

BOTANICAL BRIEFING

Myco-heterotrophy: when fungi host plants

Vincent Merckx^{1,3,*}, Martin I. Bidartondo⁴ and Nicole A. Hynson²

¹Department of Plant and Microbial Biology, and ²Department of Environmental Science, Policy & Management, University of California, Berkeley, CA 94720, USA, ³Laboratory of Plant Systematics, K.U. Leuven, Kasteelpark Arenberg 31, PO Box 2437, B-3001 Leuven, Belgium and ⁴Imperial College London and Royal Botanic Gardens, Kew TW9 3DS, UK

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- **Background** Myco-heterotrophic plants are partly or entirely non-photosynthetic plants that obtain energy and nutrients from fungi. These plants form a symbiosis with arbuscular mycorrhizal, ectomycorrhizal or saprotrophic fungi to meet their nutrient demands.
- **Scope** This Botanical Briefing summarizes current knowledge about myco-heterotrophy, discusses its controversial aspects and highlights future directions for research.
- **Conclusions** Considerable recent progress has been made in terms of understanding the evolutionary history, germination and nutrition of myco-heterotrophic plants. Myco-heterotrophic plants: (1) are diverse and often ancient lineages that have coevolved with fungi, (2) often demonstrate unusually high specificity towards fungi during germination and maturity, and (3) can either cheat common mycorrhizal networks supported by neighbouring photosynthetic plants to satisfy all or part of their energetic and nutritional needs, or recruit free-living saprotrophic fungi into novel mycorrhizal symbioses. However, several fundamental aspects of myco-heterotrophy remain controversial or unknown, such as symbiotic costs and physiology.

Key words: Cheater, common mycorrhizal network, mutualism, myco-heterotrophy, non-photosynthetic, symbiosis.

INTRODUCTION

Mycorrhizas are obligate and ubiquitous symbioses between the vast majority of plants and some members of three fungal phyla (Basidiomycota, Ascomycota and Glomeromycota). In general, mycorrhizas are mutualistic; the plant exchanges photosynthetically derived carbon for fungal-acquired soil minerals (for a comprehensive text on mycorrhizal biology see Smith and Read, 2008). Ectomycorrhizal plants can, for instance, transfer up to 30 % of their fixed carbon to their respective fungi in exchange for the majority, if not all, of their nitrogen (Smith and Read, 2008). Sustaining this costly plant–fungal exchange is vital: it allows both partners to establish, grow and complete their life cycles. Globally, the mycorrhizal mutualism is widespread and essential for the functioning of all terrestrial ecosystems.

The main diagnostic criteria for the types of mycorrhizas formed in nature are the phylogenetic identity of the fungal lineages engaged in the symbiosis and the morphology at the symbiotic interface of plant and fungus. The two dominant types of mycorrhizas are the ancestral arbuscular mycorrhizas that involve members of the Glomeromycota and most plants, and the more recent ectomycorrhizas that involve some members of the Basidiomycota and Ascomycota and several woody plants. All mycorrhizas are intimate cell-to-cell interactions, either intra- or intercellular. However, unlike other well-established examples of mutualisms such as the fig–fig wasp, yucca–yucca moth and legume–rhizobial symbioses, the majority of mycorrhizal plants and fungi show remarkably low specificity. Mycorrhizal promiscuity leads to the

widespread occurrence of multi-species mycorrhizal linkages in nature or ‘common mycorrhizal networks’. It also has led, perhaps counter-intuitively, to specialized cheating by plant lineages that evolved from mutualists. In deeply shaded forest understoreys, plants are light-limited and to cope with this limitation numerous plants have evolved to cheat mycorrhizal networks, or free-living fungi, by gaining organic carbon and other essential elements from the fungi. These plants are referred to as ‘myco-heterotrophs’. Myco-heterotrophic plants have long attracted the curiosity of biologists, and they have been the target of unabated controversies and speculation (for detailed overviews see Leake, 1994; Bidartondo, 2005). In fact, these puzzling plants dominated the very beginnings of the field of mycorrhizal biology (e.g. Kamienski, 1881).

In reference to myco-heterotrophic plants, because their targeted mycorrhizal fungi may be more prevalent, more ancient and evolutionarily tracked by the plants, the fungi may be referred to as ‘hosts’. A plant may be an ‘initial’ myco-heterotroph only during germination, a ‘partial’ myco-heterotroph (or ‘mixotroph’) with limited photosynthetic capacity as a mature plant, or a ‘full’ myco-heterotroph lacking photosynthetic capacity during its entire life span.

EVOLUTION

There are over 400 species of fully myco-heterotrophic plants (Leake, 1994; Fig. 1) and nearly 20 000 partially myco-heterotrophic plants (mostly initial myco-heterotrophs in the Orchidaceae). New species are described almost every year while others have not been collected for over a century and

* For correspondence. E-mail merckx@nature.berkeley.edu



FIG. 1 Examples of myco-heterotrophic and partially myco-heterotrophic plants from different angiosperm families: (A) *Pterospora andromeda*, (B) *Sarcodes sanguinea* (both Pterosporeae; Monotropoideae; Ericaceae), (C) *Voyria clavata* (Gentianaceae), (D) *Cephalanthera damasonium* (Orchidaceae), (E) *Kupea martinupei* (Triuridaceae), and (F) *Afrothismia hydra* (Thismiaceae).

may be extinct. Myco-heterotrophy evolved independently over 40 times within plant lineages and it has been confirmed in liverworts, monocots and eudicots. Myco-heterotrophic angiosperms comprise part of the families Burmanniaceae, Corsiaceae, Ericaceae, Gentianaceae, Iridaceae, Orchidaceae, Petrosaviaceae, Polygalaceae, Thismiaceae and Triuridaceae (Leake, 1994). The majority of fully myco-heterotrophic flowering plants are restricted to the tropics, but myco-heterotrophic Ericaceae and some Orchidaceae occur in temperate forests. Owing to the reduction or loss of key morphological characters, the taxonomic affinities of many groups of myco-heterotrophic flowering plants remained confusing for many decades. With the rise of molecular systematics, new tools for identifying the photosynthetic relatives of myco-heterotrophic plants became available. Indeed, molecular data have been successfully used to infer the phylogenetic position of many myco-heterotrophic plant clades, often with surprising results (e.g. Cameron *et al.*, 2003). However, in many myco-heterotrophs, chloroplast genes are mostly lacking, or highly divergent, and nuclear and mitochondrial substitution rates are often greatly elevated, causing biases in inferred phylogenies (Merckx *et al.*, 2009). In addition, acquiring a representative sample of taxa, a critical factor for

successful phylogenetic reconstruction, is still an obstacle for many rare fully myco-heterotrophic groups such as Corsiaceae, Triuridaceae, *Epirixanthes* (Polygalaceae), *Cheilothea* (Ericaceae) and Thismiaceae.

Many myco-heterotrophic plant genera are considered ancient because of their pantropical distribution (Leake, 1994), but there is only one series of fossils that might be assigned to an extant myco-heterotrophic lineage. These fossils are from the Upper Cretaceous (about 90 Mya) and show affinities with extant Triuridaceae (Gandolfo *et al.*, 2002). However, it remains questionable whether these fossilized plants were in fact myco-heterotrophic (Gandolfo *et al.*, 2002) and members of the Triuridaceae (Furness *et al.*, 2002). Indirect evidence from molecular clock analyses has confirmed the old age of myco-heterotrophy, at least in some groups, indicating that myco-heterotrophic lineages can persist and diversify over considerable evolutionary time (Merckx and Bidartondo, 2008; Merckx *et al.*, 2008).

HOST FUNGI

Historically, it was assumed that myco-heterotrophic plants obtained nourishment directly from soil organic matter.

Consequently, these plants were described as ‘saprophytes’, a term that (although incorrect) is still frequently used (Leake, 2005). The observations by early investigators of myco-heterotrophic plants revealed a lack of direct plant–plant connections via haustoria comparable with those found in parasitic plants. Instead, they demonstrated the presence of fungal filaments closely associated with the root systems. These initial observations ultimately led to our current understanding of the need for fungi in the establishment and growth of these plants. Owing to the lack of diagnostic morphology of mycorrhizas it was not until relatively recently with the use of molecular ecology tools that the identity of the fungi forming mycorrhizas with myco-heterotrophic plants was unambiguously revealed. It soon became clear that many full myco-heterotrophs were in fact ‘epiparasites’ involved in tripartite symbioses through shared mycorrhizal fungi with adjacent autotrophic plants. Thus, the two most common types of mycorrhizal symbioses (ectomycorrhizas and arbuscular mycorrhizas) have been repeatedly exploited by myco-heterotrophic plants. With few exceptions, myco-heterotrophic Aneuraceae, Orchidaceae and Ericaceae exploit ectomycorrhizal networks while myco-heterotrophic Burmanniaceae, Corsiaceae, Gentianaceae, Thismiaceae and Triuridaceae exploit arbuscular mycorrhizal networks (reviewed by Leake 2005). In these cases, myco-heterotrophy is thought to represent mutualistic breakdowns, i.e. epiparasitic myco-heterotrophic plants evolved from mutualistic autotrophic mycorrhizal plants. As an alternative to associations with mycorrhizal fungi, some myco-heterotrophic orchids are specialized on litter- and wood-decay fungi (Ogura-Tsujita *et al.*, 2009).

SPECIFICITY

Thinking about tri- and multi-partite symbioses involving diverse lineages is challenging for most biologists accustomed at most to bipartite symbioses, but it is central to understanding the ecology and evolution of myco-heterotrophy. Both the arbuscular and the ectomycorrhizal symbiosis are generally characterized by low specificity between plants and fungi. An autotrophic mycorrhizal plant typically associates with multiple distantly related fungi and a mycorrhizal fungus often associates simultaneously with multiple distantly related plants (Giovannetti *et al.*, 2004; Lian *et al.*, 2006). In contrast to autotrophic mycorrhizal plants, however, myco-heterotrophic plants often show high specificity towards fungi even though the fungi remain generalists (e.g. Bidartondo *et al.*, 2002). In the Monotropoideae (Ericaceae), for example, epiparasitism of ectomycorrhizal networks has led to diversification into five myco-heterotrophic plant lineages, each of which phylogenetically tracks one of five distantly related basidiomycete fungal lineages (reviewed by Bidartondo, 2005). An extreme case of phylogenetic tracking has been observed in *Afrothismia* (Thismiaceae), where specialization by five closely related plant species to five closely related lineages of arbuscular mycorrhizal fungi resulted in a delayed co-speciation pattern (i.e. fungal hosts diverged in advance of their plant parasites) (Merckx and Bidartondo, 2008; Fig. 2).

Mycorrhization is the critical life-history stage for myco-heterotrophic plants. In fact, mycorrhizal specificity is often so extreme that many myco-heterotrophic plants will not germinate or develop in the absence of their target fungal symbiont (Bruns and Read, 2000). Even if germination is triggered by a close relative of the host fungus, the seedling may not survive past the early stages of development (Bidartondo and Read, 2008). Although the process leading to this extreme level of fungal specificity is not yet understood, from an evolutionary perspective there are two mechanisms that may be involved: (1) the myco-heterotrophic plant has selected from the potential fungal community the best target to meet its nutrient demands, and (2) the myco-heterotrophic plant, because of its increasingly parasitic interaction with fungi, has been ‘denied’ access to most members of the fungal community except for a few fungal lineages that fail to detect or exclude the plant (Bruns *et al.*, 2002; Egger and Hibbett, 2004; Bidartondo, 2005). In either case, the maintenance of a carbon supply is paramount for the survival of the myco-heterotrophic plant, and it has been argued that once an appropriate fungal partner had been found, the plant fine-tunes its physiology to adapt to that particular fungus and it is therefore largely incapable of host-jumps to distantly related fungi (Bidartondo and Bruns, 2002). Thus, narrow specialization of plants towards fungi and speciation of the lineages involved may result in a pattern of evolutionary tracking and in some cases co-speciation between myco-heterotrophic plants and mycorrhizal fungi (Fig. 2). However, it does not appear that fungal specialization is a requisite for the loss of photosynthesis in myco-heterotrophic plants (Hynson and Bruns, 2009). This indicates that identifying a specific fungus that meets the plant’s demands need not be the initiating process in the subversion of the mycorrhizal mutualism.

PHYSIOLOGICAL ECOLOGY

The physiology of myco-heterotrophic plants remained nearly entirely unexplained until the recent application of stable isotope analyses. The analysis of the natural abundance of carbon ($^{13}\text{C}/^{12}\text{C}$) and nitrogen ($^{15}\text{N}/^{14}\text{N}$) stable isotopes in plants is a powerful tool by which to infer strategies of resource acquisition and metabolic pathways in plants (Dawson *et al.*, 2002; but see also Robinson, 2001). The stable isotope signatures of myco-heterotrophic plants seem best to fit a food-chain model in which the plants’ stable isotope signatures reflect those of their host fungi, their ultimate nutrient source (Trudell *et al.*, 2003). Generally, the source of a nutrient is left depleted in the heavy isotope (^{13}C or ^{15}N) compared with its sink (Fry, 2006). For instance, previous work has shown that fully myco-heterotrophic plants that associate with ectomycorrhizal fungi are significantly enriched in ^{13}C and ^{15}N compared with autotrophic understorey plants, and they have carbon and nitrogen isotope signatures similar to, or more enriched in the heavy isotopes than, ectomycorrhizal fungi (e.g. Gebauer and Meyer, 2003; Tedersoo *et al.*, 2007; Fig. 3). These findings indicate that ectomycorrhizal myco-heterotrophic plants are receiving both carbon and nitrogen through distinct pathways compared with those used by autotrophic ectomycorrhizal plants. Also, the similarity of

the carbon and nitrogen isotope signatures of myco-heterotrophs to those of ectomycorrhizal fungal fruit bodies rather than surrounding photosynthetic plants provides further evidence that the fungi are the sole nutrient source for these plants. What remains unknown is in what forms carbon and nitrogen pass from fungus to myco-heterotrophic plant and whether these nutrients are processed differently

compared with autotrophic plants which share the same fungus. In particular, most myco-heterotrophs studied to date are significantly enriched in ^{15}N compared with both their host fungi and surrounding autotrophic plants. The cause of this enrichment is unclear, but it is possibly linked to the type of nitrogen compounds that are transferred from the fungus to the myco-heterotroph, or the processing of nitrogen by the plant once it is received from the fungus (Trudell *et al.*, 2003; Nygren *et al.*, 2007).

Recently, interest has turned to determining the patterns of nutrient acquisition in putatively partially myco-heterotrophic plants that are closely related to fully myco-heterotrophic plants. Stable isotope analyses have indicated a unique trophic strategy in green Orchidaceae and Ericaceae plants where carbon stable isotope values tend to fall between those of autotrophic and myco-heterotrophic plants, while their nitrogen signatures are enriched in ^{15}N compared with surrounding autotrophic plants (e.g. Gebauer and Meyer, 2003; Tedersoo *et al.*, 2007; Zimmer *et al.*, 2007; Hynson *et al.*, 2009; Fig. 3). This pattern indicates that these partially myco-heterotrophic plants are tapping into two carbon sources, one via photosynthesis and the other via mycorrhizal fungi, and that they are acquiring N through a pathway similar to fully myco-heterotrophic plants. Thus, plants that are capable of gaining carbon through both autotrophy and myco-heterotrophy are referred to as partial myco-heterotrophs (or mixotrophs) (reviewed by Selosse and Roy, 2009). The driving physiological, ecological and evolutionary forces leading to the relative enrichment in ^{13}C and ^{15}N in partially myco-heterotrophic plants compared with neighbouring autotrophic plants remain unknown. One clue to the factors that may influence the myco-heterotrophic abilities of green plants is that individual species' enrichment in ^{13}C appears to be habitat-specific and therefore possibly influenced by light availability and/or the presence of particular mycorrhizal fungi (Bidartondo *et al.*, 2004; Julou *et al.*, 2005; Tedersoo *et al.*, 2007).

In contrast, a study that analysed the carbon and nitrogen isotope signatures of *Gastrodia confusa* (a fully myco-

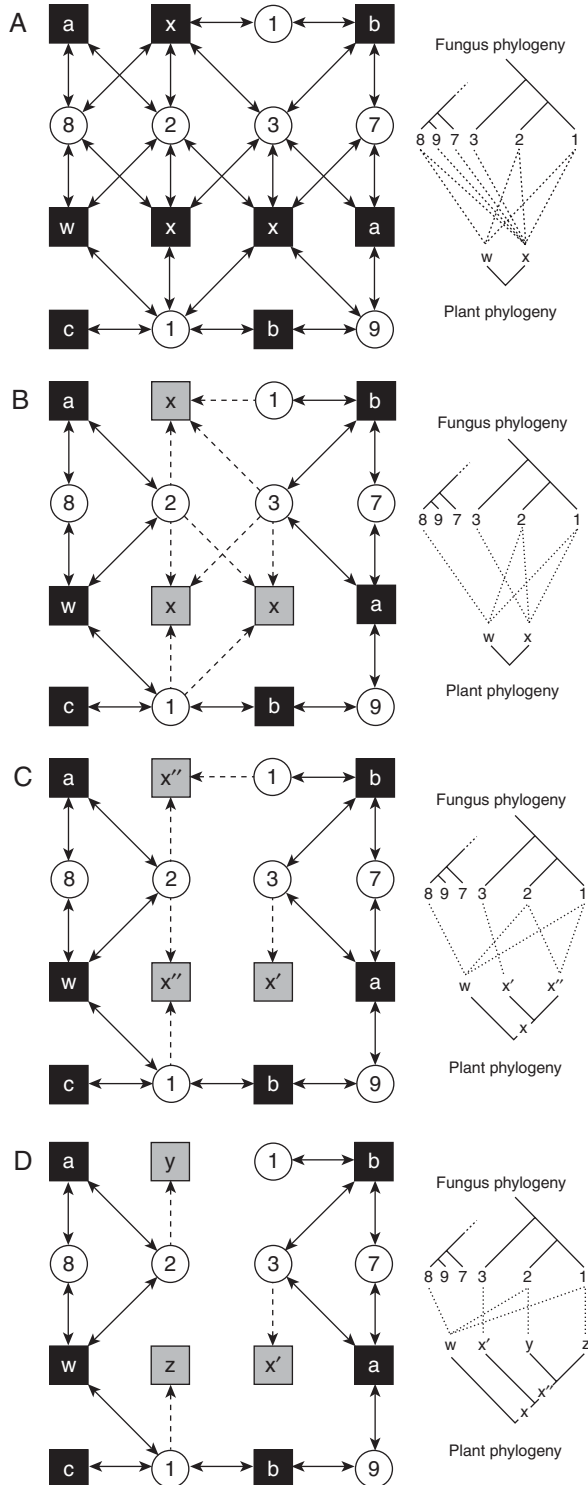


FIG. 2. A model for the evolution of myco-heterotrophic plants in which speciation of plant lineages and simultaneous specialization of plants to fungi leads to phylogenetic tracking (Merckx and Bidartondo, 2008). (A) A community of generalist mycorrhizal mutualists. Black squares represent mycorrhizal plants in two distant lineages, ((a,b),c) and (w,x). Circles represent mycorrhizal fungi in two distant lineages, ((1,2),3) and ((9,8),7). For instance, 1 and 2 are sister taxa and their closest relative is 3. All plants must be linked to fungi and all fungi must be linked to plants. Double-ended arrows show mutualistic mycorrhizal links where plants provide carbon to fungi and fungi provide mineral nutrients to plants. (B) A mycorrhizal community where plant species x (grey) has lost the ability to photosynthesize so it cannot provide carbon to fungi thereby breaking down the mycorrhizal mutualism. Myco-heterotrophic plants depend on fungi that link them to photosynthetic plants. Single-ended dashed arrows show non-mutualistic mycorrhizal links where fungi provide carbon to plants. Myco-heterotrophic plants have a reduced mycorrhizal range and only associate with related fungi 1, 2 and 3. (C) Speciation of the non-photosynthetic plant lineage x into x' and x'' leads to further specialization on fungal lineages; x' depends on fungus 3 and x'' depends on fungi 1 and 2. The fungi and the photosynthetic plants remain generalist mycorrhizal mutualists. (D) Speciation of plant lineage x' into y and z. Plant y specializes on fungal host 2 and plant z specializes on closely related fungal host 1. This form of phylogenetic tracking by myco-heterotrophic plants towards pre-existing fungal lineages produces an evolutionary pattern of delayed co-speciation.

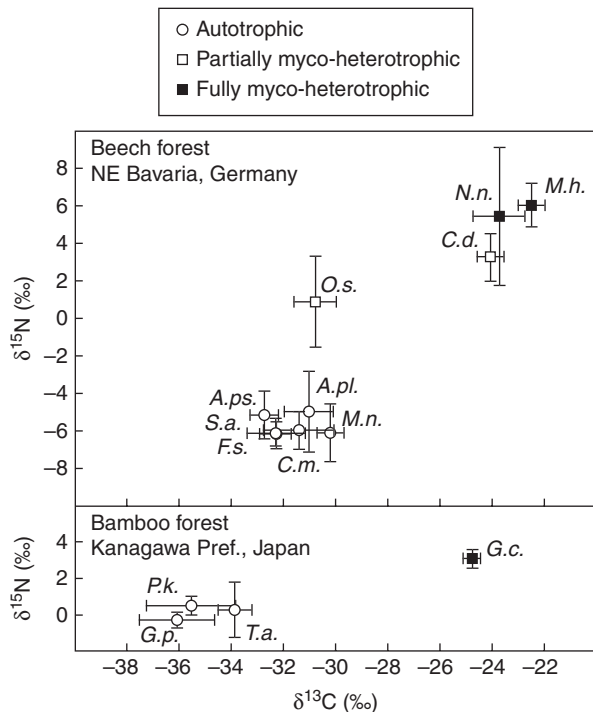


FIG. 3 Mean $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values (\pm s.d.) in leaf tissue of autotrophic, partially myco-heterotrophic and fully myco-heterotrophic plants, as indicated, from two different sites: a beech forest in Germany and a bamboo forest in Japan. Data consolidated from Gebauer and Meyer (2003), Zimmer *et al.* (2007, 2008) and Ogura-Tsujita *et al.* (2009). Fully myco-heterotrophic plants are significantly enriched in ^{15}N and ^{13}C compared with autotrophic plants at both sites. The green orchid *Cephalanthera damasonium* shows similar but slightly less pronounced enrichments in ^{15}N and ^{13}C . At the Bavarian site, *Orthilia secunda* (Ericaceae) is significantly enriched in ^{15}N , but not in ^{13}C , compared with autotrophic plants. However, at sites where ground-level irradiance is low, a significant myco-heterotrophic gain of ^{13}C was detected for this species (Zimmer *et al.*, 2008). Abbreviations: *A.pl.*, *Acer platanoides* ($n = 5$); *A.ps.*, *Acer pseudoplatanus* ($n = 9$); *C.d.*, *Cephalanthera damasonium* ($n = 5$); *C.m.*, *Convallaria majalis* ($n = 12$); *F.s.*, *Fagus sylvatica* ($n = 14$); *G.c.*, *Gastrodia confusa* ($n = 5$); *G.p.*, *Gynostemma pentaphyllum* ($n = 4$); *M.h.*, *Monotropa hypopitys* ($n = 4$); *M.n.*, *Melica nutans* ($n = 5$); *N.n.*, *Neottia nidus-avis* ($n = 14$); *O.s.*, *Orthilia secunda* ($n = 5$); *P.k.*, *Piper kadsura* ($n = 5$); *S.a.*, *Sorbus aucuparia* ($n = 7$); *T.a.*, *Thelypteris acuminata* ($n = 5$).

heterotrophic orchid from bamboo forests in Asia that depends on free-living saprotrophic *Mycena* fungi) reported an even higher enrichment in ^{13}C , but little enrichment in ^{15}N , compared with myco-heterotrophic plants that depend on ectomycorrhizal fungi of neighbouring autotrophic plants (Ogura-Tsujita *et al.*, 2009). Similar to myco-heterotrophs that associate with ectomycorrhizal fungi, this orchid has isotope signatures reflecting the signature of its nutrient source. Saprotrophic fungi, especially those found on woody substrates, are accessing a ^{13}C -enriched pool of carbon that is also enriched in ^{15}N compared with living plants, but depleted in ^{15}N compared with ectomycorrhizal fungi (Taylor *et al.*, 2003).

CONTROVERSIES AND CHALLENGES

The study of myco-heterotrophic plants is very much in its infancy, and research has been largely driven by

methodological developments in molecular and plant physiological ecology. And yet, a reluctance to accept established dogmas regarding the ecology and physiology of the mycorrhizal symbiosis relative to myco-heterotrophy has led to answers that have been provocative and unconventional, for example the epiparasitic mode of life of most myco-heterotrophs and their specialization upon particular lineages of mycorrhizal fungi (reviewed by Bidartondo, 2005), bidirectional carbon flow in a green orchid–mycorrhizal association (Cameron *et al.*, 2008) and coevolution between plants and mycorrhizal fungi (Merckx and Bidartondo, 2008). These answers in turn have prompted the emergence of novel perspectives on symbioses (Sachs and Simms, 2006; Selosse *et al.*, 2006). These new theoretical frameworks are conceptually sound because myco-heterotrophic plants stand apart from the major models of cheating within mutualisms (yucca–yucca moth, fig–fig wasp, ant–lycaenid butterfly) as the only non-animal system. Furthermore, myco-heterotrophs provide a prime example of the subversion of mutualisms in nature and offer a system with which to examine further how symbioses remain robust and exclude cheaters. It is also a practical system as it sheds light on the importance of all-too-often ‘black-box’ below-ground interactions and provides mechanistic approaches to conservation and management of biodiversity. Indeed, myco-heterotrophy shows that the biology, evolution and conservation of many plants cannot be understood without a direct focus on individual species of fungi that may, for example, determine plant distribution.

Despite considerable progress in our understanding of myco-heterotrophic plants, they continue to present major challenges in scientific investigations. Our current inferences regarding the nutrient acquisition strategies of myco-heterotrophic plants are based on a limited number of case studies with a notable bias towards myco-heterotrophic plants from temperate areas, where ectomycorrhizas are abundant. Yet to be determined are the isotope signatures of myco-heterotrophic plants that depend on the more widespread arbuscular mycorrhizal fungi. Therefore, it remains unknown whether ectomycorrhizal and arbuscular mycorrhizal myco-heterotrophic plants are physiologically convergent; and although isotope signatures give insight into the carbon and nitrogen acquisition strategies of myco-heterotrophs, the physiological mechanism of these nutrient transfers is completely unknown and thus prone to speculation. In addition to the signals involved in triggering myco-heterotrophic plant seed germination and mycorrhization, the identity of the fungal hosts of most myco-heterotrophic and closely related partial myco-heterotrophic plants, particularly during germination, also remains largely unknown. Within a robust phylogenetic context, this information will eventually uncover the evolutionary history of mycorrhizal specialization by myco-heterotrophic plants. Comparing fungal specialization between recent and old myco-heterotrophic lineages will, in turn, reveal the timing of the process.

Another prominent question is whether myco-heterotrophic plants are in fact parasites of their fungal hosts and/or the autotrophic plants that are part of common mycorrhizal networks. In other words: are there measurable costs for mycorrhizas that have been invaded by a myco-heterotrophic plant? This is a methodologically and conceptually challenging question,

particularly for tripartite symbioses, and to date there are no experimental data to address this matter. Although it may seem obvious that a myco-heterotrophic plant exploits its host fungus, there may be benefits for the hosts as well. Some myco-heterotrophic plants may stimulate the growth of their fungal partner and thus perhaps compensate for, or exacerbate, carbon loss (Bidartondo, 2005). It has been proposed that myco-heterotrophic plants specialize on mycorrhizal fungi that are particularly efficient at tapping into carbon sources from autotrophic hosts (Egger and Hibbett, 2004), so, similar to other cheaters of mutualisms (Bronstein, 2001), the carbon cost imposed by a myco-heterotrophic plant on a fungus may be negligible.

In terms of the ecological theory of plant community dynamics, myco-heterotrophic plants provide the clearest evidence for the existence of common mycorrhizal networks in nature, a controversial topic in itself. Myco-heterotrophic plants are the only obvious examples for the potentially widespread phenomenon of plant-to-plant net C transfer via shared mycorrhizal fungi. This functional role of mycorrhizal networks still remains one of the most hotly contested topics in mycorrhizal biology, owing to a combination of technical difficulties and challenging implications. Thus far, the many tests for net carbon transfer from arbuscular mycorrhizal or ectomycorrhizal fungi to green plants have either failed or, if successful, been criticized on methodological grounds (Francis and Read, 1984; Simard *et al.*, 1997; Fitter *et al.*, 1999; Lerat *et al.*, 2002; Pfeiffer *et al.*, 2004). For instance, Pfeiffer and co-workers could not detect carbon transfer *in vitro* from *Glomus intraradices* to transformed arbuscular mycorrhizal carrot roots growing on glucose. This has been recently confirmed *in vitro* with *G. intraradices* and whole plants of *Medicago truncatula* (Voets *et al.*, 2008). As previously mentioned, the only exceptions have been field studies of green orchids and Pyroleae (subfamily Monotropoideae of the family Ericaceae) that are closely related to fully myco-heterotrophic plants and generally grow in the dark understorey of forest habitats. These understorey plants, although photosynthetic, fulfil a significant proportion of their adult nutritional needs with fungal-derived carbon and nitrogen. Thus, we know there are at least partially myco-heterotrophic plants. Arbuscular mycorrhizas are by far the dominant mycorrhizas on Earth; roughly 70% of plant families depend on glomeromycete fungi to obtain soil mineral nutrients and these fungi depend entirely on host plants to obtain carbon. However, none of the photosynthetic plants closely related to non-photosynthetic arbuscular mycorrhizal plants has been examined for facultative mycorrhizal cheating, despite the fact that well over 3000 plant species may fall into this category (e.g. Polygalaceae, Gentianaceae, Dioscoreales, Iridaceae). Judging from studies of ectomycorrhizal partially myco-heterotrophic plants, one would conclude that closely related relatives of fully myco-heterotrophic plants are the best initial candidates for testing whether facultative cheating occurs within the arbuscular mycorrhizal symbiosis.

Finally, there are still many gaps in our understanding of the evolution and ecology of myco-heterotrophic plants. For some myco-heterotrophic plant genera, basic information including distribution, life history, pollination biology, dispersal agents, ecology and taxonomic position is not available. This is mainly due to the fact that species belonging to these

genera appear to be rare and ephemeral. Fieldwork is therefore an inevitable first step towards a better understanding of these remarkable plants. Because most myco-heterotrophic plants grow in threatened forest habitats and *ex-situ* conservation is currently not possible, prompt action should be undertaken to study these plants. Collections of myco-heterotrophic plants should consist of alcohol-preserved material for taxonomic identification, silica-gel-dried material of above-ground parts for DNA extraction, and lysis-buffer- or spirit-preserved root material for the molecular identification of fungi. Dried material of above-ground parts of myco-heterotrophic plants and autotrophic reference plants is necessary for the identification of carbon and nitrogen gains through isotope abundance analysis. In addition, photographs, GPS coordinates and field notes can provide critical information on the ecology of many rare species. These data are essential for the design of realistic experiments to address fundamental questions about mycorrhizal cheating both in the field and in the laboratory.

CONCLUSIONS

Technological developments have enabled significant advances in our understanding of the phylogenetic relationships, fungal symbionts and modes of nutrient acquisition in myco-heterotrophic plants. Clearly, multiple plant lineages have been able independently to shift to one extreme end of the mutualism–parasitism continuum of the mycorrhizal symbiosis in which plants parasitize fungi, thereby revealing the importance and potential of underground fungal networks in plant communities. Major challenges lie ahead for scientific investigation of myco-heterotrophs, their closely related autotrophic species, and their evolutionary, ecological and physiological pathways. There is no doubt that the outcome will lead to more exciting insights, not only in the biology of these enigmatic plants but also in our understanding of mycorrhizal networks in ecosystems.

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LITERATURE CITED

- Bidartondo MI.** 2005. The evolutionary ecology of myco-heterotrophy. *New Phytologist* **167**: 335–352.
- Bidartondo MI, Bruns TD.** 2002. Fine-level mycorrhizal specificity in the Monotropoideae (Ericaceae): specificity for fungal species groups. *Molecular Ecology* **11**: 557–569.
- Bidartondo MI, Read DJ.** 2008. Fungal specificity bottlenecks during orchid germination and development. *Molecular Ecology* **17**: 3707–3716.
- Bidartondo MI, Redecker D, Hijri I, et al.** 2002. Epiparasitic plants specialized on arbuscular mycorrhizal fungi. *Nature* **419**: 389–392.
- Bidartondo MI, Burghardt B, Gebauer G, Bruns TD, Read DJ.** 2004. Changing partners in the dark: isotopic and molecular evidence of ectomycorrhizal liaisons between forest orchids and trees. *Proceedings of the Royal Society B: Biological Sciences* **271**: 1799–1806.

- Bronstein JL. 2001. The exploitation of mutualisms. *Ecology Letters* 4: 277–287.
- Bruns TD, Read DJ. 2000. *In vitro* germination of nonphotosynthetic myco-heterotrophic plants stimulated by fungi isolated from the adult plants. *New Phytologist* 148: 335–342.
- Bruns TD, Bidartondo MI, Taylor DL. 2002. Host specificity in ectomycorrhizal communities: what do the exceptions tell us? *Integrative and Comparative Biology* 42: 352–359.
- Cameron DC, Johnson I, Read DJ, Leake JR. 2008. Giving and receiving: measuring the carbon cost of mycorrhizas in the green orchid, *Goodyera repens*. *New Phytologist* 180: 176–184.
- Cameron KM, Chase MW, Rudall PJ. 2003. Recircumscription of the monocotyledonous family Petrosaviaceae to include *Japanolirion*. *Brittonia* 55: 214–225.
- Dawson TE, Mambelli S, Plamboeck AH, Templer PH, Tu KP. 2002. Stable isotopes in plant ecology. *Annual Reviews in Ecology and Systematics* 33: 507–559.
- Egger KN, Hibbett DS. 2004. The evolutionary implications of exploitation in mycorrhizas. *Canadian Journal of Botany* 82: 1110–1121.
- Fitter AH, Daniell HA, Robinson D. 1999. Resource sharing in plant–fungus communities: did the carbon move for you? *Trends in Ecology and Evolution* 14: 70.
- Francis R, Read DJ. 1984. Direct transfer of carbon between plants connected by vesicular–arbuscular mycorrhizal mycelium. *Nature* 307: 53–56.
- Fry B. 2006. *Stable isotope ecology*, 1st edn. New York: Springer.
- Furness CA, Rudall PJ, Eastman A. 2002. Contributions of pollen and tapetal characters to the systematics of Triuridaceae. *Plant Systematics and Evolution* 235: 209–218.
- Gandolfo MA, Nixon KC, Crepet WL. 2002. Triuridaceae fossil flowers from the Upper Cretaceous of New Jersey. *American Journal of Botany* 89: 1940–1957.
- Gebauer G, Meyer M. 2003. ^{15}N and ^{13}C natural abundance of autotrophic and myco-heterotrophic orchids provides insight into nitrogen and carbon gain from fungal association. *New Phytologist* 160: 209–223.
- Giovannetti M, Sbrana C, Avio L, Strani P. 2004. Patterns of below-ground plant interconnections established by means of arbuscular mycorrhizal networks. *New Phytologist* 164: 175–181.
- Hynson NA, Bruns TD. 2009. Evidence of a myco-heterotroph in the plant family Ericaceae that lacks mycorrhizal specificity. *Proceedings of the Royal Society B: Biological Sciences* 276: 4053–4059.
- Hynson NA, Preiss K, Gebauer G, Bruns TD. 2009. Isotopic evidence of full and partial myco-heterotrophy in the plant tribe Pyroleae (Ericaceae). *New Phytologist* 182: 719–726.
- Jolou T, Burghardt B, Gebauer G, Berveiller D, Damesin C, Selosse M-A. 2005. Mixotrophy in orchids: insights from a comparative study of green individuals and nonphotosynthetic individuals of *Cephalanthera damasanum*. *New Phytologist* 166: 639–653.
- Kamienski F. 1881. Die Vegetationsorgane der *Monotropa hypopitys* L. *Botanische Zeitung* 29: 457–461.
- Leake JR. 1994. The biology of myco-heterotrophic ('saprophytic') plants. *New Phytologist* 127: 171–216.
- Leake JR. 2005. Plants parasitic on fungi: unearthing the fungi in myco-heterotrophs and debunking the 'saprophytic' plant myth. *Mycologist* 19: 113–122.
- Lerat S, Gauci R, Catford JG, Vierheilig H, Piché Y, Lapointe L. 2002. ^{14}C transfer between the spring ephemeral *Erythronium americanum* and sugar maple saplings via arbuscular mycorrhizal fungi in natural stands. *Oecologia* 132: 181–187.
- Lian C, Narimatsu M, Nara K, Hogetsu T. 2006. *Tricholoma matsutake* in a natural *Pinus densiflora* forest: correspondence between above- and below-ground genets, association with multiple host trees and alteration of existing ectomycorrhizal communities. *New Phytologist* 171: 825–836.
- Merckx V, Bidartondo MI. 2008. Breakdown and delayed cospeciation in the arbuscular mycorrhizal mutualism. *Proceedings of the Royal Society B: Biological Sciences* 275: 1029–1035.
- Merckx V, Chatrou LW, Lemaire B, Sainge M, Huysmans S, Smets E. 2008. Diversification of myco-heterotrophic angiosperms: evidence from Burmanniaceae. *BMC Evolutionary Biology* 8: 178.
- Merckx V, Bakker F, Huysmans S, Smets E. 2009. Bias and conflict in phylogenetic inference of myco-heterotrophic plants: a case study in Thismiaceae. *Cladistics* 25: 64–77.
- Nygren CMR, Edqvist J, Elfstrand M, Heller G, Taylor AFS. 2007. Detection of extracellular protease activity in different species and genera of ectomycorrhizal fungi. *Mycorrhiza* 17: 241–248.
- Ogura-Tsujita Y, Gebauer G, Hashimoto T, Umata H, Yukawa T. 2009. Evidence for novel and specialized mycorrhizal parasitism: the orchid *Gastrodia confusa* gains carbon from saprotrophic *Mycena*. *Proceedings of the Royal Society B: Biological Sciences* 276: 761–767.
- Pfeffer P, Douds DD, Bücking H, Schwartz DP, Shachar-Hill Y. 2004. The fungus does not transfer carbon to or between roots in an arbuscular mycorrhizal symbiosis. *New Phytologist* 163: 617–627.
- Robinson D. 2001. $\delta^{15}\text{N}$ as an integrator of the nitrogen cycle. *Trends in Ecology and Evolution* 16: 153–162.
- Sachs JL, Simms EL. 2006. Pathways to mutualism breakdown. *Trends in Ecology and Evolution* 21: 585–592.
- Selosse M-A, Roy M. 2009. Green plants that feed on fungi: facts and questions about mixotrophy. *Trends in Plant Science* 14: 64–70.
- Selosse M-A, Richard F, He X, Simard SW. 2006. Mycorrhizal networks: des liaisons dangereuses? *Trends in Ecology and Evolution* 21: 621–628.
- Simard SW, Perry DA, Jones MD, Myrold DD, Durali DM, Molina R. 1997. Net transfer of carbon between ectomycorrhizal tree species in the field. *Nature* 388: 579–582.
- Smith SE, Read DJ. 2008. *Mycorrhizal symbiosis*, 3rd edn. London: Academic Press.
- Taylor AFS, Fransson PM, Hogberg P, Hogberg MN, Plamboeck AH. 2003. Species level patterns in ^{13}C and ^{15}N abundance of ectomycorrhizal and saprotrophic fungal sporocarps. *New Phytologist* 159: 757–774.
- Tedersoo L, Peller P, Kõljalg U, Selosse M-A. 2007. Parallel evolutionary paths to mycoheterotrophy in understory Ericaceae and Orchidaceae: ecological evidence for mixotrophy in Pyroleae. *Oecologia* 151: 206–217.
- Trudell SA, Rygielwicz PT, Edmonfd RL. 2003. Nitrogen and carbon stable isotope abundances support the myco-heterotrophic nature and host-specificity of certain achlorophyllous plants. *New Phytologist* 160: 391–401.
- Voets L, Goubau I, Olson PA, Merckx R, Declerck S. 2008. Absence of carbon transfer between *Medicago truncatula* plants linked by a mycorrhizal network, demonstrated in an experimental microcosm. *FEMS Microbial Ecology* 65: 350–360.
- Zimmer K, Hynson NA, Gebauer G, Allen EB, Allen MF, Read DJ. 2007. Wide geographical and ecological distribution of nitrogen and carbon gains from fungi in pyrolids and monotropoids (Ericaceae) and in orchids. *New Phytologist* 175: 166–175.
- Zimmer K, Meyer C, Gebauer G. 2008. The ectomycorrhizal specialist orchid *Corallorhiza trifida* is a partial myco-heterotroph. *New Phytologist* 178: 395–400.