto-mycorrhizal
Campbell (1971)	Possibly *Clitocybe squamulosa*	Parasitic + ecto-mycorrhizal
Martin (1985)	Several species of *Tricholoma*	Ecto-mycorrhizal
Castellano and Trappe (1985)	*Elaphomyces muricatus*	Ecto-mycorrhizal
Cullings et al. (1996)	Suilloid group (including *Rhizopogon*)	Ecto-mycorrhizal
Kasuya et al. (1995)	Unknown yellow, clamped Basidiomycete	Ecto-mycorrhizal
Castellano and Trappe (1985)	*Truncocolumella citrina, Rhizopogon vinicolor, Cenococcoum geophilum*	Ecto-mycorrhizal
Castellano and Trappe (1985)	*Cenococcoum geophilum*, unknown isolate	Ecto-mycorrhizal
Cullings et al. (1996)	*Rhizopogon subcaerulescens* group	Ecto-mycorrhizal
Bruns and Read (2000)	2 Closely related *Rhizopogon* spp.	Ecto-mycorrhizal
Bruns and Read (2000)	2 Closely related *Rhizopogon* spp.	Ecto-mycorrhizal
Cullings et al. (1996)	Cantharellaceae, *Rhizopogon, Suillus*, unknown fungus	Ecto-mycorrhizal
Kretzer et al. (2000)	*Rhizopogon ellenae* complex	Ecto-mycorrhizal

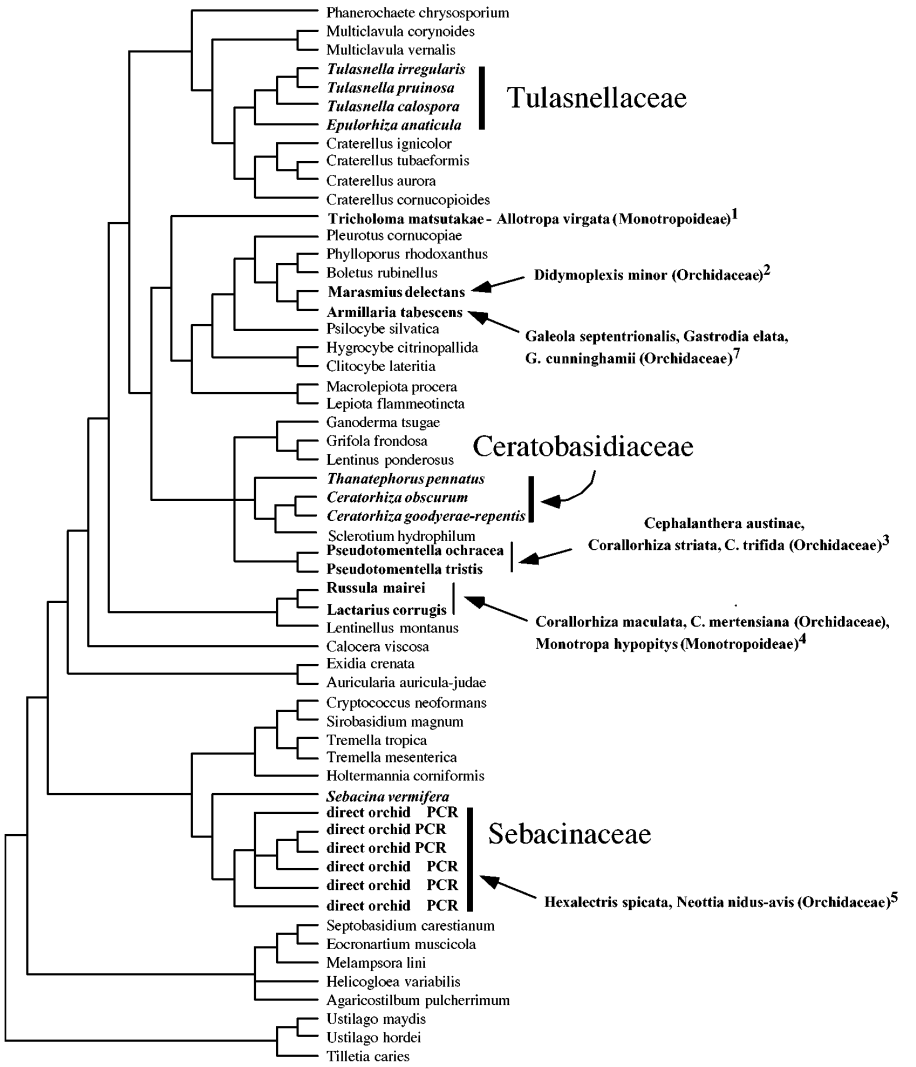
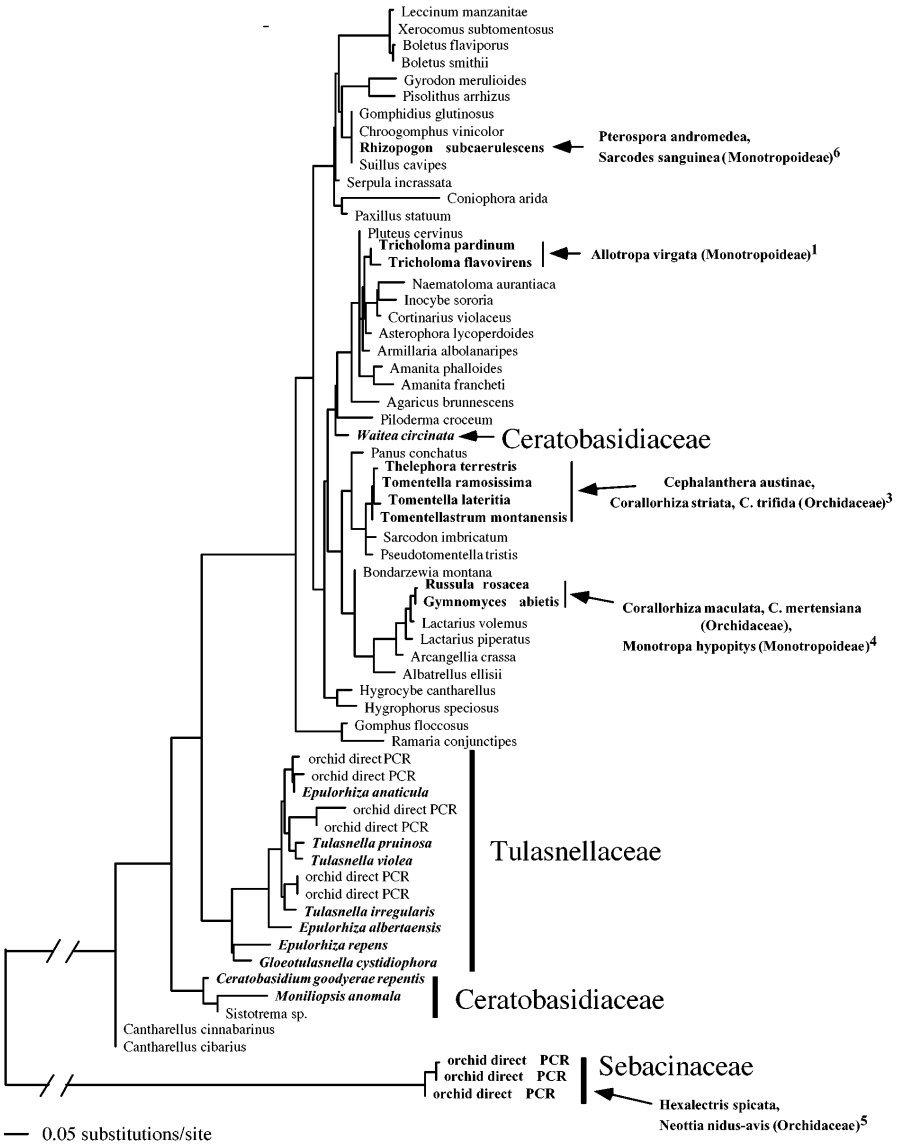


Fig. 15.1A,B. Maximum likelihood trees resulting from heuristic searches using the Hasegawa-Kishino-Yano (HKY) model of nucleotide substitution (unequal rates with gamma distribution shape parameter = 0.5 and Ti/Tv = 2.0) as implemented in PAUP*4.0 are shown. Swapping began using the most parsimonious trees; the cutoff for swapping on a tree was set at 1 % worse than the maximum likelihood. Fungi that are members of *Rhizoctonia* sensu lato are indicated in *bold-italic* and are followed by the family of the associated teleomorph. The specific myco-heterotroph-fungal pairs are documented in the following references: *1* Lefevre et al. (1998); *2* Burgeff (1932); *3* Taylor (1997); Taylor and Bruns (1997); McKendrick et al. (2000b); *4* Cullings et al. (1996); Taylor and Bruns (1997, 1999b); *5* S.L. McKendrick, J.R. Leake, D.L. Taylor and D.J. Read, in prep.; D.L. Taylor, T.D. Bruns and S.A. Hodges, in prep.; *6* Cullings et al. (1996); Bidartondo et al. (2000); Kretzer et al. (2000); *7* Kusano (1911); Hamada (1939); Campbell (1962); Terashita (1996). **A** Strict consensus of the two maximum likelihood trees (-ln L scores = 9117.43441). The data set included 96 taxa and 451 bases from the 5′ end of the nuclear 28S ribosomal gene. The GenBank sequences are from the following studies:



Berres et al. (1995); Feibelman et al. (1997); Hibbett et al. (1998); Johnson and Vilgalys (1998); Drehmel et al. (1999); Hopple and Vilgalys (1999); Mitchell and Bresinsky (1999); Taylor and Bruns (1999a) as well as D. L. Taylor (unpubl.). For ease of presentation, numerous taxa (mostly of the Agaricales) were pruned from the trees after the search was complete. The Ustilaginiomycetes were designated as a monophyletic outgroup. **B** Phylogram of one of the six maximum likelihood trees resulting from analysis of the 355 bp mitochondrial large subunit database of Bruns et al. (1998), with additional sequences from Kristiansen et al. (2001); D.L. Taylor, T.D. Bruns, S.A. Hodges (in prep.),; and D.L. Taylor (unpubl.), for a total of 133 taxa. The six trees with scores of 4776.56233 were obtained from a heuristic search. Again, numerous taxa of less relevance were pruned from the resulting trees. Midpoint rooting was used

diniomycetes), *Oliveonia*, *Scotomyces*, *Tofispora*, *Waitea* and *Ypsilonidium* (Andersen and Rasmussen 1996). The sexual structures of (1) *Sebacina,* (2) *Tulasnella,* and (3) *Ceratobasidium/Thanatephorus* differ markedly in the size, shape and septation of hypobasidia, basidia, sterigmata and basidiospores (Wells 1994; Andersen and Rasmussen 1996). In addition, the septal pore caps are unique in each of these three groups (Tu et al. 1977; Currah and Sherburne 1992; Andersen 1996; Muller et al. 1998). It is thus an unfortunate coincidence that a variety of soil-inhabiting Basidiomycetes were assigned to the same form-genus *and* were found associated with many orchids. This coincidence has caused confusion, and has likely slowed progress in understanding the specificity of orchids toward their mycorrhizal fungi.

Analysis of both nuclear (Fig. 15.1A) and mitochondrial large-subunit sequences (Fig. 15.1B) from an array of Basidiomycetes, including various orchid isolates, dramatically illustrate the large phylogenetic distances between the three major orchid *Rhizoctonia* clades, with *Waitea* comprising a fourth Hymenomycete clade that has not been found in orchids. Neither of these analyses resolves the placement of the major *Rhizoctonia* clades with confidence, which is not surprising, considering that there are very few representatives of the Corticiaceae, Aphyllophorales, or tremellaceous jelly fungi in these data sets, which were assembled primarily from available GenBank accessions (for fuller descriptions of the alignments and data sources, see Bruns et al. 1998; Taylor and Bruns 1999a). However, in addition to the wide divergences among the major *Rhizoctonia* clades, these analyses demonstrate that significant sequence variation is present among different orchid isolates *within* each major clade. Given that there are similar or lower levels of sequence divergence within other important Basidiomycete families (e.g. Russulaceae, Amanitaceae, Boletaceae; see discussion in Bruns et al. 1998), it appears that the major fungal clades associated with orchids are at least as old and diverse as these families, despite the fact that most orchid *Tulasnella* isolates have been referred to a single species, *T. calospora* (= *Rhizoctonia repens*). Hence, treating orchid *Rhizoctonia* strains as a homogeneous group is unsupportable from an evolutionary point of view.

Until recently, fungal specificity in orchids has been studied using only two approaches, the first being the analysis of seed germination and growth when paired with various fungal strains under controlled, monoxenic conditions on media in the laboratory, and the second being the morphological description and/or isolation of fungi from adult plants growing in the wild. We review the evidence about specificity obtained using these approaches in the next two sections.

15.3.1.2 Laboratory Seed Germination

Several of the most influential early workers in the field of orchid mycorrhiza, including Noel Bernard (1909) and Hans Burgeff (1932, 1936) found that only one or a few fungal strains were highly effective in promoting seed germination and growth in vitro. They interpreted these results as indicating a high degree of fungal specificity in these partially and fully MHPs. However, Curtis (1937, 1939), and a number of later workers, found that strains isolated from adults of one species were often ineffective in laboratory germination tests when paired with seeds of the same species, but stimulated germination of seeds from another orchid species (Hadley 1970; Harvais 1974; Harvais and Hadley 1967; Nishikawa and Ui 1976; Tokunaga and Nakagawa 1974). These inconsistent results led them to suggest a general lack of fungal specificity in green orchids. The few studies that have successfully obtained in vitro seed germination in achlorophyllous, fully myco-heterotrophic orchids have also suggested low specificity (Umata 1995, 1997a,b, 1998). *Ganoderma australe*, *Loweporus tephroporus*, *Microporus affinus*, *Phellinus* sp. and *Erythromyces crocicreas* all stimulated germination of the fully MHP *Galeola altissima* (Umata 1995), even though *E. crocicreas* is the only fungus known to associate with this orchid in the wild.

15.3.1.3 Isolation and Characterisation of Fungi from Wild Adults

Analyses of specificity in adult orchids based on the isolation of fungi and their identification by morphology have frequently suggested low specificity, at least in green species. Fungal isolates from single species of European, North American and Japanese terrestrial orchids often belong to several of the major *Rhizoctonia* clades (Currah et al. 1987, 1988, 1990; Curtis 1937, 1939; Harvais 1974; Harvais and Hadley 1967; Nishikawa and Ui 1976; Tokunaga and Nakagawa 1974). Furthermore, Curtis (1937) found that different orchid species growing in the same location often harboured the same *Rhizoctonia* strains, while a single orchid often harboured different *Rhizoctonia* strains in each distinct habitat in which it was found. Zelmer et al. (1996) examined squash mounts of mycorrhizal roots of 17 North American terrestrial species, and, like Curtis, observed different fungi in the same plant species from different sites. There are at least two potential limitations to these studies. First, because it has proven extremely difficult to induce fruit body formation in *Rhizoctonia* strains isolated from orchids, the identification of these isolates was based upon less reliable vegetative morphological features (see discussion in Andersen 1990; Andersen and Rasmussen 1996). Secondly, in most of these studies, isolates were obtained from slices of mycorrhizal tissue, which does not reliably discriminate between infections by mycorrhizal and other fungi.

In contrast to the above-mentioned studies, numerous green Australian orchids have yielded isolates that suggest significant specificity. Warcup and Talbot (1966, 1967, 1971, 1980) revolutionised orchid mycorrhizal research by obtaining fruit-body formation in numerous *Rhizoctonia* fungi isolated from orchids, enabling their teleomorphs to be determined. To avoid isolation of non-mycorrhizal root inhabiting fungi, individual pelotons were transferred onto isolation plates. Generally, isolates from several individuals of each orchid species were obtained, often across a wide geographic range. Several important patterns emerge from their analyses of hundreds of isolates and dozens of orchid species (Warcup 1971, 1981). First, some orchid species were associated almost exclusively with a single fungal species, while other orchids were restricted to associations within a single fungal genus, and only a few orchids consistently associated with *Rhizoctonia* species belonging to two or more of the major clades shown in Fig. 15.1A,B. Second, orchid species belonging to the same genus, or even subtribe, were often specific toward the same fungal species or genus. These patterns have been confirmed by several other groups working in Australia (Clements 1988; Perkins et al. 1995; Perkins and McGee 1995; Ramsay et al. 1986, 1987).

A further exception to the pattern of low specificity in adult green orchids was recorded in *Epipactis helleborine*. This orchid, which frequently occurs as albino forms intermixed with green plants, is consistently colonised by a morphologically distinct non-*Rhizoctonia* fungus with dark-coloured, unclamped, thick-walled verrucose hyphae over a wide geographic range in Eastern Europe (Salmia 1988, 1989). Although the dark fungus formed extensive pelotons, it could only be isolated when the most stringent sterilisation methods were employed (Salmia 1988). With less stringent sterilisation, numerous other soil inhabiting fungi appeared in culture and presumably out-competed the dark-coloured fungus.

15.3.1.4 Fully Myco-heterotrophic Orchids

While morphological studies of specificity in adult green orchids are contradictory, studies of adult achlorophyllous orchids have been more consistent, and suggest two interesting patterns. The first pattern is one of specific associations in myco-heterotrophic orchids (Table 15.1). The second pattern is that, with a few exceptions, the fungi involved are not *Rhizoctonia* species. Examples of these specific associations with non-*Rhizoctonia* fungi that have emerged from morphological identification methods include *Gastrodia elata* and *Galeola septentrionalis* with *Armillaria* spp. (Kusano 1911; Terashita 1996), *Galeola altissima* with *Erthryomyces crocicreas* (Hamada and Nakamura 1963), *Galeola hydra* with *Fomes* sp. (Burgeff 1959), *Didymoplexis minor* with *Marasmius coniatus*, and another *Gastrodia* sp. with a *Xerotus* sp. (Burgeff 1932, 1959). Campbell (1962, 1963, 1964, 1970a,b) also suggested spe-

cific fungal associations in a number of MHPs, although the reported identities of several of these fungi appear to be incorrect based upon later studies.

Studies in the last few years have applied two additional approaches to measuring and understanding specificity, namely the analysis of seed germination in the field, and the direct molecular identification, without isolation, of the fungi associated with both seedlings and adults from the field.

15.3.1.5 Seedling Germination in the Field

Gaku Masuhara and co-workers performed some of the earliest studies of seed germination in the field, and obtained evidence that the expression of specificity under laboratory conditions can differ strikingly from its expression in the field. As an aside, *Rhizoctonia* strains whose hyphae will fuse (anastomose) on Petri plates can potentially mate, while strains whose hyphae do not fuse appear to be reproductively isolated. Hence, the sorting of strains into "anastomosis groups" provides a useful technique for identifying related strains, especially within the large *Rhizoctonia solani* complex.

Masuhara et al. (1993) showed that in vitro germination and growth of the partial MHP *Spiranthes sinensis* was stimulated by *Rhizoctonia* tester strains from all but one of 23 multinucleate and binucleate anastomosis groups (mostly members of the *Ceratobasidium/Thanatephorus* clade), as well as *Rhizoctonia repens* (*Tulasnella*). In contrast, seeds that were planted into a field site in a rectangular array were colonised in 26 out of 27 cases by *Rhizoctonia repens* (Masuhara and Katsuya 1994). A similar predominance of *R. repens* was found in adults from the same site. Parsley stem baits were placed in the soil across the same grid, and isolations from these baits showed that seven different *Rhizoctonia* anastomosis groups were widely distributed in the field. Isolates of anastomosis group G were especially common, even though this fungus was never found in *Spiranthes* plants, while *R. repens* was not a dominant coloniser of the baits. The isolates obtained from parsley baits also induced *Spiranthes* germination and growth under sterile laboratory conditions, despite the fact that they were not associated with plants in the field. These authors proposed that the patterns in the field represent 'ecological specificity' while the in vitro results represent a much broader 'potential specificity'. Other studies have shown that the outcome of particular plant-fungus interactions in vitro are highly dependent on the exact nutrient conditions of the media (Beyrle and Smith 1993), and that fungal isolates tend to loose their symbiotic potential over time when maintained in culture (Ramsbottom 1922; Hadley 1982). These factors may have contributed to inconsistencies in laboratory germination studies.

15.3.1.6 Molecular studies of wild plants

Recent studies of achlorophyllous orchids have improved our understandings of specificity by employing geographically widespread sampling and molecular tools for fungal identification. Zelmer and Currah (1995) showed that the leafless terrestrial orchid *Corallorhiza trifida* associates with a non-*Rhizoctonia* fungus displaying yellow, clamped hyphae in both Europe and Canada. Nuclear ribosomal internal transcribed spacer (ITS) sequence analysis has since shown that the specific symbionts of *C. trifida* are members of the Thelephoraceae (McKendrick et al. 2000b). Similar molecular methods, coupled with widespread sampling, demonstrated that *Cephalanthera austinae*, like *Corallorhiza trifida*, associates only with fungi in the Thelephoraceae, while *Corallorhiza maculata* and *C. mertensiana* associate only with fungi in the Russulaceae (Taylor and Bruns 1997, 1999b). Although these associations are not one-to-one, they indicate specificity toward fungi falling into single genera or families. The restriction of associations of achlorophyllous (and perhaps also green) orchids to discrete Basidiomycete families or genera represents narrow mycorrhizal specificity, in contrast to typical photosynthetic ecto-mycorrhizal plant species, which associate with numerous fungi representing wide phylogenetic arrays of Basidiomycetes and Ascomycetes (Molina et al. 1992).

While the trend of non-*Rhizoctonia* associations in achlorophyllous orchids is striking, there are some exceptions. The specific associates of the well-known achlorophyllous orchid *Neottia nidus-avis* are members of the *Sebacina* clade of *Rhizoctonia* fungi (S.L. McKendrick, J.R. Leake, D.L. Taylor and D.J. Read, in prep.). Similarly, a study of the achlorophyllous orchid *Hexalectris spicata* showed that the orchid was consistently associated with a *Sebacina*-like fungus on both the east and west coast of the United States, but that some east coast individuals also harboured *Thanatephorus pennatus* in both roots and non-mycorrhizal rhizomes (D.L. Taylor, T.D. Bruns and S.A. Hodges, in prep.).

The mixture of fungi found in *Hexalectris* serves to illustrate the potential dangers of evaluating specificity using isolation alone. The *Sebacina*-like fungi were slow growing and difficult to isolate, even from densely colonised roots, while the fast-growing *Thanatephorus* was easily isolated from roots and scantily colonised rhizomes. The authors suggest that the *Sebacina*-like fungi, which gave the dominant ITS restriction fragment length polymorphism (RFLP) patterns from peloton-filled roots of plants containing both fungi, are the specific associates of this orchid, while *Thanatephorus* occurs sporadically as a parasite or endophyte. Similarly, fast growing *Ceratorhiza* strains were isolated from *Corallorhiza maculata* (Zelmer et al. 1996), but considerable molecular evidence points to unculturable *Russula* species as the specific associates of this orchid (Taylor and Bruns 1997, 1999b). Orchids, like most plants, are probably hosts to numerous epiphytic and endophytic fungi (Carroll 1995; Clay 1993; Jumpponen and Trappe 1998; Petrini et al.

1995; Stone et al. 1996). Indeed, when isolations were carried out on roots and leaves of several epiphytic and lithophytic species of *Lepanthes*, very similar arrays of fungi, predominantly *Xylaria* and *Rhizoctonia* species, were isolated from *both* organs (Bayman et al. 1997). Since these fungi did not form pelotons in leaves, it seems that they were present as endophytes. Based on the patterns of isolation from *Lepanthes, Hexalectris* and *Corallorhiza*, we suggest that aggressive *Rhizoctonia* species from the *Ceratobasidium/Thanatephorus* clade may sometimes colonise orchids in an endophytic or parasitic manner while other fungi simultaneously function as the mycorrhizal symbionts of these orchids.

While narrow fungal specificity appears to be the rule in adult achlorophyllous orchids, generalisations cannot yet be made concerning specificity in green orchids due to the conflicting results of various studies. Molecular techniques may help to overcome the difficulties in identifying *Rhizoctonia*-like fungi and in dealing with mixed infections by both mycorrhizal and non-mycorrhizal fungi. Molecular techniques have so far been applied only to achlorophyllous species. However, a recent report demonstrated PCR-amplification of diagnostic fungal genes directly from single pelotons dissected from the green orchid *Dactylorhiza majalis* (Kristiansen et al. 2001). Such precise techniques offer hope for improved understandings of mycorrhizal interactions even in situations where multiple fungal species colonise a single plant.

15.3.2 Overview of Specificity in the Monotropoideae

The literature on specificity in the Monotropoideae is much less extensive than that on orchids. Monotropoid mycorrhizae are unique due to the penetration of plant epidermal cells by individual hyphal "pegs" which eventually lyse and eject their cytoplasm into the plant cell (Dudderidge and Read 1982). Monotropoid fine-roots are surrounded by a fungal mantle and contain Hartig-nets as in typical ecto-mycorrhizae (Smith and Read 1997).

Molecular studies have shown that individual species within the Monotropoideae associate with phylogenetically narrow groups of fungi, and thus far, all of these fungi have been ecto-mycorrhizal taxa. In the first such report, Cullings et al. (1996) used ITS-RFLPs, mitochondrial taxon-specific probes, and partial ITS sequence to show that *Pterospora andromedea*, throughout its range in western North America, was associated exclusively with fungi in the *Rhizopogon subcaerulescens* species group. They also examined three other species of monotropes, and, with the exception of *Sarcodes sanguinea*, found that all appeared to be specialists. However, their reports for species other than *P. andromedea* are in need of confirmation, as they were based on small sample sizes and no ITS data.

Two more recent reports confirm specificity for an additional species and remove the only apparent exception to specificity within the monotropes.

Lefevre et al. (1998) used molecular characterisation and morphology to show that *Allotropa virgata* associates exclusively with *Tricholoma magnivelare*. Kretzer et al. (2000) re-examined the associates of *Sarcodes sanguinea* from a much larger sample (76 plants versus 12) with a combination of ITS-RFLP and ITS sequence data obtained directly from roots; cultures were also isolated from the *Sarcodes* roots and used to synthesise mycorrhizae with pine. All sequences, RFLP patterns and cultures belonged to the *Rhizopogon ellenae* species complex. Additional sampling throughout most of the geographic range of *Sarcodes sanguinea* confirms this result (M. Bidartondo, pers. comm.). Interestingly, the *R. ellenae* complex is closely related to, but distinct from, the *Rhizopogon* species that associate with *P. andromedea*.

Kretzer et al. (2000) noted that the roots of *S. sanguinea* seemed to turn over fairly quickly, and those that were discoloured and had fragmented mantles were often difficult or impossible to amplify by PCR. They also noted, however, that fragmented mantles and associated rhizomorphs seen on older roots looked like those of *R. ellenae*. We suspect that the conflict with earlier molecular identification may have been based, at least in part, on amplifications from older roots by Culling et al. (1996). Nevertheless, six of the 12 mitochondrial large subunit (mt-LSU) ML5–6 region ribosomal gene sequences reported by Cullings et al. (1996) were suilloid, and are therefore consistent with a *Rhizopogon* identification. The other six are not good matches to any taxa in a fairly extensive database of ecto-mycorrhizal Basidiomycetes, although five of the six (reported as Cantharellaceae) are now known to be close to *Clavulina* (Bruns et al. 1998). Whatever the cause of the conflict, these earlier reports should now be viewed with skepticism given the greater sample size and higher resolution approach (ITS versus mt-LSU) used in the Kretzer et al. (2000) study.

All of the recent molecular studies have demonstrated specificity, and their results are therefore at odds with Castellano and Trappe (1985), who listed a different and more diverse set of fungal associates for several members of the Monotropoideae on the basis of attempts to trace mycelial connections between sporocarps and roots. It is noteworthy that the descriptions of the two fungal isolates from *Pterospora andromedea*, although not identified, resemble a *Rhizopogon* species. Using the same approach, Campbell (1971) claimed to have traced *Armillaria* rhizomorphs to *Monotropa uniflora* root balls, but the current molecular evidence suggests that the associates fall within the Russulaceae (Cullings et al. 1996). In contrast, morphological characterisation of the associates of *Monotropa uniflora* and *M. hypopithys* by Martin (1985, 1986) are compatible with recent molecular identifications. He reported that *M. uniflora* associated exclusively with several *Russula* species and that European collections of *M. hypopithys* associated with *Tricholoma* species. This agrees with the Cullings et al. (1996) report for *M. uniflora* and with recent unpublished work on *M. hypopithys* from M. Bidartondo (pers. comm.).

15.4 Influences on Specificity

In the preceding sections, we have summarised data showing that, unlike most autotrophic plants, a number of fully myco-heterotrophic species in the Orchidaceae and Monotropoideae are fungal specialists. We next consider the influences of genotype and environment on patterns of fungal specificity, and show how specificity has evolved in orchids and monotropes. We discuss evidence concerning the relative contributions of fungal distribution, habitat and genotype to the expression of specificity, and then take up the issue of whether changes in specificity occur through different developmental stages of the plants.

15.4.1 Local Distribution of Fungi

At present, the limited data available suggest that some MHPs that associate with specific fungi do so, even when surrounded by numerous fungal species. For example, a variety of fungal species, identified both as ecto-mycorrhizae and as fruit bodies, were observed in close proximity to, or intermingled with, the root balls of *Monotropa hypopitys, Allotropa virgata,* and *Pterospora andromedea* (Castellano and Trappe 1985). Combined with later evidence that these plants associate exclusively with single genera or species of fungi (Cullings et al. 1996; Lefevre et al. 1998), these observations are contrary to the hypothesis that specificity is simply due to an absence of alternate fungi. The studies described above of the *Rhizoctonia* fungi occurring in soil alongside the partial MHP *Spiranthes sinensis* (Masuhara and Katsuya 1994) repeat this pattern. Similarly, tree roots growing within a few centimetres of *Cephalanthera austinae* flower spikes were colonised by a variety of ecto-mycorrhizal fungi, often including species in the Russulaceae, while the orchid was associated exclusively with fungi in the Thelephoraceae (Taylor and Bruns 1997). Perhaps even more striking was the observation that every sampled individual of *Corallorhiza maculata*, growing in an area of several hundred square metres, was associated with a single *Russula* species which was never found fruiting on the plot, while mushrooms of six other *Russula* species were collected throughout the plot (Taylor and Bruns 1999b). These studies did not objectively quantify the diversity and abundance of fungal species at the sites, but do clearly show that numerous other fungi were present that were not associated with the specialised MHPs.

The only quantitative, belowground study of the fungal community surrounding a MHP revealed an immense diversity of ecto-mycorrhizal fungi and an intriguing fine-scale spatial patterning of these fungi (Bidartondo et al. 2000). ITS RFLP analysis revealed the presence of 80 different species of ecto-mycorrhizal fungi colonising red fir roots in only 36 soil cores harvested

near *Sarcodes sanguinea* plants (Monotropoideae) at a single site. Within *Sarcodes* root balls, and in soil cores 10 cm away from the root balls, the exclusive *Sarcodes* symbiont, *Rhizopogon ellenae*, was the dominant fungus colonising fir roots (by mycorrhizal biomass). In contrast, *R. ellenae* was not a dominant fungus on fir roots in cores 100 cm from *Sarcodes* and was never found in cores 500 cm away. The authors argue that the *Sarcodes* plants must be promoting colonisation of fir roots by *Rhizopogon*, rather than occupying microsites already containing dense *Rhizopogon* mycorrhizae, due to the fact that the fir ecto-mycorrhizae occur surrounding the expanding *Sarcodes* root ball, rather than in its centre. This surprising observation is consistent with Bjorkman's finding that extracts from *Monotropa* plants stimulate growth of the *Monotropa* fungus, in vitro (Bjorkman 1960). Even more striking is the observation that fir root densities increase dramatically very close to *Sarcodes* plants (Bidartondo et al. 2000), suggesting that these plants are able to locally stimulate the growth of both the autotrophic host tree and the fungus. It would appear that these plants are able to significantly alter ecto-mycorrhizal community structure, at least on a fine spatial scale.

15.4.2 Habitat and Genetic Influences on Specificity

Studies of green Australian orchids have contributed to our understandings of geographic and habitat influences over specificity. Ramsay et al. (1987) isolated binucleate *Rhizoctonia* fungi (presumably *Ceratobasidium* spp.) from several *Pterostylis* species over a wide geographic area and from a variety of habitats, then determined the anastomosis group to which each isolate belonged. They found that one anastomosis group occurred over a wide area and range of habitats, while two others were common in dry habitats, and a fourth occurred only in a wetter coastal region. A fifth anastomosis group was also a dominant colonist, but only of *Pterostylis nana* and other species when they were growing in plantations of exotic pines, suggesting that this fungus is a habitat specialist, while isolates belonging to the first group were recovered from all *Pterostylis nana* plants growing outside the plantations in native plant communities. This appears to be a case where the pattern of fungal distribution across plant communities has impacts upon the observed patterns of specificity. Similar habitat and geographic patterns were uncovered by molecular analysis of the ecto-mycorrhizal *Russula* species associated with the fully MHP *Corallorhiza maculata* (Taylor and Bruns 1997, 1999b). Certain *Russula* species, defined by RFLP analysis of the ITS region, were the dominant symbionts of orchids growing in *Quercus* forests, but were never found in samples from nearby coniferous forests, thus, demonstrating a strong correlation between specificity and plant community. In addition, a single *Gymnomyces* species (Russulaceae) was found in all samples collected above 2000 m in elevation, but was never found at lower elevations.

An important outstanding issue concerns the determination of causation, as opposed to correlation, in producing the habitat and geographic patterns described above. Additional studies of *Corallorhiza maculata* have shown that much of the correlation between *Russula* species occurrences, in planta, and environmental factors could be due to co-variation with another critical parameter: plant genotype. Several sequence-characterised amplified region (SCAR) markers were developed for *C. maculata* and multilocus genotypes were determined for 122 plants (D.L. Taylor, T.D. Bruns and S.A. Hodges, in prep.). The *Russula* species associated with each of these plants was also determined, and placed in a phylogenetic context by sequencing of the fungal ITS region. Four of the six plant genotypes turned out to associate with their own separate clades of fungal species within the Russulaceae. The authors conclude that the genetic constituency of *C. maculata* individuals has a stronger influence on the occurrence of particular fungi, in planta, than does habitat, because different plant genotypes never shared a *Russula* species, even when growing together. Instead, each plant genotype was associated with a distinct fungal lineage, regardless of the presence of other plant genotypes and other *Russula* species at the same site. The correlations between fungal occurrences in *C. maculata* and habitat variables appear to be due to preferences of the various plant genotypes for different habitats. These subspecialised plant genotypes appear to be analogous to the "host-races" commonly seen in herbivorous insects and phytopathogenic fungi (Price 1980; Farrell et al. 1992; Thompson 1994).

15.4.3 Ontogenetic Influences on Specificity

Symbiotic interactions often change in an organised fashion through the life of an organism. This concept is best illustrated by the many parasites that switch hosts between discrete life-cycle stages, such as host-alternating rust fungi. Due to the minute size of the seeds in most MHPs – a striking evolutionary convergence pointed out by Leake (1994) – the ontogenetic stages prior to the emergence of aboveground organs have been exceedingly difficult to observe in the field (Rasmussen and Whigham 1998). These difficulties have been largely overcome by encasing seeds in mesh bags or packets with pore sizes that retain the seeds but permit the entry of fungal hyphae (Rasmussen and Whigham 1993). This technique has been used to compare fungal associations in young protocorms, under nearly natural conditions, with fungal associations in wild adults, and to ask questions about the timing and stages of seedling development and the distribution of compatible fungi in the field.

The results from the majority of these seed-packet studies suggest that mycorrhizal specificity in MHPs is expressed from the very earliest stages of ontogeny. In the initial seed packet studies of Rasmussen and Whigham

(1993), they found that the achlorophyllous orchid *Corallorhiza odontorhiza* germinated only at sites where adults occurred naturally. Seeds that had germinated contained pelotons formed by a slow-growing, dark colored fungus bearing clamp connections. Similar fungi have been consistently observed in adults of this species, and found to belong to the Thelephoraceae based upon ITS sequence analysis (Taylor 1997). Thus, it seems likely that *C. odontorhiza* seedlings and adults are both specific toward fungi in the Thelephoraceae. Direct, PCR-based analyses of fungal ITS sequences in planta revealed that both minute seedlings and adults of *Corallorhiza trifida* from two continents associate exclusively with fungi in the Thelephoraceae (McKendrick et al. 2000b). Germination of *Corallorhiza maculata* seeds in packets occurred 2 years after planting, and involved the same *Russula* species that was found in most adults at that site (D.L. Taylor, unpubl. data). Molecular analyses also showed that seedlings from packets and wild adults of the fully MHP *Neottia nidus-avis* were associated with identical *Sebacina*-like species (S.L. McKendrick, J.R. Leake, D.L. Taylor and D.J. Read, in prep.). While these studies have found fungi belonging to the same genus or family in both seedlings and adults, seedlings were sometimes associated with fungal species that were not found in adults (McKendrick et al. 2000b). It is conceivable that a greater diversity of related species colonise wild seedlings than colonise adults, but the sample sizes of the present studies are too small to demonstrate this conclusively. An in vitro study has recently shown that *Pterospora* and *Sarcodes* (Monotropoidea) seeds germinate when challenged with closely related, but 'incorrect' species of *Rhizopogon*, while the seeds were unresponsive when challenged with an array of more distantly related ecto-mycorrhizal Basidiomycetes (Bruns and Read 2000). If similar phenomena occur in nature, it implies that specificity may narrow as plants mature subsequent to germination, due to compatibility phenomena. This narrowing could be accomplished by the replacement of incorrect with correct fungi during the life of the plant, or simply by the death of plants associated with the wrong fungus. The latter possibility is especially interesting because it would imply a large selection coefficient favouring 'targeting' by the plant of the most compatible fungi at the earliest possible stage. It will also be important to determine what role the fungi play in shaping observed patterns of specificity, e.g. whether fungal rejection of the plant could be responsible for incompatibility such as that seen in *Pterospora* seedlings associated with the wrong *Rhizopogon* species.

Since the seedlings of photosynthetic terrestrial orchids are initially heterotrophic, but become autotrophic at a later stage in ontogeny, it is worthwhile to ask whether changes in specificity coincide with the heterotrophic to autotrophic transition. Unfortunately, there is very little data on this subject. The field germination studies of *Spiranthes sinensis* clearly showed that this photosynthetic orchid targets the same fungus at seed germination and in adulthood (Masuhara and Katsuya 1994). In contrast, seedlings of the green orchids *Cypripedium calceolus, C. passerinum, Platanthera hyperborea* and

Spiranthes romanzoffiana from out-planted packets sometimes contained pelotons with clamp connections, while adults of these species never contained clamped hyphae, suggesting the occurrence of different fungal species in seedlings and adults (Zelmer et al. 1996). Furthermore, fungal hyphal morphologies in seedlings of *Platanthera hyperborea, Cypripedium passerinum* and *Spiranthes romanzoffiana* differed across sites (Zelmer et al. 1996). As with the studies of achlorophyllous orchids, larger sample sizes are needed to statistically test the apparent differences between seedlings and adults. Molecular identification of the fungi should also be pursued, as many of the fungi associated with seedlings in that study were not identified.

15.5 Evolution of Specificity

Molecular-phylogenetic studies are beginning to reveal evolutionary patterns of specialisation in MHPs, in addition to the ecological patterns described above. Representatives of only a few of the known specific associates of MHPs were available for inclusion in the phylogenetic trees presented in Fig. 15.1A,B. Yet, even these few examples demonstrate that a wide phylogenetic diversity of Basidiomycete fungi have become the targets of various orchids and monotropes. Furthermore, even closely related plants, such as different *Corallorhiza* species, target distantly related fungi (in this case the Russulaceae versus the Thelephoraceae), indicating that major "host-shifts" have occurred rapidly as measured on an evolutionary time-scale. Possibly, processes analogous to host-race formation have contributed to the evolution of mycorrhizal specialisation in other MHPs, as well as *Corallorhiza*. The recent finding of genetic variation in specificity within *Corallorhiza maculata* suggests that evolutionary changes in specificity are ongoing in this species. Such intraspecific genetic variation opens the way for studies of the selective pressures that are acting to shape specificity and other aspects of the mycorrhizal interaction.

15.6 Fungal Trophic Niches and Mycorrhizal Carbon Dynamics

The trophic niches (i.e. the major sources of energy) of the fungal symbionts of MHPs are of interest for several reasons. First, to the degree to which these plants are fungal-specific, they offer an indirect means of identifying, and perhaps quantifying, carbon flows through hyphal networks in soil. Second, the carbon sources of the fungi are likely to have strong impacts upon the ecology of these plants. Put more simply, if the fungus depends on a particu-

lar resource, the plant must also depend on it. Third, determination of fungal trophic niches may offer clues about the selective pressures that have shaped the evolution of specificity in MHPs. Of particular interest are differences in the trophic niches of fungi associated with autotrophic versus partially myco-heterotrophic versus fully myco-heterotrophic plants. It may be that one function of specificity in fully MHPs is to restrict associations to taxa belonging to a particularly suitable trophic niche. The trophic niches of fungal associations from several green orchids, achlorophyllous orchids and achlorophyllous monotropes are given in Table 15.1. The niches of ecto-mycorrhizal fungi and the impact of environmental factors on ecto-mycorrhizal fungi are discussed by Erland and Taylor (Chap. 7, this Vol.).

The *Rhizoctonia* species that have been found in wild orchid seedlings are of interest, since protocorms are non-photosynthetic, regardless of the later photosynthetic status of the plant. Fungi from each of the three *Rhizoctonia* clades found in adult orchids have also been reported in orchid protocorms from the field.

The *Ceratobasidium/Thanatephorus* clade has been most extensively studied due to the economic importance of the ubiquitous pathogen *Rhizoctonia solani* (*Thanatephorus cucumeris*). *Ceratobasidium* species have been isolated from numerous adult green orchids, and have also been found in a few wild seedlings (Zelmer et al. 1996; Hayakawa et al. 1999), most notably of orchids in the genus *Goodyera*. An elegant study by Downie demonstrated that the *Ceratobasidium* fungus of *Goodyera* occurred preferentially on dead pine needles in both the soil litter layer, and aboveground in the forest canopy (Downie 1943). Hence, it seems likely that seedlings of *Goodyera* acquire carbon indirectly from pine needles via the saprotrophic activities of the associated fungi. However, closely related or conspecific *Ceratobasidium* strains form endophytic associations with conifer roots (Sen et al. 1999).

The rare, achlorophyllous underground Australian orchids *Rhizanthella gardneri* and *R. slateri* form mycorrhizal associations with fungi which are also capable of forming ecto-mycorrhizal structures, including a mantle and Hartig net, on various photosynthetic hosts (Warcup 1985, 1991). Two isolates fruited in culture, but their taxonomic placement was problematic. Warcup eventually placed them in *Thanatephorus*, but stated that they have several atypical characters for that genus. Ecto-mycorrhiza formation has not been reported for any other *Thanatephorus* species. Further phylogenetic and ecological studies of these fungi are needed before concluding that *Thanatephorus* is a genus characterised by the ability to form ecto-mycorrhizae.

Rhizoctonia repens (*Tulasnella* clade) has been recorded in numerous wild orchid seedlings (Rasmussen and Whigham 1993; Masuhara and Katsuya 1994; Rasmussen 1995; Zelmer et al. 1996; Hayakawa et al. 1999). Strains of *R. repens* that were tested did have some cellulose degrading capabilities (Smith 1966), but several members of the genus *Tulasnella* were found to lack lignolytic enzymes (Worrall et al. 1997). There is no evidence that they form

ecto-mycorrhizae. In summary, the trophic activities of fungi in this clade are almost completely unknown, which is unfortunate given their prominence as orchid mycorrhizal symbionts.

The last *Rhizoctonia* clade, which includes *Sebacina vermifera*, forms mycorrhizal associations with several genera of green orchids in Australia, and has recently been found to include specific associates of the achlorophyllous orchids *Neottia nidus-avis* (both seedlings and adults) and *Hexalectris spicata* (S.L. McKendrick, J.R. Leake, D.L. Taylor and D.J. Read, in prep.; D.L. Taylor, T.D. Bruns and S.A. Hodges, in prep). As with *Tulasnella*, the trophic activities of these fungi are unknown. The only clue is provided by the fact that *Sebacina vermifera* and related fungi have been isolated as secondary colonists of both ecto-mycorrhizae and arbuscular mycorrhizae of various autotrophs (Williams 1985; Milligan and Williams 1988; Warcup 1988; Williams and Thilo 1989). Whether they are plant root endophytes, plant parasites, saprotrophic rhizosphere fungi, or myco-parasites remains unclear.

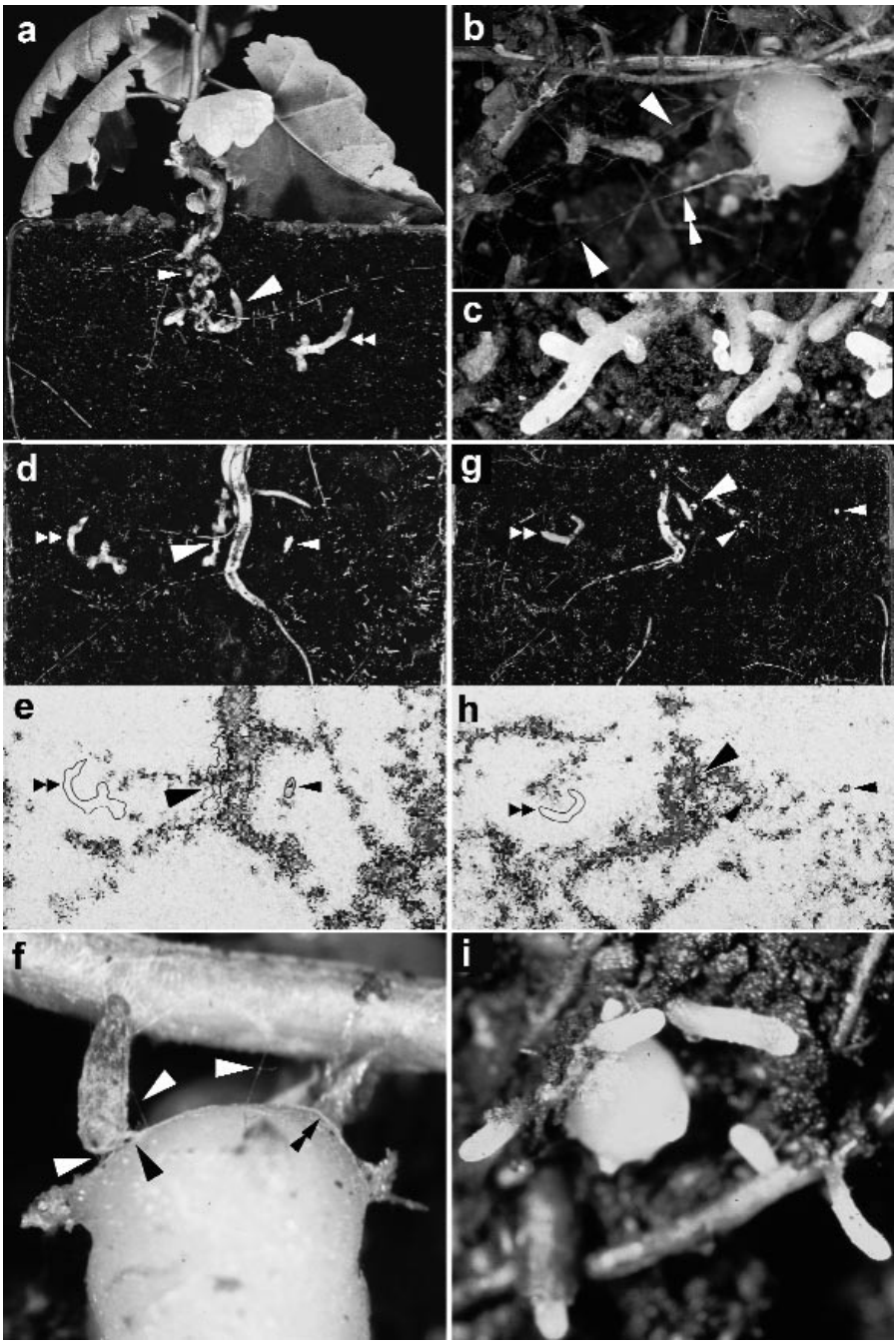
As mentioned above, most achlorophyllous orchids do not associate with fungi that fall into the *Rhizoctonia* clades (Furman and Trappe 1971). These non-*Rhizoctonia* fungi are phylogenetically diverse (see Fig. 15.1A,B), but appear to share the attribute of exclusive access to large and reliable sources of fixed carbon. These fungi are aggressive pathogens, wood-decay fungi and ecto-mycorrhizal symbionts. It has been hypothesised that these fungi are preferred over *Rhizoctonia* species as targets for specificity by fully myco-heterotrophic plants due to their linkages to larger carbon sources (Taylor and Bruns 1997; McKendrick et al. 2000a). This hypothesis is currently difficult to evaluate given the uncertainty concerning the trophic activities of many *Rhizoctonia* species. Kusano (1911) made the radical claim that *Gastrodia elata* associates specifically with a species of *Armillaria*, a well-known genus of tree-killers and saprotrophs, and supported his claim with extremely detailed anatomical observations. Recent work in Japan has revealed the exact identities of several *Armillaria* species associated with another achlorophyllous orchid, *Galeola septentrionalis* (Cha and Igarashi 1996; Terashita 1996). *Gastrodia cunninghamii* in New Zealand has also been reported to associate with an *Armillaria* species (Campbell 1962). The only confirmed case of association with a wood-decay fungus occurs in the achlorophyllous liana, *Galeola altissima*, whose putative specific associate, *Erythromyces crocicreas,* decomposes the dead tree trunks on which the orchid is found (Hamada and Nakamura 1963; Umata 1995). There are two additional, unconfirmed cases. Burgeff reports the isolation of a *Xerotus* species, which could fall into the wood-decay category, from another *Galeola* species (Burgeff 1959), and Campbell believed the specific associate of *Gastrodia sesamoides* to be the wood-decay fungus *Fomes mastoporus* (Campbell 1964).

Several reports document specific associations between achlorophyllous orchids and ecto-mycorrhizal Basidiomycetes. Zelmer and Currah showed that isolates from *Corallorhiza trifida* produced ecto-mycorrhizae on *Pinus*

contorta (Zelmer and Currah 1995). Molecular analyses were conducted on field-collected roots and rhizomes of the achlorophyllous orchids *Cephalanthera austinae* and *Corallorhiza maculata* and surrounding ecto-mycorrhizal tree roots (Taylor and Bruns 1997). PCR-amplified fungal ITS RFLP patterns and/or single strand conformational polymorphism (SSCP) "fingerprints" were identical in paired orchid and tree root samples, despite the strikingly different endomycorrhizal versus ecto-mycorrhizal anatomies observed.

A recent microcosm study (McKendrick et al. 2000a) using *C. trifida* provided the first confirmation both of the ability of this myco-heterotrophic orchid to act as a source of inoculum which enables ecto-mycorrhizae to form on the roots of autotrophs, and to facilitate the subsequent transfer of carbon between the plants through linking mycelium (Fig. 15.2a–i). *Betula pendula* plants were transferred to soil supporting naturally germinated seedlings of the orchid. Ecto-mycorrhizae formed rapidly on roots of *Betula* (Fig. 15.2a–c),

Fig. 15.2. A myco-heterotrophic orchid is linked to an ecto-mycorrhizal tree seedling through a shared fungal partner. **a–i** Microcosms in which the mycorrhizal fungus of the myco-heterotrophic orchid, *Corallorhiza trifida,* formed ecto-mycorrhizae on roots of *Betula pendula*. **a** The upper half of a representative microcosm containing *Corallorhiza* plants which were added as seedlings (*large arrow*), control plants in which hyphal connections to *Betula* were broken (*double arrow*), and a recruit of the orchid which developed from seed in situ (*small arrow*). Note the clusters of ecto-mycorrhizal tips on the *Betula* roots. **b** Detail of the "recruit" shown in **a**: fungal hyphal bridge (*large arrows*) between the developing orchid plant and adjacent mycorrhizal root tips; hyphae can be seen passing from the end of a rhizoid on the orchid seedling (*double arrow*) to adjacent ecto-mycorrhizal root tips. **c** Detail of the branched ecto-mycorrhizal root tips of *Betula* which developed in association with the mycorrhizal fungus of *C. trifida*. **d** The upper portion of a second replicate microcosm in which mycorrhizal links were established between *Corallorhiza* plants and *Betula* seedlings. For a key see **a**. Note the extensive cluster of mycorrhizal root tips in the lower right-hand quarter of the image. **e** Digital autoradiograph of the area shown in (d): counts detected in each pixel (0.25 mm^2) are depicted on a linear 12-shade colour scale (0–23 counts per pixel; pixels with >23 counts displayed in the brightest red). Significant counts are seen in the recruit (*small arrow*), original orchid (*large arrow*) and in the ecto-mycorrhizal root tips of *Betula right hand quarter*. **f** Details of area in **d** indicated by the *large arrow*, showing hyphal bridges between the original *Corallorhiza* plant and the *Betula* root which has grown horizontally across it. The *Betula* root has developed ecto-mycorrhizal short-roots linked to the orchid both by individual hyphal bridges (*white arrows*) and by multi-stranded rhizomorphs (*large black arrow*). One of the rhizomorphs (*double black arrow*) appears to connect to the group of rhizoids on the extreme right of the orchid rhizome. **g** The upper portion of a third replicate microcosm: the original orchid (*large arrow*) is partly buried in the soil but emerges in three other places below the *arrow*. Two recruits can be seen (*small arrows*). **h** Digital autoradiograph of the area shown in **g**: radioactivity can be visualised in the two recruits (*small arrows*), and in the original orchid (*large arrow* and *three circled areas* below this *arrow*) but none can be seen in the control orchid (*double arrow*). **i** Detail of the recruit shown in **g** and **h**, which is near the original plant, showing the developing orchid surrounded by young ecto-mycorrhizal roots tips of *Betula*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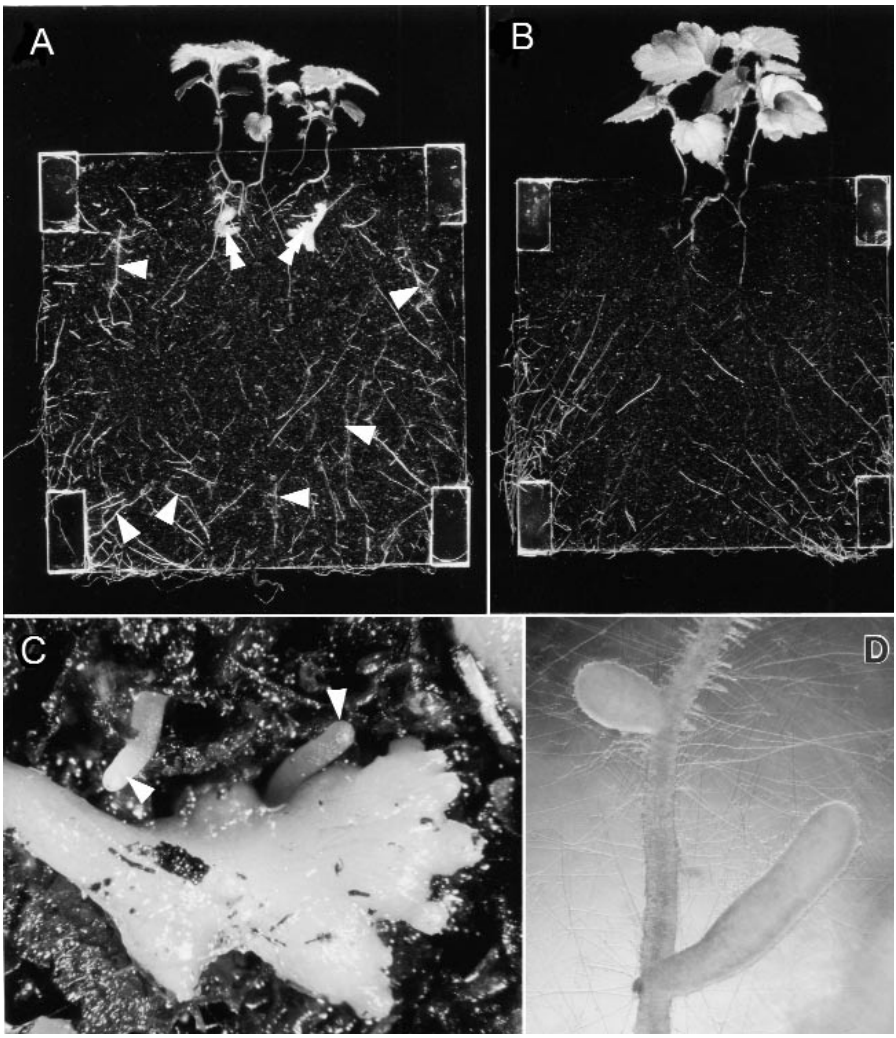

Fig. 15.3. A myco-heterotrophic, achlorophyllous hepatic is linked to an ecto-mycorrhizal tree seedling through a shared fungal partner. **A–D** Microcosms in which the mycorrhizal fungus of the achlorophyllous hepatic, *Cryptothallus mirabilis,* formed ecto-mycorrhizae with *Betula pendula*. **A** Microcosm supporting *Betula* seedlings growing on *Sphagnum* peat with plants of *C. mirabilis double arrows*. Mycorrhizal fungal hyphae (*single arrows*) growing from *C. mirabilis* to colonise the peat. **B** A parallel microcosm with *Betula* grown on the same medium as in **A**, but without *Cryptothallus*. Note absence of fungal mycelia. **C** Close-up of *C. mirabilis* thallus grown with *B. pendula* as in **A** showing the conversion of roots of *Betula* to ecto-mycorrhizae (*single arrows*) in the vicinity of the hepatic. **D** Ecto-mycorrhizal laterals formed on monoxenically grown *Betula* seedlings inoculated with a pure culture of the mycorrhizal fungus of *C. mirabilis*. Sections of such roots (not shown) reveal a Hartig net and mantle which are typical ecto-mycorrhizal structures

and hyphae linking the myco-heterotroph to the autotroph could be readily observed (Fig. 15.2b,f). When shoots of the *Betula* plants linked in this way were fed $^{14}CO_2$ it was revealed, using digital autoradiography (Fig. 15.2e–h) followed by liquid scintillation counting that significant quantities of carbon were transferred from the autotroph to the linked heterotroph. No transfer was detected in control systems lacking hyphal connections. When these experiments were repeated using *Salix repens* rather than *Betula* as the autotrophic associate, similar results were obtained demonstrating that the fungus lacked narrow specificity with regard to its autotrophic partners. The ecology of interplant carbon transport is further discussed in Chapter 2.

A similar relationship has also been demonstrated between the only known myco-heterotrophic bryophyte, the hepatic *Cryptothallus mirabilis*, and the autotroph *Betula*, with which *C. mirabilis* is consistently associated in nature (Read et al. 2000). Rhizotrons of the kind employed by McKendrick et al. (2000a) were again used. It was shown that hyphae emerging from the hepatic formed ecto-mycorrhizal associations with the *Betula* seedlings (Fig. 15.3A,C) while no such symbioses were produced in rhizotrons without the liverwort (Fig. 15.3B). Using fungal isolates obtained from pelotons found within the tissues of *C. mirabilis* thalli it was confirmed, under aseptic conditions that the symbiont of the hepatic was able to produce mycorrhizae on *Betula* seedlings (Fig. 15.3D). Again, after exposing the autotroph to $^{14}CO_2$, significant quantities of carbon transfer to *C. mirabilis* were demonstrated (Read et al. 2000).

15.7 Conclusions and Future Goals

In this review, we have highlighted three novel patterns that have emerged from recent studies, primarily concerning members of the Orchidaceae and Monotropoideae. The first pattern is a consistently narrow specificity of these plants towards selected fungal families, genera and even species. In several of these taxa, this specificity has been shown to hold from the very earliest stages of ontogeny. The second pattern is that many of these specific associations are formed with fungi which simultaneously form ecto-mycorrhizae with neighboring autotrophic plants. This pattern is especially surprising in orchids and *Cryptothallus*, in view of the fact that their mycorrhizal structures involve internal penetration of cells in contrast to ecto-mycorrhizae in which the fungi are extracellular. The specificities shown between myco-heterotrophs of the Orchidaceae and Monotropoideae and their fungal partners are particularly striking, given the accumulating evidence that these plants often grow in the midst of diverse communities of ecto-mycorrhizal fungi. The third pattern is that carbon is often supplied to myco-heterotrophs from autotrophic plants through a shared mycorrhizal mycelial network. These patterns prompt two related questions, namely, *how* and *why* has specificity evolved in these plants?

Studies of the physiological mechanisms of recognition and rejection, on the parts of both the plant and the fungus, are needed in order to begin to understand *how* specificity is controlled at the cellular level. Recognition and rejection may be mediated by specific signal molecules and receptor genes, as with many plant-pathogen interactions. Comparative and phylogenetic studies are needed in order to determine *how* specificity has arisen from an evolutionary point of view. For example, it will be important to determine whether changes in specificity over evolutionary time are consistent and directional, (i.e., progressive narrowing) or chaotic, and to determine the frequency of host-jumps of different magnitudes (i.e., jumps between sister species, between genera, between families, etc.).

An understanding of *why* specificity exists will require rigorous analyses of the selective forces that act upon its expression. Tight specificity appears to be a more consistent feature of full MHPs than partial MHPs, suggesting that a plant's position on the autotrophy–myco-heterotrophy continuum may help to predict its place on the specificity continuum. While far more data are needed to test this pattern, if the pattern holds, it could help direct inquiries into the selective advantages of specificity. Experimental manipulations of MHPs in the field and microcosm should help to reveal the conditions under which different targets (i.e. different fungi) or levels of specificity (i.e. narrower or wider phylogenetic groups) are favoured. To understand selective pressures, it will be imperative to determine whether MHPs interact with their fungi, and their indirect autotrophic hosts, as parasites or as mutualists. The fact that MHPs acquire carbon from their fungi suggests that the plants may be acting as parasites. If so, the fungi would be expected to evolve defenses, thus setting the stage for an arms race which could easily favour specificity. On the other hand, it has long been postulated that MHPs supply some desirable metabolite to their fungi and thus act as mutualists. The stimulation of what we now believe to be *Tricholoma* by *Monotropa* extracts, and the proliferation of *Rhizopogon* mycorrhizae and fir roots around *Sarcodes*, support this view. However, stimulation does not necessarily imply benefit, as many parasites induce damaging proliferation (hypertrophy) of host tissues via hormonal manipulation.

Finally, we suggest that the question of broadest relevance concerning MHPs is whether they interact with fungal associates in a markedly different way than do photosynthetic plants, or whether they have simply modified existing interactions in ways that enable them to extract more carbon, and perhaps cheat the system? If the latter is the case, then MHPs represent only extremes in a continuum, and photosynthetic plants may also, at times, act as cheaters. Such a phenomenon would cast the mycorrhizal symbiosis in a less universally beneficent light, and demand more detailed analyses of costs and benefits, detection and regulation of cheaters, and the maintenance of fair exchanges.

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References

Andersen TF (1990) A study of hyphal morphology in the form genus *Rhizoctonia*. Mycotaxon 37:25–46

Andersen TF (1996) A comparative taxonomic study of *Rhizoctonia* sensu lato employing morphological, ultrastructural and molecular methods. Mycol Res 100:1117–1128

Andersen TF, Rasmussen HN (1996) The mycorrhizal species of *Rhizoctonia*. In: Sneh B, Jabaji-Hare S, Neate S, Dijst G (eds) Rhizoctonia species: taxonomy, molecular biology, ecology, pathology and disease control, Second International Symposium on *Rhizoctonia*, Noordwijkerhout, Netherlands, 27–30 June 1995. Kluwer, Norwell, Massachusetts, pp 379–390

Bayman P, Lebron LL, Tremblay RL, Lodge DJ (1997) Variation in endophytic fungi from roots and leaves of *Lepanthes* (Orchidaceae). New Phytol 135:143–149

Berbee ML (1996) Loculoascomycete origins and evolution of filamentous ascomycete morphology based on 18S rRNA gene sequence data. Mol Biol Evol 13:462–470

Berenbaum MR (1996) Introduction to the symposium: on the evolution of specialization. Am Nat 148:S78–S83

Bernard N (1909) L'évolution dans la symbiose. Les orchidées et leur champignons commensaux. Ann Sci Nat Bot 9:1–196

Bernays EA (1988) Host specificity in phytophagous insects: Selection pressure from generalist predators. Entomol Exp Appl 49:131–140

Berres ME, Szabo LJ, McLaughlin DJ (1995) Phylogenetic relationships in auriculariaceous basidiomycetes based on 25S ribosomal DNA sequences. Mycologia 87:821–840

Beyrle HF, Smith SE (1993) Excessive carbon prevents greening of leaves in mycorrhizal seedlings of the terrestrial orchid *Orchis morio*. Lindleyana 8:97–99

Bidartondo MI, Kretzer AM, Pine EM, Bruns TD (2000) High root concentration and uneven ecto-mycorrhizal diversity near *Sarcodes sanguinea* (Ericaceae): a cheater that stimulates its victims? Am J Bot 87:1783–1788

Bjorkman E (1960) *Monotropa hypopitys* L. – an epiparasite on tree roots. Physiol Plant 13:308–327

Bruns TD, Read DJ (2000) Germination of *Sarcodes sanguinea* and *Pterospora andromedea* seeds is stimulated by the *Rhizopogon* species associated with the roots of adult plants. New Phytol 148:335–342

Bruns TD, Vilgalys R, Barns SM, Gonzalez D, Hibbett DS et al (1992) Evolutionary relationships within the fungi: analyses of nuclear small subunit rRNA sequences. Mol Phylogenet Evol 1:231–241

Bruns TD, Szaro TM, Gardes M, Cullings KW, Pan JJ et al (1998) A sequence database for the identification of ecto-mycorrhizal basidiomycetes by phylogenetic analysis. Mol Ecol 7:257–272

Burgeff H (1932) Saprophytismus und Symbiose. Studien an tropischen Orchideen. Fischer, Jena, 249 pp

Burgeff H (1936) Die Samenkeimung der Orchideen. Fischer, Jena, 312 pp

Burgeff H (1959) Mycorrhiza of orchids. In: Withner CL (ed) The orchids: a scientific survey. Ronald Press, New York, pp 361–395

Campbell EO (1962) The mycorrhiza of *Gastrodia cunninghamii* Hook Trans R Soc N Z 1:289–296
Campbell EO (1963) *Gastodia minor* Petrie, an epiparasite of Manuka. Trans R Soc N Z 2:73–81
Campbell EO (1964) The fungal association in a colony of *Gastrodia sesamoides* R. Br Trans R Soc N Z 2:237–246
Campbell EO (1970a) The fungal association of *Yoania australis*. Trans R Soc N Z Biol Sci 12:5–12
Campbell EO (1970b) Morphology of the fungal association in three species of *Corallorhiza* in Michigan. Mich Bot 9:108–113
Campbell EO (1971) Notes on the fungal association of two *Monotropa* species in Michigan. Mich Bot 10:63–67
Carroll G (1995) Forest endophytes: pattern and process. Can J Bot 73:S1316–S1324
Castellano M, Trappe J (1985) Mycorrhizal associations of five species of Monotropoidae in Oregon. Mycologia 77:499–502
Cha JY, Igarashi T (1996) *Armillaria jezoensis*, a new symbiont of *Galeola septentrionalis* (Orchidaceae) in Hokkaido. Mycoscience 37:21–24
Clay K (1993) The ecology and evolution of endophytes. Agric Ecosyst Environ 44:39–64
Clements MA (1988) Orchid mycorrhizal associations. Lindleyana 3:73–86
Cullings KW, Szaro TM, Bruns TD (1996) Evolution of extreme specialization within a lineage of ecto-mycorrhizal epiparasites. Nature 379:63–66
Currah RS, Sherburne R (1992) Septal ultrastructure of some fungal endophytes from boreal orchid mycorrhizas. Mycol Res 96:583–587
Currah RS, Sigler L, Hambleton S (1987) New records and new taxa of fungi from the mycorrhizae of terrestrial orchids of Alberta. Can J Bot 65:2473–2482
Currah RS, Hambleton S, Smreciu A (1988) The mycorrhizae and mycorrhizal fungi of *Calypso bulbosa* (Orchidaceae). Am J Bot 75:737–750
Currah RS, Smreciu EA, Hambleton S (1990) Mycorrhizae and mycorrhizal fungi of boreal species of *Platanthera* and *Coeloglossum* (Orchidaceae). Can J Bot 68:1171–1181
Currah RS, Zelmer CD, Hambleton S, Richardson KA (1997) Fungi from orchid mycorrhizas. In: Arditti J, Pridgeon AM (eds) Orchid biology: reviews and perspectives, vol VII. Kluwer, Boston, pp 117–170
Curtis JT (1937) Non-specificity of orchid mycorrhizal fungi. Proc Soc Exp Biol Med 36:43–44
Curtis JT (1939) The relation of specificity of orchid mycorrhizal fungi to the problem of symbiosis. Am J Bot 26:390–398
Downie DG (1943) Source of the symbiont of *Goodyera repens*. Trans Bot Soc Edinb 33:383–390
Drehmel D, Moncalvo J-M, Vilgalys R (1999) Molecular phylogeny of *Amanita* based on large-subunit ribosomal DNA sequences: implications for taxonomy and character evolution. Mycologia 91:610–618
Dressler RL (1993) Phylogeny and Classification of the Orchid family. Dioscorides Press, Portland, 314 pp
Dudderidge JA, Read DJ (1982) An ultrastructural analysis of the development of mycorrhizas in *Monotropa hypopitys* L. New Phytol 92:203–214
Farrell BD, Mitter C, Futuyma DJ (1992) Diversification at the insect-plant interface. Bioscience 42:34–42
Feibelman TP, Doudrick RL, Cibula WG, Bennett JW (1997) Phylogenetic relationships within the Cantharellaceae inferred from sequence analysis of the nuclear large subunit rDNA. Mycol Res 101:1423–1430
Furman TE, Trappe JM (1971) Phylogeny and ecology of mycotrophic achlorophyllous angiosperms. Q Rev Biol 46:219–225

Gardes M, Bruns TD (1996) Community structure of ecto-mycorrhizal fungi in a *Pinus muricata* forest: above- and below-ground views. Can J Bot 74:1572–1583

Hadley G (1970) Non-specificity of symbiotic infection in orchid mycorrhiza. New Phytol 69:1015–1023

Hadley G (1982) Orchid Mycorrhiza. In: Arditti J (ed) Orchid biology: reviews and perspectives, vol II. Cornell Univ Press, Ithaca, pp 83–118

Hamada M (1939) Studien über die Mykorrhiza von *Galeola septentrionalis* Reichb. f. neuer Fall der Mykorrhiza-Bildung durch intraradicale Rhizomorpha. Jpn J Bot 10:151–211

Hamada M, Nakamura SI (1963) Wurzelsymbiose von *Galeola altissima* Reichb. F., einer chlorophyllfreien Orchidee, mit dem holzzerstörenden Pilz *Hymenochate crocicreas*. Berk Et Br Sci Rep Tohoku Univ Ser IV (Biol) 29:227–238

Harvais G (1974) Notes on the biology of some native orchids of Thunder Bay, their endophytes and symbionts. Can J Bot 52:451–460

Harvais G, Hadley G (1967) The relation between host and endophyte in orchid mycorrhiza. New Phytol 66:205–215

Hayakawa S, Uetake Y, Ogoshi A (1999) Identification of symbiotic rhizoctonias from naturally occurring protocorms and roots of *Dactylorhiza aristata* (Orchidaceae). J Fac Agric Hokkaido Univ 69:129–141

Hibbett DS, Pine EM, Langer E, Langer G, Donoghue MJ (1997) Evolution of gilled mushrooms and puffballs inferred from ribosomal DNA sequences. Proc Natl Acad Sci USA 94:12002–12006

Hibbett DS, Hansen K, Donoghue MJ (1998) Phylogeny and biogeography of Lentinula inferred from an expanded rDNA dataset. Mycol Res 102:1041–1049

Hopple JS, Vilgalys R (1999) Phylogenetic relationships in the mushroom genus Coprinus and dark-spored allies based on sequence data from the nuclear gene coding for the large ribosomal subunit RNA: divergent domains, outgroups, and monophylly. Mol Phylogenet Evol 13:1–19

Jaenike J (1990) Host specialization in phytophagous insects. Annu Rev Ecol Syst 21:243–273

Johnson J, Vilgalys R (1998) Phylogenetic systematics of *Lepiota* sensu lato based on nuclear large subunit rDNA evidence. Mycologia 90:971–979

Jumpponen A, Trappe JM (1998) Dark septate endophytes: a review of facultative biotrophic root-colonizing fungi. New Phytol 140:295–310

Karen O, Nylund J-E (1997) Effects of ammonium sulphate on the community structure and biomass of ecto-mycorrhizal fungi in a Norway spruce stand in southwestern Sweden. Can J Bot 75:1628–1642

Kasuya MCM, Masaka K, Igarashi T (1995) Mycorrhizae of *Monotropastrum globosum* growing in a *Fagus crenata* forest. Mycoscience 36:461–464

Kretzer AM, Bidartondo MI, Grubisha L, Spatafora JW, Szaro TM, Bruns TD (2000) Regional specialization of *Sarcodes sanguinea* (Ericaceae) on a single fungal symbiont from the *Rhizopogon ellenae* (Rhizopogonaceae) species complex. Am J Bot 87:1778–1782

Kristiansen KA, Taylor DL, Kjoller R, Rasmussen HN, Rosendahl S (2001) Identification of mycorrhizal fungi from *Dactylorhiza majalis* (Orchidaceae) based on PCR, SSCP and sequencing of Mitochondrial ribosomal LsDNA from single pelotons. Mol Ecol (in press)

Kusano S (1911) *Gastrodia elata* and its symbiotic association with *Armillaria mellea*. J Coll Agric Jpn 9:1–73

Leake JR (1994) The biology of myco-heterotrophic ('saprophytic') plants. Tansley review no 69. New Phytol 127:171–216

Lefevre CK, Carter CM, Molina R (1998) Morphological and molecular evidence of specificity between *Allotropa virgata* and *Tricholoma magnivelare*. In: Second Interna-

tional Conference on Mycorrhiza, Poster Presentation, Program and Abstracts. Uppsala, Sweden, p 107
Martin JF (1985) Sur la Mycorhization de *Monotropa hypopithys* par quelques espèces du genre *Trichloma*. Bull Soc Mycol France 101:249–256
Martin JF (1986) Mycorhization de *Monotropa uniflora* L. par des Russulaceae. Bull Soc Mycol Fr102:155–159
Masuhara G, Katsuya K (1994) *In situ* and *in vitro* specificity between *Rhizoctonia* spp. and *Spiranthes sinensis* (Persoon) Ames. var. *amoena* (M. Bieberstein) Hara (Orchidaceae). New Phytol 127:711–718
Masuhara G, Katsuya K, Yamaguchi K (1993) Potential for symbiosis of *Rhizoctonia solani* and binucleate *Rhizoctonia* with seeds of *Spiranthes sinensis* var. *amoena* in vitro. Mycol Res 97:746–752
McKendrick SL, Leake JR, Read DJ (2000a) Symbiotic germination and development of myco-heterotrophic plants in nature: transfer of carbon from ecto-mycorrhizal *Salix repens* and *Betula pendula* to the orchid *Corallorhiza trifida* through shared hyphal connections. New Phytol 145:539–548
McKendrick SL, Leake JR, Taylor DL, Read DJ (2000b) Symbiotic germination and development of myco-heterotrophic plants in nature: ontogeny of *Corallorhiza trifida* Chatel and characterisation of its mycorrhizal fungi. New Phytol 145:523–537
Milligan MJ, Williams PG (1988) The mycorrhizal relationship of multinucleate rhizoctonias from non-orchids with *Microtis* (Orchidaceae). New Phytol 108:205–209
Mitchell AD, Bresinsky A (1999) Phylogenetic relationships of *Agaricus* species based on ITS-2 and 28S ribosomal DNA sequences. Mycologia 91:811–819
Molina R, Massicotte H, Trappe JM (1992) Specificity phenomena in mycorrhizal symbioses: community-ecological consequences and practical implications. In: Allen MF (ed) Mycorrhizal functioning: an integrative plant-fungal process. Chapman and Hall, New York, pp 357–423
Moore RT (1987) The genera of *Rhizoctonia*-like fungi: *Ascorhizoctonia, Ceratorhiza* gen. nov., *Epulorhiza* gen. nov., *Moniliopsis,* and *Rhizoctonia.* Mycotaxon 29:91–99
Moore RT (1996) The dolipore/parenthesome septum in modern taxonomy. In: Sneh B, Jabaji-Hare S, Neate S, Dijst G (eds) Rhizoctonia species: taxonomy, molecular biology, ecology, pathology and disease control. Second international symposium on *Rhizoctonia*, June 1995, The Netherlands. Kluwer, Boston, pp 13–34
Muller WH, Stalpers JA, Van Aelst AC, Van Der Krift TP, Boekhout T (1998) Field emission gun-scanning electron microscopy of septal pore caps of selected species in the *Rhizoctonia* s.1. complex. Mycologia 90:170–179
Nishikawa T, Ui T (1976) Rhizoctonias isolated from wild orchids in Hokkaido. Trans Mycol Soc Jpn 17:77–84
O'Donnell K, Cigelnik E, Weber NS, Trappe JM (1997) Phylogenetic relationships among ascomycetous truffles and the true and false morels inferred from 18S and 28S ribosomal DNA sequence analysis. Mycologia 89:48–65
Ogoshi A (1987) Ecology and pathogenicity of anastomosis and intraspecific groups of *Rhizoctonia solani* Kuhn. Annu Rev Phytopathol 25:125–143
Perkins AJ, McGee PA (1995) Distribution of the orchid mycorrhizal fungus, *Rhizoctonia solani*, in relation to its host, *Pterostylis acuminata*, in the field. Aust J Bot 43:565–575
Perkins AJ, Masuhara G, McGee PA (1995) Specificity of the Associations Between *Microtis parviflora* (Orchidaceae) and its mycorrhizal fungi. Aust J Bot 43:85–91
Peterson RL, Uetake Y, Zelmer C (1998) Fungal symbiosis with orchid protocorms. Symbiosis 25:29–55
Petrini O, Petrini LE, Rodrigues KF (1995) Xylariaceous endophytes: an exercise in biodiversity. Fitopatol Brasil 20:531–539
Price PW (1980) Evolutionary biology of parasites, vol 15. Princeton Univ Press, Princeton

Ramsay RR, Dixon KW, Sivasithamparam K (1986) Patterns of infection and endophytes associated with western Australian orchids. Lindleyana 1:203–214
Ramsay RR, Sivasithamparam K, Dixon KW (1987) Anastomosis groups among rhizoctonia-like endophytic fungi in southwestern Australian *Pterostylis* species (Orchidaceae). Lindleyana 2:161–166
Ramsbottom J (1922) Orchid Mycorrhiza. Trans Br Mycol Soc 12:28–61
Rasmussen HN (1995) Terrestrial orchids: from seed to mycotrophic plant. Cambridge Univ Press, New York
Rasmussen HN, Whigham DF (1993) Seed ecology of dust seeds *in situ*: a new study technique and its application to terrestrial orchids. Am J Bot 80:1374–1378
Rasmussen HN, Wigham DF (1998) The underground phase: A special challenge in studies of terrestrial orchid populations. Bot J Linnean Soc 126:49–64
Read DJ, Duckett JG, Francis R, Ligrone R, Russell A (2000) Symbiotic fungal associations in 'lower' land plants. Philos Trans R Soc Lond B 355:815–831
Robinson D, Fitter A (1999) The magnitude and control of carbon transfer between plants linked by a common mycorrhizal network. J Exp Bot 50:9–13
Salmia A (1988) Endomycorrhizal fungus in chlorophyll-free and green forms of the terrestrial orchid *Epipactis helleborine*. Karstenia 28:3–18
Salmia A (1989) Features of endomycorrhizal infection of chlorophyll-free and green forms of *Epipactis helleborine* (Orchidaceae). Ann Bot Fenn 26:15–26
Schmid E, Oberwinkler F (1993) Mycorrhiza-like interaction between the achlorophyllous gametophyte of *Lycopodium clavatum* L. and its fungal endophyte studied by light and electron microscopy. New Phytol 124:69–81
Schmid E, Oberwinkler F (1994) Light and electron microscopy of the host-fungus interaction in the achlorophyllous gametophyte of *Botrychium lunaria*. Can J Bot 72:182–188
Schmid E, Oberwinkler F (1995) A light- and electron-microscopic study on a vesicular-arbuscular host-fungus interaction in gametophytes and young sporophytes of the Gleicheniaceae (Filicales). New Phytol 129:317–324
Schmid E, Oberwinkler F (1996) Light and electron microscopy of a distinctive VA mycorrhiza in mature sporophytes of *Ophioglossum reticulatum*. Mycol Res 100:843–849
Schmid E, Oberwinkler F, Gomez LD (1995) Light and electron microscopy of a host-fungus interaction in the roots of some epiphytic ferns from Costa Rica. Can J Bot 73:991–996
Sen R, Hietala AM, Zelmer CD (1999) Common anastomosis and internal transcribed spacer RFLP groupings in binucleate *Rhizoctonia* isolates representing root endophytes of *Pinus sylvestris*, *Ceratorhiza* spp. from orchid mycorrhizas and a phytopathogenic anastomosis group. New Phytol 144:331–341
Smith SE (1966) Physiology and ecology of orchid mycorrhizal fungi with reference to seedling nutrition. New Phytol 65:488–499
Smith SE, Read DJ (1997) Mycorrhizal symbiosis, 2nd edn. Academic Press, San Diego
Stone JK, Sherwood MA, Carroll GC (1996) Canopy microfungi: function and diversity. Northwest Sci 70:37–45
Swann EC, Taylor JW (1993) Higher taxa of Basidiomycetes: an 18S rRNA gene perspective. Mycologia 85:923–936
Taylor DL (1997) The evolution of myco-heterotrophy and specificity in some North American orchids. PhD Thesis, Univ California, Berkeley
Taylor DL, Bruns TD (1997) Independent, specialized invasions of ecto-mycorrhizal mutualism by two non-photosynthetic orchids. Proc Natl Acad Sci USA 94:4510–4515
Taylor DL, Bruns TD (1999a) Community structure of ecto-mycorrhizal fungi in a *Pinus muricata* forest: minimal overlap between the mature forest and resistant propagule communities. Mol Ecol 8:1837–1850

Taylor DL, Bruns TD (1999b) Population, habitat and genetic correlates of mycorrhizal specialization in the 'cheating' orchids *Corallorhiza maculata* and *C. mertensiana*. Mol Ecol 8:1719–1732

Terashita T (1982) Fungi inhabiting wild orchids in Japan (II). Isolation of symbionts from *Spiranthes sinensis* var. *amoena.* Trans Mycol Soc Jpn 23:319–328

Terashita T (1996) Biological species of Armillaria symbiotic with *Galeola septentrionalis*. Nippon Kingakukai Kaiho 37:45–49

Thompson JN (1994) The coevolutionary process. Univ Chicago Press, Chicago

Tokunaga Y, Nakagawa T (1974) Mycorrhiza of orchids in Japan. Trans Mycol Soc Jpn 15:121–133

Tu CC, Kimbrough JW, Aldrich HC (1977) Cytology and ultrastructure of *Thanatephorus cucumeris* and related taxa of the *Rhizoctonia* complex. Can J Bot 55:2419–2436

Uetake Y, Ishizaka N (1996) Cytochemical localization of adenylate cyclase activity in the symbiotic protocorms of *Spiranthes sinensis*. Mycol Res 100:105–112

Uetake Y, Peterson RL (1997) Changes in actin filament arrays in protocorm cells of the orchid species, *Spiranthes sinensis*, induced by the symbiotic fungus *Ceratobasidium cornigerum*. Can J Bot 75:1661–1669

Uetake Y, Peterson RL (1998) Association between microtubules and symbiotic fungal hyphae in protocorm cells of the orchid species, *Spiranthes sinensis*. New Phytol 140:715–722

Uetake Y, Kobayashi K, Ogoshi A (1992) Ultrastructural changes during the symbiotic development of *Spiranthes sinensis* (Orchidaceae) protocorms associated with binucleate *Rhizoctonia* anastomosis group C. Mycol Res 96:199–209

Uetake Y, Farquhar ML, Peterson RL (1997) Changes in microtubule arrays in symbiotic orchid protocorms during fungal colonization and senescence. New Phytol 35:701–709

Umata H (1995) Seed germination of *Galeola altissima*, an achlorophyllous orchid, with aphyllophorales fungi. Mycoscience 36:369–372

Umata H (1997a) Formation of endomycorrhizas by an achlorophyllous orchid, *Erythrorchis ochobiensis*, and *Auricularia polytricha*. Mycoscience 38:335–339

Umata H (1997b) In vitro germination of *Erythrorchis ochobiensis* (Orchidaceae) in the presence of *Lyophyllum shimeji*, an ecto-mycorrhizal fungus. Mycoscience 38:355–357

Umata H (1998) A new biological function of shiitake mushroom, *Lentinula edodes*, in a myco-heterotrophic orchid, *Erythrorchis ochobiensis*. Mycoscience 39:85–88

Warcup JH (1971) Specificity of mycorrhizal association in some Australian terrestrial orchids. New Phytol 70:41–46

Warcup JH (1981) The mycorrhizal relationships of Australian orchids. New Phytol 87:371–381

Warcup JH (1985) *Rhizanthella gardneri* (Orchidaceae), its rhizoctonia endophyte and close association with *Melaleuca uncinata* (Myrtaceae) in Western Australia. New Phytol 99:273–280

Warcup JH (1988) Mycorrhizal associations of isolates of *Sebacina vermifera.* New Phytol 110:227–231

Warcup JH (1991) The *Rhizoctonia* endophytes of *Rhizanthella* (Orchidaceae). Mycol Res 95:656–659

Warcup JH, Talbot PHB (1966) Perfect states of some rhizoctonias. Trans Br Mycol Soc 49:427–435

Warcup JH, Talbot PHB (1967) Perfect states of rhizoctonias associated with orchids. New Phytol 66:631–641

Warcup JH, Talbot PHB (1971) Perfect states of rhizoctonias associated with orchids. II. New Phytol 70:35–40

Warcup JH, Talbot PHB (1980) Perfect states of rhizoctonias associated with orchids. III. New Phytol 86:267–272
Wells K (1994) Jelly fungi, then and now. Mycologia 86:18–48
Williams PG (1985) Orchidaceous rhizoctonias in pot cultures of vesicular-arbuscular mycorrhizal fungi. Can J Bot 63:1329–1333
Williams PG, Thilo E (1989) Ultrastructural evidence for the identity of some multinucleate rhizoctonias. New Phytol 112:513–518
Worrall JJ, Anagnost SE, Zabel RA (1997) Comparison of wood decay among diverse lignicolous fungi. Mycologia 89:199–219
Zelmer CD, Currah RS (1995) Evidence for a fungal liaison between *Corallorhiza trifida* (Orchidaceae) and *Pinus contorta* (Pinaceae). Can J Bot 73:862–866
Zelmer CD, Cuthbertson L, Currah RS (1996) Fungi associated with terrestrial orchid mycorrhizas, seeds and protocorms. Mycoscience 37:439–448