

The evolutionary implications of exploitation in mycorrhizas¹

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Abstract: Some views of mutualism, where the fitness of two symbiotic partners is higher in association than when apart, assume that they necessarily evolve towards greater benefit for the partners. Most mutualisms, however, seem prone to conflicts of interest that destabilize the partnership. These conflicts arise in part because mutualistic outcomes are conditional, depending upon complex interactions between environmental, developmental, and genotypic factors. Mutualisms are also subject to exploitation or cheating. Although various compensating mechanisms have been proposed to explain how mutualism can be maintained in the presence of exploiters, none of these mechanisms can eliminate exploitation. In this paper we explore various compensating mechanisms in mycorrhizas, examine the evidence for exploitation in mycorrhizas, and conclude that mycorrhizal mutualisms exhibit characteristics that are more consistent with a concept of reciprocal parasitism. We propose that researchers should not assume mycorrhizas are mutualistic based upon structural characteristics or limited functional studies showing bilateral exchange and should view mycorrhizas as occupying a wider range on the symbiotic continuum, including commensalism and antagonism. We recommend that comparative studies of mycorrhizas incorporate other types of root associations that have traditionally been considered antagonistic.

Key words: mycorrhizas, mutualism, exploiters, compensating mechanisms, symbiotic continuum.

Résumé : Certaines perceptions du mutualisme, où l'adaptation des deux partenaires symbiotiques est plus grande dans l'association que dans l'isolement, assument qu'ils évoluent nécessairement en faveur du plus grand bénéfice des partenaires. La plupart des mutualismes semblent cependant enclins à des conflits d'intérêts qui déstabilisent les partenaires. Ces conflits surviennent en partie parce que les issues du mutualisme sont conditionnelles et dépendent d'interactions complexes dans l'environnement ainsi que de facteurs développementaux et génotypiques. Les mutualismes sont aussi sujets à l'exploitation et à la tricherie. Bien qu'on ait proposé différents mécanismes de compensation pour expliquer comment le mutualisme peut être maintenu en présence d'exploiteurs, aucun de ces mécanismes peut éliminer l'exploitation. Les auteurs explorent différents mécanismes de compensation chez les mycorrhizes, en examinant les preuves d'exploitation chez les mycorrhizes, et concluent que les mutualismes mycorrhiziens montrent des caractéristiques qui correspondent mieux au concept de parasitisme réciproque. Les auteurs proposent que les chercheurs ne devraient pas assumer que les mycorrhizes sont mutualistes, sur la base de caractéristiques structurales ou d'études limitées sur le fonctionnement montrant des échanges bilatéraux, et devraient considérer les mycorrhizes comme occupant une part plus importante du continuum symbiotique, incluant le commensalisme et l'antagonisme. Ils recommandent que les études comparatives sur les mycorrhizes incorporent d'autres types d'associations mycorrhiziennes traditionnellement considérées comme antagonistes.

Mots clés : mycorrhizes, mutualisme, exploiters, mécanismes de compensation, continuum symbiotique.

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Introduction

In this paper, we use the term mutualism in the sense of Boucher et al. (1982), "an interaction between species that is

beneficial to both", with benefit measured as an increase in relative fitness. We use symbiosis in the original sense of de Bary (1887), as applying to any association of dissimilar organisms living closely together, whether antagonistic or mutualistic.

Based upon simple models whereby fitness of both partners is perpetually enhanced in association, early theory suggested that mutualisms should evolve to "benefit the association" (Law and Lewis 1983; Law 1985), resulting in tighter, more obligate mutualisms that represent stable evolutionary end points (Price 1991). Although some mutualisms may progress in this manner, mycorrhizal mutualisms are better characterized as "reciprocal exploitations" (Herre et al. 1999) or reciprocal parasitism, where conflicts of interest arise that destabilize the partnership. Reasons for this instability include the conditionality of outcomes of the

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interaction, which may range from positive to negative depending upon the context (Bronstein 1994), and the presence of exploiters that degrade or invade mutualisms (Bronstein 2001).

Conditional outcomes

Outcomes of symbiotic interactions are highly dependent upon their context and thus vary considerably in space and time. Bronstein (1994) reviewed “conditional outcomes” in mutualistic associations and discussed their implications for maintenance of mutualisms. She proposed that interactions be viewed as a continuum (Fig. 1) upon which outcomes may vary depending upon biotic and abiotic factors. Many examples have been cited of outcomes of mycorrhizal colonization that vary depending upon developmental, environmental, and genotypic factors (Francis and Read 1995; Johnson et al. 1997; Tuomi et al. 2001; Jones and Smith 2004), and models have been developed that predict different conditional outcomes depending upon density-dependent factors (Gange and Ayres 1999). Francis and Read (1995) noted that arbuscular mycorrhiza (AM) fungi were most antagonistic to plants normally found in disturbed, ruderal environments and speculated that these plants may be restricted to such environments because they are more susceptible to parasitic interactions with AM fungi. Kytöviita et al. (2003) also noted conditional outcomes depending upon whether seedlings were integrated into common mycelial networks with mature plants; seedlings that were grown without mature plants obtained a growth benefit from mycorrhizal colonization, while those that were grown with mature plants did not. The sum of such conditional outcomes over the lifetime of the partners will determine individual fitness and evolutionary trajectories. The implication of conditionality is that mycorrhizal symbioses cannot be assumed to be mutualistic; under some conditions they are antagonistic, including parasitism by the fungus on the plant or parasitism by the plant on the fungus.

Exploitation

An exploiter of a mutualism is “an individual that obtains a benefit offered to mutualists, but that does not reciprocate” (Bronstein 2001). Bronstein (2001) argues that “exploiter” is a more neutral term than others that have been applied, such as “cheaters”, “parasites of mutualisms”, and “defectors”. For example, the term “cheater” implies that symbionts that act to maximize their fitness at the expense of their partner are somehow not following the rules of the game, when in fact they are following the same evolutionary rules as other organisms, even if the outcome is no longer mutualism. In theory, exploiters obtain a fitness advantage over cooperators if they redeploy resources that would have been expended on their partner to enhance their own fitness. Recognizing that this definition subsumes several kinds of exploitation strategy, Bronstein (2001) classified exploiters into three categories: “exploiter species”, “pure exploiters”, and “conditional exploiters”.

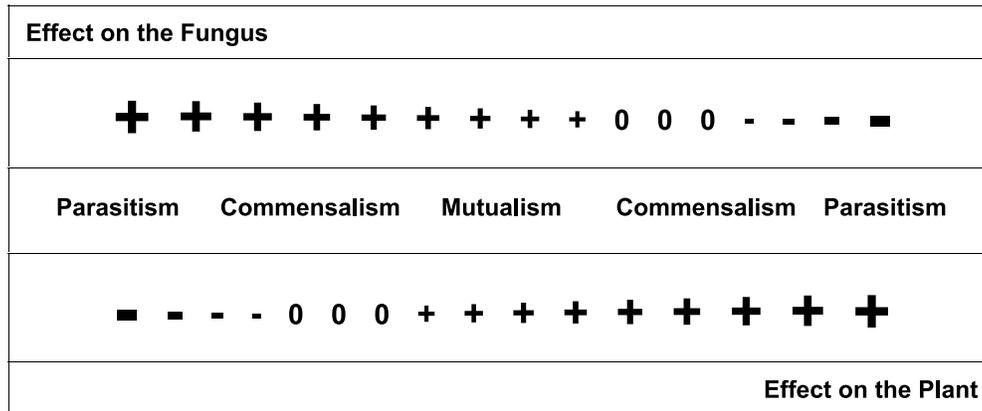
In “exploiter species” (“aprovechados” sensu Soberon Mainero and Martinez del Rio 1985) exploitation is a pure species-level behaviour (the trait is fixed within all members

of the population). Examples given by Bronstein (2001) include yucca wasps that do not transfer pollen (Pellmyr et al. 1996) and orchid floral mimics that do not produce nectar (Dafni 1984). In the yucca pollinator symbiosis, exploiter lineages have been shown to be related to mutualistic lineages, but not to sister groups (Pellmyr et al. 1996), which would be the expected outcome if fungi that form mutualistic associations with one host are preadapted to invade mutualisms of another host as exploiter species. Mycoheterotrophic plants, which obtain carbon from autotrophic plants via their shared mycorrhiza fungal partners (Leake 1994), have been proposed to be plant exploiter species (Bidartondo et al. 2002, 2003). To our knowledge, no case of fungal exploiter species has been conclusively demonstrated. Possible fungal exploiter species include dark septate endophyte fungi (Jumpponen and Trappe 1998), which are usually characterized as parasitic (but see Jumpponen 2001) but form typical ectomycorrhizas (ECM) on some conifers. Little is known about the mechanisms by which they evade host defenses or whether they mimic signals produced by mycorrhizal fungi, but if further research indicates similarities, then this would provide evidence for exploitation.

The term “pure exploiters” applies to individuals within species (Bronstein 2001) and is a population-level phenomenon. They have also been called “free riders” (Denison et al. 2003) and “defectors” (see Wilkinson and Sherratt 2001). Examples include automimicry, in which some individuals in a population mimic those that provide a benefit (Bronstein 2001), and *Rhizobium* mutants that form nodules but do not fix nitrogen (Denison et al. 2003). Pure exploiters may be nearly genetically identical to their parental strain, as in the case of a transposon-induced mutant of *Bradyrhizobium japonicum*, which accumulates carbon at a higher rate than cooperative nitrogen-fixing strains, suggesting potential for a fitness advantage (Hahn and Studer 1986). Perhaps the best example of variability among individual mycorrhizal fungal isolates in traits that could potentially be subject to exploitation is *Laccaria bicolor*. Isolates of *L. bicolor* express a range of symbiotic potentials, including differences in the extent of colonization of roots, fungal biomass, and acid phosphatase activity (Kropp and Fortin 1988; Wong et al. 1989; Kropp 1990), and the effects of different isolates on plant biomass accumulation ranged from positive to negative. Kropp (1997) showed considerable range in ability of isolates to colonize short roots and determined that there was a heritable genetic component. Since control of colonization was shown to be polygenic, this would facilitate selection of intermediate genotypes by stabilizing selection (Kropp 1997). This example suggests that it would not be difficult to generate exploitative genotypes, but we do not know if such genotypes would be competitive in the environment or if stabilizing selection would eliminate them from the population. Little research has been done on pure exploiters of mycorrhizal systems, in part because of the difficulty in determining the genetic basis of the interaction and in measuring lifetime fitness for individuals within populations.

Recognizing the importance of conditional outcomes in determining the lifetime fitness of individuals, Bronstein (2001) created a class of “conditional exploiters” for cases

Fig. 1. The symbiotic continuum showing the relationship between mutualism, commensalism, and parasitism based upon fitness effects on plant and fungal partners. (From Bronstein 1994, reproduced with permission of Trends Ecol. Evol., Vol. 9, p. 215, © 1994 Elsevier Trends Journals.)



where individuals act mutualistically on occasion. She predicted that conditional exploiters would be common where slight differences in the ecological context are sufficient to shift interactions from mutualistic to antagonistic, which is likely to apply in a wide range of mycorrhizal symbioses.

The concepts above were formulated to apply to species and individuals, but can also be applied to other genetically coherent units, including populations or even nuclei. Thus, these concepts can be applied to organisms such as AM fungi, which have genetically heterogeneous, multinucleate thalli (Sanders et al. 2003). However, exploitation operating at different hierarchical levels does make it difficult to conclusively delineate members of the “conditional exploiter” group.

Exploiters have been found in all mutualisms that have been studied in detail (Bronstein 2001), regardless of the type or tightness of the association. If mutualisms are best characterized as reciprocal parasitism (i.e., both partners will always be trying to minimize their contribution to the association and maximize their own benefit), then conflicts of interest between partners are unavoidable, since benefit to one partner more or less translates into a cost for the other (Bronstein 2001). Given that exploitation has considerable potential to erode mutualistic associations, much attention has turned to identifying mechanisms that compensate for the theoretical fitness advantage obtained by exploiters.

Compensating mechanisms

It has been suggested that mutualisms could be stabilized by “compensating mechanisms”, which act to nullify the fitness benefits that would otherwise be realized by exploiters. Examples of compensating mechanisms that have been proposed include retaliation, parcelling, trait-group selection, genetic uniformity, vertical transmission, neighbourhood interactions, filtering, hostage trading, and by-products (Herre et al. 1999; Yu 2001). Here, we suggest an additional mechanism, which we call “promiscuity”, to describe the potentially stabilizing effect of multiple hosts. Yu (2001) suggests that since retaliation and parcelling require partners to “remember” past behaviours, they are unlikely to apply, and he states that trait group selection has yet to be conclusively demonstrated. In the following sections we discuss the re-

maining mechanisms and consider whether there is evidence for the occurrence of these compensating mechanisms in mycorrhizal fungi.

Genetic uniformity

Law and Lewis (1983) argued that it is more advantageous for progeny of mutualists to be like their parent(s) (e.g., asexual) than to be genetically variable (e.g., sexual). Therefore, low rates of genetic exchange should stabilize mutualism. Two arguments why genetic uniformity is important have been developed: it may require fewer resources to maintain homogeneous symbionts within an individual host, and genetic uniformity counters the tendency for exploiter genotypes to arise (Wilkinson and Sherratt 2001).

Law (1985) examined mycorrhizal associations and concluded that arbuscular mycorrhizas and ericoid mycorrhizas (ERM) largely fit the prediction of low rates of genetic exchange, but that ECM did not, which he attributed to their lack of conformity to an inhabitant or exhabitant structure (see Vertical transmission). However, conclusions that ERM and AM fungi have reduced genetic exchange, and thus higher genetic uniformity, are countered by observations that at least some ERM have sexual stages. Recent modelling by Flatt et al. (2001) suggests that even low levels of sexual reproduction can stabilize population dynamics to a degree that is comparable to exclusively sexual populations. Even AM fungi, which are considered to be asexual, exhibit some evidence of recombination (Gandolfi et al. 2003) and much higher than expected levels of genetic diversity (Sanders et al. 2003). Dark septate endophytes of roots (Jumpponen and Trappe 1998), which comprise a diverse group of mostly asexual species affiliated with the Helotiales (Ascomycota), also appear to be quite genetically variable (Grunig et al. 2001, 2002). In summary, it is difficult to make a case that any mycorrhizal mutualisms show the impaired levels of genetic exchange or high levels of genetic uniformity that according to Law and Lewis (1983) would promote mutualism.

Vertical transmission

Vertical transmission of symbionts from parents to offspring links the fitness of symbiotic partners (i.e., the reproductive success of one partner depends upon the other)

(Herre et al. 1999) and may even drive antagonistic associations toward mutualism (Law and Dieckmann 1998). Vertical transmission is generally invoked for organisms that spread vegetatively, such as endosymbionts (Law and Lewis 1983), because horizontal transmission of progeny would nullify the effects of linkage by allowing new genotype–genotype combinations to form. Whether vertical transmission necessarily promotes mutualism is controversial (Douglas and Smith 1989; Faeth and Fagan 2002, 2003). Even *Neotyphodium* grass endophytes, which have been widely presented as a model of vertically transmitted mutualism, have been proposed to act more commonly as parasites, only providing mutualistic benefit under extreme conditions (Faeth and Fagan 2003). If so, then these would be examples of conditional exploiters sensu Bronstein (2001).

Dark septate endophyte fungi are the most likely candidates for vertical transmission in roots. They may be transmitted vertically when plants propagate vegetatively, but there is no evidence that they are transmitted in seed, although recent suggestions that they are also endophytes in leaves (Aaltonen and Barrow 2003) makes this possibility more plausible. Like grass endophytes, many studies report the interaction to be parasitic (Jumpponen 2001), but they also can provide a growth response under extreme conditions, such as at highly acidic pH (Wilcox and Wang 1987). The fungi that form AM are apparently asexual (but see Genetic uniformity) and could be transmitted by vegetative plant growth. Again, there is little evidence that they are transmitted in seed, and most new generations of plants appear to be colonized via AM spores or mycelium, which would lead to horizontal transmission. The other classes of mycorrhizas are dominated by sexually reproducing basidiomycetes and ascomycetes and are horizontally transmitted.

It isn't obvious that vertical transmission applies sufficiently in any root symbiosis to counteract exploitation. However, Wilkinson and Sherratt (2001) argue that vertical transmission may not be necessary and that horizontal transmission can lead to similar outcomes in spatially structured populations where there are strong neighbourhood interactions.

Neighbourhood interactions

The impact of exploitation can be reduced if exploiters and cooperators interact mostly with each other (Yu 2001). This is a density-dependent effect. As exploiters increase in density their fitness decreases, because exploitation generates local selection for hosts that retaliate. If neighbourhood interactions are strong, then exploitative genotypes will be more likely to encounter progeny of retaliatory hosts, and the spread of exploiters will be self-limiting (Wilkinson and Sherratt 2001). Neighbourhood interactions require strong spatial structuring, a condition that seems to apply in many natural mycorrhizal systems.

Neighbourhood interactions have been argued to apply in mycorrhizal hosts that have large seeds that limit dispersal, because progeny establish close to their parents and are thus more likely to be colonized by their parents' mycorrhizal assemblage (Wilkinson 1997; Brundrett 2002). If ECM basidiospore nuclei largely migrate into local established mycelia, as suggested by Kytöviita (2000), this might also

promote neighbourhood interactions by reducing migration of genotypes and facilitating local selection. However, Stenlid (2000) has pointed out that no one has demonstrated substantial spread of a third nuclear type into an established dikaryotic mycelium, although new dikaryons may grow out of existing mycelia. Neighbourhood interactions would also be accentuated if fungi can monopolize patches. There is evidence for territorial clones in some mycorrhizal species (Dahlberg and Stenlid 1994, 1995; Dahlberg 1997) and for extensive production of mycorrhizal mats (Griffiths et al. 1996) with unique chemical and biotic characteristics (Griffiths et al. 1991, 1994; Aguilera et al. 1993) that could impede invasion by other fungi. Such territoriality could facilitate neighbourhood interactions by forcing establishment of seedlings into local fungal patches, particularly if there is strong competition with fungi that invade mats. Whether these dynamics are sufficient to significantly stabilize mycorrhizal mutualisms is an open question that bears further investigation.

Filtering

Filtering is a partner-choice mechanism that differentially sanctions exploiters or rewards cooperators (Yu 2001). If partners can recognize exploiters and block their access to nutrients, or recognize more cooperative genotypes and increase their nutrient access, then the fitness advantage of exploiters can be nullified and mutualism promoted. Filtering can be an effective mechanism to maintain mutualism, although to be effective it must occur before significant exchange takes place between the symbionts (Yu 2001).

Filtering mechanisms can have a physiological basis, such as where plants control root-nodule senescence depending upon nitrogen-fixing potential of the nodule, which would allow them to filter exploitative nonfixing rhizobia (West et al. 2002; Denison et al. 2003). A similar model could be applied in mycorrhizal systems if the plant controls senescence or longevity of different portions of the root system independently. There is some evidence that plants can allocate more carbon to mycorrhizal versus nonmycorrhizal roots (Cairney et al. 1989). There is also variation in longevity of short roots; short roots in conifers have a defined life-span before degeneration takes place, and this period varies widely from 30 to 85 d, with a predicted maximum age of 170–240 d for spruce (Downes et al. 1992). It is possible that plants can sanction exploitative fungi by controlling root mortality, as discussed by Hoeksema and Kummel (2003). If so, this would provide a mechanism by which plants could filter exploiters and thus enforce mutualism.

Alternately, filtering could be analogous to resistance to plant-pathogenic fungi (Heath 1991) or the *nod* recognition genes in azotrophic bacterium–legume symbioses (Denarie et al. 1992; Debelle et al. 2001). Heath (1991) argues that basic resistance is the norm in plants and that defense mechanisms may have evolved more rapidly in aerial plant parts because of the need to maintain mycorrhizal mutualisms. Mycorrhizal fungi do elicit basic defense responses in plants, but resistance is incomplete, and further mycorrhiza development is unimpeded (Blee and Anderson 2000). In plant pathogenic symbioses, specific resistance develops in response to elicitors that allow plants to recognize virulent pathogens (Heath 1996). To date, there is little direct evi-

dence that mycorrhizal fungi elicit specific resistance, so it is unclear whether the specificity observed in mycorrhizal associations could be a manifestation of specific resistance. Whatever the mechanism, filtering could stabilize mutualisms characterized by horizontal transmission (Wilkinson and Sherratt 2001).

Hostage trading

Hostage trading is based upon the observation that exploiters can take advantage of time delays between a cooperative act and its reciprocation (Yu 2001). Therefore, cooperation can be enforced by exchanging benefits simultaneously. In mycorrhizas, this would be facilitated by tightly coupling nutrient exchange between the partners at the membrane interface, as suggested by Woolhouse (1975) and Brundrett (2002). There is no good evidence that plants and fungi can impose hostage trading by controlling nutrient exchange at the membrane interface, but the potential for this mechanism should be explored further as the interface and exchange mechanisms are understood in greater detail (see Peterson and Massicotte 2004; Jones and Smith 2004).

By-products

Benefits associated with by-products allow individuals to maximize their own fitness without negatively impacting their partners. This is achieved if exploitation produces a by-product benefit that enhances the other partner's fitness. This contrasts with the other types of exploitation discussed, where individuals maximize their own fitness at the expense of their partner. Yu (2001) gives the example of ants that parasitize plants by tending aphids on them. If a by-product of their activity is that they deter other herbivores that would cause even greater damage, then the plant has received a net benefit. Connor (1995) extended this concept to include investment by one partner into another to trigger a by-product benefit, which he called "investing in pseudo-reciprocity".

Several examples of by-products might operate in mycorrhizal systems. Many studies have suggested that mycorrhizal colonization suppresses root pathogens (Fitter and Garbaye 1994; Newsham et al. 1995a; Azcon-Aguilar and Barea 1996; Schelkle and Peterson 1996). If mycorrhizal genotypes that extract more carbon resources are more effective at suppressing root pathogens that would cause even greater harm to the host, then the plant would receive a net benefit from supporting more "exploitative" mycorrhizas. Another example is stimulation of nitrogen fixation in the rhizosphere. Many free-living or mantle-associated bacteria that are capable of nitrogen fixation occur in the rhizosphere (Fitter and Garbaye 1994; Perotto and Bonfante 1997; Rozycki et al. 1999). Enhanced growth of mycorrhizal fungi could stimulate nitrogen fixation by providing nutrients and a scaffold for bacterial growth and by scavenging of oxygen via fungal respiration that would otherwise inhibit nitrogenase function. If fungi that act exploitatively by extracting more carbon provide increased levels of nitrogen fixation as a by-product, then the plant could receive a net benefit under nitrogen-limiting conditions. Other possible examples include the stimulation of decomposition processes in the rhizosphere that feed back to plant growth (Pankow et al. 1991; Read and Perez-Moreno 2003; Sen 2003). Mycorrhizas might also provide by-product benefits by detoxifying

heavy metals (Wilkinson and Dickinson 1995; Hartley et al. 1997; Meharg and Cairney 2000; Vralstad et al. 2002a).

Although this area has received little attention from researchers, compelling evidence for the importance of by-products in mycorrhizal mutualisms is given by Newsham et al. (1995a, 1995b), who summarized several elegant experiments that suggest that AM fungi which densely colonize the root cortex provide the by-product benefit of suppression of root pathogenic fungi. Variation in the extent of ectomycorrhizal colonization of roots by *Laccaria bicolor* isolates has also been observed (Kropp and Fortin 1988; Wong et al. 1989). It would be interesting to know if this variation is also correlated with protection from root pathogens.

An important aspect of by-product trading is that it is only susceptible to exploitation once the cost to the plant exceeds the net benefit of the by-product. Thus, by-products could be important factors in stabilizing mutualisms and may provide mechanisms by which even seemingly parasitic associations could be mutualistic. Mycorrhizal biologists need to look beyond patterns of nutrient exchange when assessing the relative benefits to mycorrhizal plants and fungi.

Promiscuity

Promiscuity, the ability to associate with multiple partners, provides another mechanism to stabilize mutualisms. Mycorrhizal symbionts typically form associations with multiple partners, often simultaneously, which has led to considerable speculation about the importance of mycorrhizal networks (Wilkinson 1998). It has been demonstrated that mycorrhizal fungi that interact with multiple hosts vary in their ability to provide benefits on different plants (see Bever et al. 2001) to the extent that they may be forming mutualistic associations with some and antagonistic associations with others. Maynard Smith (1998) argued that multiple symbionts should facilitate parasitism, because a symbiont that is less pathogenic gains little fitness over the long term if the host is killed by a more virulent symbiont. However, this is not the case if selection on different hosts is asymmetrical, such that exploitation of one host increases the mutualistic benefits to another host. The work by Newsham et al. (1995a, 1995b) suggests that AM fungi are selected for phosphorus uptake in hosts with poorly developed root systems and for suppression of parasites in those with well-developed roots. Since suppression of pathogens is a function of the degree of root colonization (Newsham et al. 1995a), this may be a case where a by-product benefit acts asymmetrically on different hosts; genotypes that colonize roots more heavily may act as exploiters on hosts with poorly developed root systems but as mutualists on alternate hosts with more well-developed root systems. This would be a powerful mechanism to stabilize mycorrhizal mutualisms in the face of exploitation.

Evidence for exploitation

Compensating mechanisms that limit the exploitation of one partner by another have not been conclusively demonstrated in mycorrhizal symbioses. However, many categories of compensating mechanisms, such as hostage trading and by-product effects, have yet to be specifically investigated in mycorrhizas. Compensating mechanisms may be important

in stabilizing mutualisms, although none of them can prevent invasion by exploiters. According to Law (1985), mutualism creates a selective environment characterized by reduced genetic exchange, lower speciation rates, and decreased specificity. In contrast, if mycorrhizas have evolved in dynamic systems that are prone to exploitation, then mutualisms are more likely to be unstable, readily transforming into antagonistic interactions. In the following sections, we consider the evidence for, and predictions of, exploitative behavior in mycorrhizal symbioses.

Instability

If exploitation is a significant factor in mycorrhizal associations, we should see evidence of instability as a consequence of conflicts of interest between the partners. Hibbett et al. (2000) performed a phylogenetic analysis with approximately 160 homobasidiomycetes, including putatively mycorrhizal and saprotrophic forms, and estimated the pattern of transformations between these forms using parsimony and maximum-likelihood methods. The results suggested that there have been multiple gains as well as losses of mycorrhizal symbioses. The results of Hibbett et al. (2000) should be viewed with caution, because the analysis is quite sensitive to tree topology (they performed their analysis with only one tree) and to character coding (some taxa coded as mycorrhizal could be saprotrophic). Nevertheless, their results suggest that mycorrhizal associations can be disrupted under some conditions. Their results are consistent with those of Lutzoni et al. (2001), whose studies of ascomycete phylogeny suggest that the lichen mutualism is characterized by multiple gains or losses and is also reversible.

In addition to reconstructing the pattern of transitions between mycorrhizal and free-living forms, Hibbett et al. (2000) estimated a model of evolution of nutritional modes in homobasidiomycetes using maximum likelihood (Pagel 1999). This model had two parameters that specify rates of change between two states. Using likelihood-ratio tests, Hibbett et al. (2000) were able to reject a model in which transitions from saprotrophic to mycorrhizal forms were irreversible. They were not able to reject a model in which transitions in both directions have the same rate. The analysis by Hibbett et al. (2000) suggested that escapes from mycorrhizal associations have led to saprotrophic lifestyles but not parasitic lifestyles. They proposed that reversal to saprotrophy was possible because fungi retained degradative enzymes that permitted them to resume a saprotrophic nutritional mode. Thus, reversal may depend upon the evolutionary origin of the mutualism, since it may be easier to revert to a preadapted state. This is consistent with the hypothesis of Malloch (1987) that the origin of mycorrhizal symbioses is via saprotrophism. Alternately, escape to a parasitic nutritional mode in ECM may be either infrequent or characterized by higher rates of extinction.

Collectively, these results suggest that mycorrhizal symbioses are very dynamic over macroevolutionary time scales and are not irreversible, which is consistent with expectations under a scenario of reciprocal parasitism.

Speciation rates

Antagonistic symbioses are generally thought to result in accelerated evolution (Bergstrom and Lachmann 2003)

based upon the “Red Queen” hypothesis, which predicts that an “arms race” between symbionts will select for increased genetic variability to overcome the partner’s defenses. Law (1985) predicted that this would result in higher speciation rates in antagonistic compared with mutualistic symbioses. Bergstrom and Lachmann (2003) present a supporting model that suggests that slowly evolving species can benefit more in mutualistic symbioses, which they dub the “Red King” effect. However, this runs counter to arguments that mutualisms are susceptible to exploitation and need to evolve rapidly to avoid being exploited by their partners (Herre et al. 1999). Whether evolutionary rates are higher in antagonistic compared with mutualistic associations is unclear. It is also unclear whether higher evolutionary rates necessarily lead to higher speciation rates.

Theory suggests that sympatric speciation can occur in both antagonistic and mutualistic symbioses. Doebeli and Dieckmann (2000) developed a model for evolutionary branching for mutualistic and antagonistic symbioses, although they did not explore the issue of relative branching rate differences between mutualistic and antagonistic symbioses. Their model suggests that branching of mutualistic lineages can occur and that it will result in a “mutualistic” branch and an “exploitative” branch. They based their model upon asexual organisms, but showed that speciation can occur in sexual organisms under conditions of assortative mating, such as would be expected to occur in spatially structured populations (Brauchli et al. 1999; Johst et al. 1999) or along environmental gradients (Doebeli and Dieckmann 2003). Recently, Yu et al. (2001) described an empirical model in the ant–plant symbiosis, where species coexistence seems to have been stabilized by a spatially structured environment. Other mechanisms for sympatric speciation that have been proposed include host switching (Pellmyr et al. 1996) and reversal of mutualism (Pellmyr and Leebens-Mack 2000), both of which could operate in mycorrhizal systems.

It is generally accepted that while speciation rates are low in AM fungi, they are not low in genetic variation (see Sanders 2002), although this perception could be a consequence of our poor understanding of the diversity of AM fungi and the difficulty in applying species concepts. It is unclear whether low morphospecies diversity is a consequence of selection in mutualistic systems for low rates of genetic exchange or whether it is the result of AMF genetic constraints (e.g., limited recombination) or coevolutionary constraints (high diversity of their partners). Speciation in ERM fungi, such as *Hymenoscyphus ericae*, also appears to be low, although there is considerable evidence for population subdivision and specialization within *H. ericae* strains (Vralstad et al. 2002b), which could indicate nascent speciation in a recently evolved mutualism. Within ECM fungi there appears to be a considerable range in speciation rates, with some groups showing evidence of recent rapid radiations (i.e., high morphospecies diversity coupled with low molecular diversity), including suilloid fungi (Bruns et al. 1989), Russulaceae, and Cortinariaceae. If we are to use speciation rates as a surrogate for evolutionary rates, then it would be useful to compare related ECM groups with different speciation rates to see whether this is correlated with relative differences in exploitation. An interesting question is

whether groups with high speciation rates exhibit a wider range of interactions on the symbiotic continuum and a higher proportion of exploitative species. However, this would have to be combined with assessments of evolutionary rate by comparisons of nucleotide substitution rates in homologous genes, as suggested by Herre et al. (1999).

In summary, the relationship between mutualism, antagonism, and speciation rates is difficult to predict. The Red Queen hypothesis suggests that rates of evolution should be high in antagonistic symbioses, and the Red King suggests that mutualists may have slower rates. However, some argue that the Red Queen should also apply to mutualists. Even if one accepts that antagonism should lead to increased rates of evolution, it is not clear that this would result in increased rates of speciation. Using speciation rates as a surrogate for evolutionary rates is dangerous, as speciation rates depend upon the life cycle of the fungi, the underlying population structure, and the mode of genetic exchange, as well as on constraints imposed by coevolution with symbiotic partners. These are not necessarily comparable between different mycorrhiza types. It would be valuable to determine if there are shifts in speciation rates in clades of symbiotic organisms and whether they are correlated with shifts between mutualism and antagonism. Such analyses would face many challenges. A number of tests have been devised for localizing shifts in diversification rates on phylogenetic trees (Sanderson and Donoghue 1996), but these are not without controversy. Even if one accepts the validity of these kinds of tests, they are sensitive to a number of sources of error, including error in phylogenetic reconstruction, incorrect coding of symbioses as mutualism or antagonism, and inaccurate reconstruction of ancestral states. An additional source of error is our limited understanding of the actual diversity of fungal species, especially cryptic taxa.

Specificity

Specificity in mycorrhizal associations could be a manifestation of filtering strategies, although specificity could also be due to other factors that constrain distributions, such as ecological conditions. Specificity is a universal aspect of fungal parasitic associations (Heath 1987, 1991, 1996), where benefits to the host from recognizing and resisting plant parasites can be readily demonstrated. Plant resistance comprises basic and specific resistance. Heath (1991) argues that the basic resistance is nonspecific and protects the plant from stresses caused by random parasites; specific resistance only develops when there is sufficient parasitism to have significant effects on plant fitness. Basic resistance mechanisms are triggered by mycorrhizal fungi (Blee and Anderson 2000), but it is generally thought that they do not trigger specific resistance, since interactions are assumed to be mutualistic. Law (1985) hypothesized that resistance would not develop in mutualistic associations, since specificity would limit access to partners that could potentially provide a benefit. If mycorrhizal mutualisms are evolving primarily under mutualistic conditions, then we would not expect to see evidence of specificity due to filtering. However, if they are evolving in dynamic systems prone to exploitation, then specificity is more likely to evolve. In an excellent paper on specificity by Bruns et al. (2002), many advantages to specificity are discussed, but they conclude that “the advantages

of specificity remain unclear”. Specificity would be easier to explain if some mycorrhizal associations are exploitative, since it would favour filtering mechanisms and evolution of specific resistance.

Specificity is complex in mycorrhizal systems, even in apparently low-specificity arbuscular mycorrhizas (Bever 2002a; Helgason et al. 2002). ERM fungi, such as *H. ericae*, appear to exhibit little specificity on the surface, colonizing a wide variety of plants, from Ericaceae (Smith and Read 1997) and Epacridaceae (Cairney and Ashford 2002) to Pinaceae (Vralstad et al. 2000). However, even this group shows considerable population subdivision that has been correlated with host differences (Vralstad et al. 2002b). ECM fungal genera that show the highest specificity (>65% in the narrow or intermediate category, according to Molina et al. (1992)) include Boletaceae (*Suillus*, *Leccinum*, *Alpova*, *Rhizopogon*); Cortinariaceae (*Cortinarius*, *Dermocybe*, *Hebeloma*, *Inocybe*, *Naucoria*, *Hymenogaster*, *Thaxterogaster*, *Gomphidius*, *Chroogomphus*, *Brauniellula*, *Gomphogaster*); Hygrophoraceae (*Hygrophorus*); Russulaceae (*Lactarius*, *Russula*, *Gymnomyces*, *Macowanites*, *Martellia*); Sclerodermataceae (*Scleroderma*); and Tricholomataceae (*Tricholoma*, *Hydnangium*). Genera showing the broadest host specificity (>35% in the broad host range category) include Amanitaceae (*Amanita*), Boletaceae (*Boletus*), and Tricholomataceae (*Laccaria*) (Molina et al. 1992).

If specificity seen in mycorrhizal symbioses is due to filtering to sanction exploitative genotypes, then we would expect the greatest host specificity in those groups exhibiting the most exploitative mycorrhizal associations. Either physiological specificity (i.e., limiting carbon to fungi that provide little benefit) or genetic specificity (evolution of host resistance) would be favoured if confronted by exploiters. It may be significant that specificity is highest in the groups that appear to have high speciation rates (previous section) (e.g., *Russula*, suilloid fungi, Cortinariaceae). As specificity increases, there may be a greater likelihood of sympatric speciation due to host switching and reversal of mutualism, analogous to speciation in plant parasites, which often occurs by host switching.

If the initial stages of mycorrhizal symbioses typically encounter basic host resistance, then specificity due to filtering would only develop as fungal symbionts shift to exploitation and plants respond by developing specific resistance. If early associations encounter only limited basic resistance, then there would be few barriers to host switching, compared with later stages, when greater specificity and specialization have developed.

Negative feedback

Bever et al. (1997) provided a framework for incorporating positive and negative feedbacks from soil communities into plant population ecology. The dynamics of these feedback interactions are complex, since they are governed by the net interaction between plant competitors rather than by whether direct feedbacks upon individual plants are positive or negative. Negative feedbacks, where plants grown in soils cultured with conspecifics perform more poorly than when grown in soils that were cultured with other plant species, appear to be common phenomena (Bever et al. 1997). Some of this phenomenon can be attributed to parasitic fungi

(Mills and Bever 1998; Packer and Clay 2000, 2003), but there is evidence that mycorrhizal fungi also play a role (Bever 1994, 2002a, 2002b; Castelli and Casper 2003).

Negative feedback occurs in situations where a particular plant–fungus pairing is optimal for one partner, but not both. That is, a fungus that is the best at promoting the growth of one particular plant may itself experience the greatest benefit in association with a different plant. One prediction of negative feedback is that a diversity of plant–fungus pairings will be maintained (i.e., each plant–fungus pairing is unstable). If positive feedback is operating (i.e., there is an optimal plant–fungus pairing from the perspective of both partners), then the diversity of pairings should decrease. The occurrence of negative feedback challenges the view that natural selection should favor the evolution of balanced mutualisms, in which the fitness of both partners (and hence the symbiosis) is optimal. Negative feedback is more compatible with the view that there is an underlying conflict of interest between plant and fungal partners. This conflict is manifested as a lack of reciprocity in optimal fitness responses of plants and fungi. Put more simply, in some pairings the fungus gains the upper hand and plant growth is not optimal, whereas in other pairings the plant comes out on top.

Negative feedback in natural systems could be partially explained by selection for exploiter genotypes. Negative-feedback effects in highly managed systems are consistent with exploitation, since certain management practices, such as fertilization and disruption of neighbour interactions by tillage (Johnson et al. 1992; Johnson 1993; Treseder and Allen 2002), could also lead to an increase in exploiters. In fact, such disruption may be sufficient to shift the association from mutualism to parasitism, as may have happened with a pathogenic isolate of *Glomus macrocarpum* in an extensively managed agricultural system with high phosphorus amendment (Modjo and Hendrix 1986). The degree to which negative feedback is explained by exploitation needs further examination.

Above- and below-ground distributions

The early work of Gardes and Bruns (1996) presented a paradox: The most abundant fungi above ground were generally not the most abundant below ground and vice versa. An interpretation of this phenomenon is that fungi with high abundance above ground represent more exploitative species, on the assumption that abundantly fruiting fungi utilize more autotrophic carbon to generate their fruit-body biomass. A good example of an abundant fruiting, host-specific fungus is *Suillus pungens*, which fruits almost exclusively with *Pinus muricata* and *Pinus radiata* (Gardes and Bruns 1996; Bruns et al. 2002). *Suillus pungens* exhibits high abundance above ground and forms dense mycelial mats, but is rarely found on roots (Gardes and Bruns 1996), a pattern that would be expected if this mycorrhizal symbiosis has shifted to exploitation. *Suillus pungens* is part of the suilloid group that is mostly restricted to Pinaceae and exhibits high speciation rates, although measures of carbon uptake do not take into account by-product benefits, which in suilloid fungi may include high drought tolerance (Horton 2003).

The best cost–benefit ratio for the plant would arise from highly beneficial symbiotic fungi that exact little carbon cost in terms of sporocarp production. This is consistent with ob-

servations that fungi found most frequently on roots have a wide host range (Bruns et al. 2002), since restricted host range could be a signature of exploitation. We need to look more closely to see if fruit-body production is correlated with other potential signatures of exploitation.

Mycoheterotrophism

Mycoheterotrophic plants are achlorophyllous and obtain their carbon heterotrophically via association with mycorrhizal, parasitic, or saprotrophic fungi (Leake 1994). Mycoheterotrophs have been characterized as exploiters (cf., cheaters) of autotrophic plant lineages (Taylor and Bruns 1997; Bidartondo et al. 2002, 2003). Given that the fungal associates of mycoheterotrophs are acquiring carbon for their own growth as well as the growth of the mycoheterotroph, it would be expected that they are highly efficient at obtaining carbon, which raised the question as to whether they too are exploiters of their autotrophic hosts.

Available evidence thus far is that mycoheterotrophs are highly specific regarding the fungi they associate with (Bidartondo and Bruns 2001, 2002; Bidartondo et al. 2002, 2003). Suilloid fungi and members of the Russulaceae are common ECM associates of mycoheterotrophic plants. Both of these groups exhibit high host-plant specificity and fall into the narrow or intermediate categories of Molina et al. (1992). Since the fitness of mycoheterotrophs depends entirely upon their fungal associate, it is not surprising that selection would drive these associations to high levels of specificity, since even small variations in ability of fungal associates to procure carbon from their autotrophic hosts would have large fitness consequences for the mycoheterotroph. Also of interest are the nonmutualistic fungal associates of mycoheterotrophs, such as *Armillaria*. Clearly such fungi have no conflict of interest with their partners, as they do not act mutualistically. However, what links parasitic fungi with supposedly mutualistic mycorrhizal fungi in mycoheterotrophic associations is high efficiency of carbon uptake, suggesting that mycorrhizal fungi in these associations are parasitic. Interestingly, suilloid fungi have high evolutionary rates (Bruns et al. 1989), and suilloid fungi and Russulaceae exhibit high speciation rates, both of which have been argued to be possible signatures of exploitation.

Fungal associates of mycoheterotrophic plants appear to be strong candidates for the role of exploiters. If associations characterized by mycoheterotrophism are exploitative, they may be nascent stages of breakdown leading to escape (host switching or reversal of mutualism) or extinction. However, it is worth noting that our expectations about the relative benefits and costs of associations are based solely on patterns of nutrient flow. We have no idea about possible by-products or other benefits that might be provided to the fungi or autotrophic plants. For example, if mycoheterotrophs somehow amplified the benefits of the mycorrhizal relationship for the plants, then the dynamic of the symbiosis and its exposure to selection could change dramatically.

Conclusions: a return to the symbiotic continuum

Although there is not much direct evidence that mycorrhizal symbioses are extensively characterized by exploita-

tion, there is considerable circumstantial evidence that mycorrhizal mutualisms, like all other mutualisms, include exploiters. Some candidates that we suggest be examined further for evidence of exploitation are members of the suilloid group, Russulaceae, and Cortinariaceae, as all have signatures that could indicate a wide range of interactions on the symbiotic continuum.

There is scant evidence that mycorrhizal partners are under selective pressure to be better mutualists, what Law (1985) described as evolving to "benefit the association". Indeed, models that incorporate positive-feedback loops generated by continuously positive effects on each other's respective fitness offer unrealistic outcomes (Wilkinson and Sherratt 2001). Rather, the evidence suggests that mycorrhizal partners are under selection to be as parasitic as possible given the context, or what Wilkinson and Sherratt (2001) describe as "the best of a bad job". It may be that the exchange of carbon and nutrients is not the only factor that determines whether mycorrhizal associations are mutualistic or antagonistic. By-product benefits, or benefit under periods of high plant stress, could play more important roles. If so, selective pressures on mycorrhizas would oscillate between mutualism and antagonism, which may explain why there is so little evidence of evolution to benefit the association as described by Law (1985).

Observations of conditional outcomes in mycorrhizal symbioses are consistent with the suggestions by several authors (e.g., Wilcox 1983; Francis and Read 1995; Newsham et al. 1995b; Johnson et al. 1997) that root associations must be viewed as a continuum from mutualism to parasitism. In this context, reciprocal parasitism provides a unifying framework within which we can consider how mycorrhizas, root endophytes, root pathogens, and mycoheterotrophs arise. We should focus our efforts on understanding the factors that cause these symbioses to shift along the mutualism-parasitism continuum, with awareness that there is always a fundamental conflict of interest between the partners. We should also consider the conditions that cause the complete dissolution of symbioses, as may have occurred in both mycorrhizal and lichenized fungi (Hibbett et al. 2000; Lutzoni et al. 2001). We should not assume that mycorrhizas are mutualistic based solely upon structural or functional criteria. Instead, we should critically seek evidence for positive or negative fitness effects, whether mediated by nutrient exchange or by-product effects. Finally, we need to broaden our perspective to incorporate all root associations into our structural, functional, and phylogenetic studies to gain a better understanding of how mycorrhizal symbioses originate and evolve.

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