

Editorial

Introduction to a *Virtual Special Issue* on mycoheterotrophy: *New Phytologist* sheds light on non-green plants

Mycoheterotrophic plants obtain all of their carbon requirements through symbiotic associations with fungi, and, while achlorophyllous, they are not directly parasitic on other plants. Research into the biology of mycoheterotrophy has a long history in *New Phytologist*, perhaps because of the integration of plant physiology and plant–fungal interactions, two main research fields interesting the audience of *New Phytologist*. Indeed, the word mycoheterotrophy itself was first coined in the journal by Jonathan Leake (1994) in his Tansley Review. This was a founding step in the research on nongreen plants, previously and incorrectly termed ‘saprophytic’ on the assumption that they derived all of their carbon requirements directly from rotting vegetation, a myth still largely propagated in floras to the present day. The story of mycoheterotrophy begins even earlier, as mycoheterotrophic plants have fascinated botanists from the 19th century to the present day. Indeed, Luxford (1841) sparked intense debate in *The Phytologist* (the precursor of *New Phytologist*) as to the trophic strategy employed by *Monotropa hypopitys*, now known to be a mycoheterotroph (see **Leake, 1994** for further discussion).

In the last decade, *New Phytologist* has published 26 out of 123 (21%) of the papers containing the word ‘mycoheterotrophy’ in their title or keywords (source: ISI Web of Knowledge, October 2009). We thus decided to publish a *Virtual Special Issue* of *New Phytologist* (<http://www.newphytologist.com/view/0/virtspecissues.html>), putting together the contributions on mycoheterotrophy published in the journal since 1999, plus the groundbreaking, introductory review paper by **Jonathan Leake (1994)**. Furthermore, we investigate the most recent advances in the field thanks to three short Letters that are published alongside this Editorial. **Nicole Hynson and Tom Bruns (pp. 598–601; this issue)** report on ‘Fungal hosts for mycoheterotrophic plants: a nonexclusive, but highly selective club’, **Jonathan Leake and Duncan Cameron (pp. 601–605; this issue)** discuss

the ‘Physiological ecology of mycoheterotrophy’ and **Vincent Merckx and John Freudenstein (pp. 605–609; this issue)** investigate the ‘Evolution of mycoheterotrophy in plants: a phylogenetic perspective’. In all, these papers reflect the diversity and evolution of the research into mycoheterotrophy published over the past 15 yr. Moreover, these Letters encompass a diverse range of methods that have been employed to shed light on the biology of mycoheterotrophs and their associated fungi, ranging from detailed anatomical studies of the plant–fungus interaction (e.g. **Imhof, 1999; Domínguez et al., 2006**) to physiological methods (**McKendrick et al., 2000a,b; Gebauer & Meyer, 2003; Julou et al., 2005; Cameron et al., 2009**) and molecular methods (see **Bidartondo, 2005; Hynson & Bruns, 2010; Merckx & Freudenstein, 2010**).

Recent advances in our understanding of the mycoheterotrophic symbiosis were tightly linked to the rise of new technical and methodological advances. A major obstacle to the identification of fungal partners in mycoheterotrophic symbioses has been their unculturability *in vitro*, a problem that has been circumvented through the application of molecular methods (e.g. **McKendrick et al., 2000a, 2002; Selosse et al., 2002; Bidartondo et al., 2004**) that have resolved the identity of the fungal partners of many mycoheterotrophic plant species. It turned out that the overwhelming majority of these plants are associated with the mycorrhizal partners of other green plants, such as those surrounding the mycoheterotrophic plant (Selosse et al., 2002). The association often proved to be highly specific with few (in some cases only one) fungal partners, but generalist mycoheterotrophs have also been identified (**Martos et al., 2009; Roy et al., 2009**), raising a number of questions underpinning the *raison d’être* of the contrasting scenarios. The application of natural abundance stable isotope profiles as markers of the origin of the organic matter (**Gebauer & Meyer, 2003; Trudell et al., 2003; Zimmer et al., 2007, 2008**) further substantiated the fungal origin of the carbon and the fact that mycorrhizal fungi, and thus the carbon from nearby autotrophic plants, was often used by mycoheterotrophs. More recently, this method substantiated the use of organic matter by way of association with saprotrophic wood and litter decay fungi (e.g. **Martos et al., 2009**), in orchids at least. Lastly, direct investigations of carbon and water exchanges (**Leake et al., 2004; Julou et al., 2005; Cameron et al., 2009**) also contributed to refining our knowledge of the physiology of mycoheterotrophs. Moreover, radioactive labelling experiments with $^{14}\text{C}\text{O}_2$ provided the first definitive evidence of

Papers included in this *Virtual Special Issue* are indicated by their citations set in bold type.

fungus-to-plant carbon transfer for the orchid *Corallorhiza trifida* (McKendrick *et al.*, 2000b).

Mycoheterotrophic plants have been considered as botanical curiosities and, although they arose repeatedly in plant evolution (Leake, 1994), fully mycoheterotrophic plants are quite rare. Research on these fascinating organisms may, at first glance, appear to represent a disproportionate focus on the minutiae. However, recent research on mycoheterotrophs has revealed they are far from mere botanical curiosities. First, recent evidence has revealed that some green plants are partially mycoheterotrophic (a form of mixotrophy), utilizing carbon gained via both photosynthesis and mycoheterotrophy (Gebauer & Meyer, 2003; Julou *et al.*, 2005; Hynson *et al.*, 2009; reviewed in Selosse & Roy, 2009). Moreover, the extent of this strategy, a probable adaptation to living in dark, understory habitats, deserves further investigation in the many biomes in which mycoheterotrophs are found. Second, many plants may exhibit cryptic mycoheterotrophy; in excess of 10% of plants, from liverworts and ferns (Leake *et al.*, 2008; Winther & Friedman, 2008) to orchids (Leake, 1994), rely on mycoheterotrophy at some point in their life cycle, especially at germination. The true extent of plant dependency on fungal carbon across the kingdom is unclear and, together with ecological implications such as adaptation to living in shade forest or germinating under a dense plant cover, it represents an exciting challenge for future research.

Research on mycoheterotrophs opens numerous other future perspectives (Leake & Cameron, 2010); more research is awaited on physiology and carbon exchange between mycoheterotrophs and fungi, and into the evolution of specificity (and nonspecificity) of association (Gardes, 2002; Taylor, 2004). Mycoheterotrophs offer powerful models for understanding the evolution to achlorophylls to be paralleled with haustorial parasitic plants that feed directly on the vascular system of other plants, given the striking morphological and physiological convergence between these two groups of achlorophyllous plants (Cameron & Leake, 2007; Klimesnová, 2007; Irving & Cameron, 2009; Selosse & Roy, 2009). Many overlooked traits of mycoheterotrophs, such as the evolution of plastid genomes or reproductive biology, could be investigated in a comparative approach.

In the coming years, *New Phytologist* looks forward to welcoming the exciting papers on these and other aspects of research into mycoheterotrophy!

Marc-André Selosse

Editor, *New Phytologist*
(email ma.selosse@wanadoo.fr)

Duncan D. Cameron

Department of Animal and Plant Sciences, University of
Sheffield, Western Bank, Sheffield S10 2TN, UK

References

- Bidartondo MI. 2005. The evolutionary ecology of myco-heterotrophy. *New Phytologist* 167: 335–352.
- 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 of London. Series B, Biological Sciences* 271: 1799–1806.
- Cameron DD, Leake JR. 2007. A different kind of parasitic plant: a brief history of mycoheterotrophy and epi-parasitism. *Haustorium* 50: 4–6.
- Cameron DD, Preiss K, Gebauer G, Read DJ. 2009. The chlorophyll containing orchid *Corallorhiza trifida* derives little carbon through photosynthesis. *New Phytologist* 183: 358–364.
- Domínguez L, Sérsic A, Melville L, Peterson RL. 2006. 'Prepackaged symbioses': propagules on roots of the myco-heterotrophic plant *Arachnitis uniflora*. *New Phytologist* 169: 191–198.
- Gardes M. 2002. An orchid–fungus marriage – physical promiscuity, conflict and cheating. *New Phytologist* 154: 4–7.
- Gebauer G, Meyer M. 2003. ¹⁵N and ¹³C natural abundance of autotrophic and myco-heterotrophic orchids provides insight into nitrogen and carbon gain from fungal association. *New Phytologist* 160: 209–223.
- Hynson N, Bruns TD. 2010. Fungal hosts for mycoheterotrophic plants: a non-exclusive, but highly selective club. *New Phytologist* 185: 598–601.
- 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.
- Imhof S. 1999. Anatomy and mycotrophy of the achlorophyllous *Afrothimia winkleri*. *New Phytologist* 144: 533–540.
- Irving LJ, Cameron DD. 2009. You are what you eat: interactions between root parasitic plants and their hosts. *Advances in Botanical Research* 50: 87–138.
- Julou 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 damasonium*. *New Phytologist* 166: 639–653.
- Klimesnová J. 2007. Root-sprouting in myco-heterotrophic plants: prepackaged symbioses or overcoming meristem limitation? *New Phytologist* 173: 8–10.
- Leake JR. 1994. The biology of myco-heterotrophic ('saprophytic') plants. *New Phytologist* 127: 171–216.
- Leake JR, Cameron DD. 2010. Physiological ecology of mycoheterotrophy. *New Phytologist* 185: 601–605.
- Leake JR, McKendrick SL, Bidartondo MI, Read DJ. 2004. Symbiotic germination and development of the myco-heterotroph *Monotropa hypopitys* in nature and its requirement for locally distributed *Tricholoma* spp. *New Phytologist* 163: 405–423.
- Leake JR, Cameron DD, Beerling DJ. 2008. Fungal fidelity in the myco-heterotroph-to-autotroph life cycle of Lycopodiaceae: a case of parental nurture? *New Phytologist* 177: 572–576.
- Luxford G. 1841. Botanical notes. *Monotropa hypopitys*. *The Phytologist* 3: 43–44.
- Martos F, Dulormne M, Pailler T, Bonfante P, Faccio A, Fournel J, Dubois M-P, Selosse M-A. 2009. Independent recruitment of saprotrophic fungi as mycorrhizal partners by tropical achlorophyllous orchids. *New Phytologist* 184: 668–681.
- McKendrick SL, Leake JR, Taylor DL, Read DJ. 2000a. Symbiotic germination and development of myco-heterotrophic plants in nature: ontogeny of *Corallorhiza trifida* and characterization of its mycorrhizal fungi. *New Phytologist* 145: 523–537.
- McKendrick SL, Leake JR, Taylor DL, Read DJ. 2000b. Symbiotic germination and development of myco-heterotrophic plants in nature: transfer of carbon from ectomycorrhizal *Salix repens* and *Betula pendula* to the orchid *Corallorhiza trifida* through shared hyphal connections. *New Phytologist* 145: 539–548.
- McKendrick SL, Leake JR, Taylor DL, Read DJ. 2002. Symbiotic germination and development of the myco-heterotrophic orchid *Neottia nidus-avis* in nature and its requirement for locally distributed *Sebacina* spp. *New Phytologist* 154: 233–247.

- Merckx V, Freudenstein J. 2010. Evolution of mycoheterotrophy in plants: a phylogenetic perspective. *New Phytologist* 185: 605–609.
- Roy M, Watthana S, Stier A, Richard F, Vessabutr S, Selosse M-A. 2009. Two mycoheterotrophic orchids from Thailand tropical dipterocarpacean forests associate with a broad diversity of ectomycorrhizal fungi. *BMC Biology* 7: 51.
- Selosse M-A, Roy M. 2009. Green plants eating fungi: facts and questions about mixotrophy. *Trends in Plant Sciences* 14: 64–70.
- Selosse M-A, Weiß M, Jany JL, Tillier A. 2002. Communities and populations of sebacinoid basidiomycetes associated with the achlorophyllous orchid *Neottia nidus-avis* (L.) L.C.M. Rich. and neighbouring tree ectomycorrhizae. *Molecular Ecology* 11: 1831–1844.
- Taylor DL. 2004. Myco-heterotroph–fungus marriages – is fidelity overrated? *New Phytologist* 163: 217–221.
- Trudell SA, Rygielwicz PT, Edmonds 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.
- Winther JL, Friedman WE. 2008. Arbuscular mycorrhizal associations in Lycopodiaceae. *New Phytologist* 177: 790–801.
- 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 pyrolroids 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.

Key words: (partial) mycoheterotrophy, carbon, epi-parasitism, fungi, mixotrophy, mycorrhiza, stable isotope.

Commentary

A novel lipid signal in the arbuscular mycorrhizal symbiosis within eyesight?

Plants grow in the company of myriads of other organisms: many are direct or indirect competitors, or even pathogenic, others can be beneficial. To differentiate between friend and foe is therefore essential for a plant's survival. Chemical signalling often precedes and enables consideration as to whether a foreign organism is a friend. Plant cells detect extracellular chemical cues often before physical contact, which when perceived, trigger the generation of secondary signals and downstream events. Decoding this complexity subsequently leads to the plant's response enabling a friendly relationship. In the arbuscular mycorrhizal symbiosis (AMS) of most terrestrial plants with soil fungi of the Glomeromycota, presymbiosis (i.e. the chemical phase before physical interaction) is prerequisite for reprogramming plant cells towards accommodation of the microbial symbiont. In this issue of *New Phytologist*, Kuhn *et al.* (pp. 716–733) report new results suggesting that steroids may act as early arbuscular mycorrhizal (AM) signals.

'...steroids could indeed play an important role in many aspects of AMS including hyphal penetration and arbuscule formation. Steroids would then join the club of lipids as mycorrhizal signals ...'

Epidermal responses and cellular accommodation

Presymbiosis is a prerequisite for hyphopodium (or appressorium) formation, which is the first morphological element in the AMS. The formation of an AM fungal hyphopodium on host roots is probably induced upon contact through thigmotropic signals specifically from the host rhizodermal cell wall (Giovannetti *et al.*, 1993; Nagahashi & Douds, 1997); it cannot be induced on artificial surfaces or on nonhost roots. How do AM fungi subsequently penetrate root cells? There are two options. Penetration of the epidermal layer has been shown to occur via opening of a rhizodermal cleft between two cells through which the fungus enters (Parniske, 2004). Alternatively, the AM fungus can cross epidermal cells directly through a tunnel-like intracellular structure that forms subsequent to hyphopodium formation and before fungal penetration. The tunnel, named the prepenetration apparatus (PPA) (Genre *et al.*, 2005), is formed as a result of massive membrane restructuring and biosynthesis throughout hyphal colonization and arbuscule formation in roots (Genre *et al.*, 2008). Kuhn *et al.* used an elegant approach to identify marker genes for the early developmental phase in the AMS of *Medicago truncatula*, a widely used model species for root symbiosis research. An *in vitro* system allowed harvesting of root segments containing contact points between fungus and host plants or even developing hyphopodia. Gene expression profiling using RNA extracted from these segments yielded, among others, a candidate gene encoding the membrane-localized steroid-binding protein 1 (MtMSBP1). The results further indicated that MtMSBP1 gene expression was stimulated by a diffusible fungal signal(s). This work agrees with that of other groups indicating that perception of diffusible fungal signals precedes the transcriptional cellular reprogramming of host epidermal cells.

The enigmatic 'Myc factor'

These attempts, destined to identify soluble compounds involved in early signal perception mechanisms during AMS establishment, postulate the existence of a 'Myc factor', an AM fungal signal analogous to the rhizobial Nod factor that induces molecular responses in the host root. After many years, its chemical nature is still enigmatic. Kosuta *et al.* (2003) demonstrated that the symbiosis-specific *M. truncatula* early nodulin ENOD 11 (MtENOD11) gene is induced in the roots in absence of physical contact between both symbiotic partners. Overall, evidence was provided for a diffusible AM fungus-specific signal of < 3500 Da (Chabaud *et al.*, 2002). The Bonfante laboratory later showed that spores of *Gigaspora margarita*, as well as of two *Glomus* isolates, released diffusible molecules into the culture medium, which were perceived by plant roots via Ca²⁺-mediated signalling (Navazio *et al.*, 2007). The fungal molecules were found to be thermostable, to have a molecular mass of > 3000 Da and to be amphiphilic. Moreover, a diffusible AM fungal factor from *Gigaspora* and *Glomus* species was found to stimulate lateral root formation in *M. truncatula* (Olah *et al.*, 2005). It is unknown whether the *MtENOD11* promoter-activating factor, the described fungal spore signal, the diffusible AM fungal factor and the *MtMSBP1* gene-inducing factor are the same, or different, 'Myc factors'. It is certainly of outstanding interest to characterize the chemical nature of these factors and underlying processes.

Biosynthesis and transport of plant steroids

Steroids consist of a sterane core (saturated tetracyclic hydrocarbon 1,2-cyclopentanoperhydrophenanthrene), which is a carbon structure of four fused rings, three cyclohexane rings and one cyclopentane ring that are partially or completely hydrogenated. This core is generally substituted by additional functional groups at distinct C positions (Fig. 1). Natural steroids are derived from squalene and may thus be considered as modified triterpenes.

Knowledge of steroid biosynthesis is based upon groundbreaking discoveries made by the three Nobel laureates, Otto Wallach (1910), Leopold Ruzicka (1939) and Feodor Lynen (1964). Steroid biosynthesis is an anabolic metabolic pathway that produces steroids from isopentenylpyrophosphate, which is generated in plants via two different isoprenoid (or terpenoid) biosynthetic pathways: (1) with two molecules of acetyl-CoA as the original substrate in the cytosol or (2) with pyruvate and glyceraldehyde-3-phosphate in the plastids. Steroids are made from two molecules of farnesyl pyrophosphate forming the triterpene molecule squalene (C₃₀). In plants, steroids are basic products for membranes and defense compounds; they are also recognized as essential hormones in plants as well as in animals.

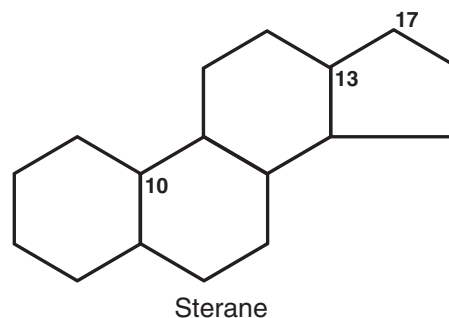


Fig. 1 Chemical diversity within steroids. The sterane core is generally substituted by functional groups attached to the four rings, that is, methyl groups at positions C10 and C13. A ketone or hydroxyl-group or an alkyl side-chain may also be present at C17.

Among numerous plant steroids, brassinolide (BL) is the most active form of the known growth-regulating brassinosteroids (BRs). The structure of BL was determined by Grove *et al.* (1979) and showed similarity to animal steroid hormones. As a result of its low concentration, the identification of BL took 10 yr of dedicated work (Mandava, 1988). MSBP1 from *Arabidopsis* is involved in the inhibition of cell elongation (Yang *et al.*, 2005) possibly through affecting vesicle trafficking and auxin distribution (Yang *et al.*, 2008). MSBP1 was shown to bind to multiple steroid molecules, including BR, with different affinities (Yang *et al.*, 2005). Sterols (i.e. steroid alcohols) of AM fungal origin are used as biochemical markers to determine the abundance of AM fungi and AM fungal biomass (see Olsson *et al.*, 2003). Only recently, the first gene involved in the steroid biosynthetic pathway, so far described from an AM fungus has been described (Oger *et al.*, 2009). Overall, the knowledge on steroid biosynthetic pathways and the complexity of steroid chemical diversity, in mycorrhizal roots, is rather poor (Campagnac *et al.*, 2008; Oger *et al.*, 2009).

It is uncertain whether steroids can act as diffusible 'Myc factors'. Steroids are relatively nonpolar molecules. Thus, whilst short-distance intracellular or intercellular transport of steroids appears possible, it seems unlikely that it occurs through the simple passive diffusion of free bioactive steroids within and between cells. As a consequence, it is likely that short-distance steroid transport involves carrier mechanisms, which allows these molecules to move through the diverse cellular environments of a mycorrhiza. Kuhn *et al.* subcellularly localized the MtMSBP1 protein to the plant endoplasmic reticulum (ER) where steroids may be produced on membranes then enter the cytosol and move to the plasma membrane, cross the plasma membrane and enter the extracellular environment, where they are perceived at the surface of surrounding cells. Along this scenario, steroids, instead of being an extracellular fungal signal, could be of plant origin and act as secondary signals. Indeed, membrane-bound, ATP-binding cassette (ABC)

transporters facilitate the movement of steroids out of animal cells, and it is possible that homologous mechanisms may exist in plants (Young & Fielding, 1999).

Provided that the function of MtMSBP1 follows sequence homology, a striking mycorrhizal phenotype, demonstrated in Kuhn *et al.* as a result of an *MtMSBP1* gene knockdown in *M. truncatula*, indicated that steroids could indeed play an important role in many aspects of the AMS, including hyphal penetration and arbuscule formation. Steroids would then join the club of lipids as mycorrhizal signals, namely the lysolipid lysophosphatidylcholine (LPC) originating from phosphatidylcholine, a common lipid in eukaryotic membranes. Lysophosphatidylcholine was shown to trigger phosphate transporter gene expression in a mycorrhiza-specific manner (Drissner *et al.*, 2007).

Life requires membranes, and AMS may require lipid signals. These signals could be generated whilst membrane invaginations during PPA formation early, and arbuscule development late, occur in AM development. They would thus pave the way towards symbiotic exchange of goods in this friendly partnership. Overall, the broadening horizon of lipid research in AMS is opening up exciting vistas for the future.

Marcel Bucher

University of Cologne, Institute of Botany,
Centre for Biosciences, Otto-Fischer-Strasse 6
50674 Cologne, Germany
(tel +49 (0)221 4702481; email m.bucher@uni-koeln.de)

References

- Campagnac E, Fontaine J, Sahraoui AL-H, Laruelle F, Durand R, Grandmougin-Ferjani A. 2008. Differential effects of fenpropimorph and fenhexamid, two sterol biosynthesis inhibitor fungicides, on arbuscular mycorrhizal development and sterol metabolism in carrot roots. *Phytochemistry* **69**: 2912–2919.
- Chabaud M, Venard C, Defaux-Petras A, Becard G, Barker DG. 2002. Targeted inoculation of *Medicago truncatula* *in vitro* root cultures reveals *mtnod11* expression during early stages of infection by arbuscular mycorrhizal fungi. *New Phytologist* **156**: 265–273.
- Drissner D, Kunze G, Callewaert N, Gehrig P, Tamasloukht MB, Boller T, Felix G, Amrhein N, Bucher M. 2007. Lyso-phosphatidylcholine is a signal in the arbuscular mycorrhizal symbiosis. *Science* **318**: 265–268.
- Genre A, Chabaud M, Timmers T, Bonfante P, Barker DG. 2005. Arbuscular mycorrhizal fungi elicit a novel intracellular apparatus in *Medicago truncatula* root epidermal cells before infection. *Plant Cell* **17**: 3489–3499.
- Genre A, Chabaud M, Faccio A, Barker DG, Bonfante P. 2008. Prepenetration apparatus assembly precedes and predicts the colonization patterns of arbuscular mycorrhizal fungi within the root cortex of both *Medicago truncatula* and *Daucus carota*. *Plant Cell* **20**: 1407–1420.
- Giovannetti M, Avio L, Sbrana C, Citernesi AS. 1993. Factors affecting appressorium development in the vesicular–arbuscular mycorrhizal fungus *Glomus mosseae* (Nicol & Gerd.) Gerd. & Trappe. *New Phytologist* **123**: 115–122.
- Grove MD, Spencer GF, Rohwedder WK, Mandava N, Worley JF, Warthen JD, Steffens GL, Flippen-Anderson JL, Cook JC. 1979. Brassinolide, a plant growth-promoting steroid isolated from *Brassica napus* pollen. *Nature* **281**: 216–217.
- Kosuta S, Chabaud M, Lougnon G, Gough C, Denarie J, Barker DG, Becard G. 2003. A diffusible factor from arbuscular mycorrhizal fungi induces symbiosis-specific *mtnod11* expression in roots of *Medicago truncatula*. *Plant Physiology* **131**: 952–962.
- Kuhn H, Küster H, Requena N. 2010. Membrane steroid binding protein 1 induced by a diffusible fungal signal is critical for mycorrhization in *Medicago truncatula*. *New Phytologist* **185**: 716–733.
- Mandava NB. 1988. Plant growth-promoting brassinosteroids. *Annual Review of Plant Physiology and Plant Molecular Biology* **39**: 23–52.
- Nagahashi G, Douds DD. 1997. Appressorium formation by am fungi on isolated cell walls of carrot roots. *New Phytologist* **136**: 299–304.
- Navazio L, Moscaticello R, Genre A, Novero M, Baldan B, Bonfante P, Mariani P. 2007. A diffusible signal from arbuscular mycorrhizal fungi elicits a transient cytosolic calcium elevation in host plant cells. *Plant Physiology* **144**: 673–681.
- Oger E, Ghignone S, Campagnac E, Fontaine J, Grandmougin-Ferjani A, Lanfranco L. 2009. Functional characterization of a c-4 sterol methyl oxidase from the endomycorrhizal fungus *Glomus intraradices*. *Fungal Genetics and Biology* **46**: 486–495.
- Olah B, Briere C, Bécard G, Denarie J, Gough C. 2005. Nod factors and a diffusible factor from arbuscular mycorrhizal fungi stimulate lateral root formation in *Medicago truncatula* via the *dmi1/dmi2* signalling pathway. *Plant Journal* **44**: 195–207.
- Olsson PA, Larsson L, Bago B, Wallander H, Aarle IMV. 2003. Ergosterol and fatty acids for biomass estimation of mycorrhizal fungi. *New Phytologist* **159**: 7–10.
- Parniske M. 2004. Molecular genetics of the arbuscular mycorrhizal symbiosis. *Current Opinion in Plant Biology* **7**: 414–421.
- Yang X-H, Xu Z-H, Xue H-W. 2005. Arabidopsis membrane steroid binding protein 1 is involved in inhibition of cell elongation. *Plant Cell* **17**: 116–131.
- Yang X, Song L, Xue HW. 2008. Membrane steroid binding protein 1 (*msbp1*) stimulates tropism by regulating vesicle trafficking and auxin redistribution. *Molecular Plant* **1**: 1077–1087.
- Young SG, Fielding CJ. 1999. The ABCs of cholesterol efflux. *Nature Genetics* **22**: 316–318.

Key words: arbuscular mycorrhiza, chemical signalling, Myc factor, plant steroids, symbiosis.

Changing juvenile growth patterns in tropical trees: selective effects, history, or both?

A tree seed germinates in a forest and begins to grow. Given the right combination of environmental conditions, and a bit of luck, the seedling might one day become a fully grown tree. The quiet process of seed dispersal, germination and establishment occurs trillions of times each year across the world's forests, but only a vanishingly small fraction of seedlings ever survive to become adult trees. Because individual germination events occur on such large numerical

and spatial scales, and because trees are long-lived, understanding what makes some seedlings successful (and most not), may have important ramifications for regional and global-scale carbon cycling for decades or centuries to come. Several studies have demonstrated increased growth and biomass accumulation in tropical and temperate forests in recent decades and have suggested that these changes may be caused by increased atmospheric CO₂ concentrations (e.g. Phillips *et al.*, 1998; Johnson & Abrams, 2009). The focus, however, has been squarely on mature trees (usually > 10 cm diameter at breast height (dbh)). Long-term trends in the early growth rates of trees are poorly documented. This represents a double-barrelled gap in our knowledge because early growth rates determine both the composition and future structure of the forest (and therefore future carbon storage potential). In large part, this problem is caused by methodological challenges. Evaluating temporal variability in the early growth of trees is a tricky business. On the one hand, longitudinal studies that directly measure seedling growth are typically short (i.e. years to (rarely) decades) relative to the potential life span of the trees. On the other hand, retrospective studies based on tree-ring analyses, which estimate early growth of trees that were established decades or centuries in the past, represent a biased sample – the trees that survived to the present. Conducted independently, neither provides a complete picture of long-term variability in early growth rates.

'In tropical forests, where few tree species have annual growth rings, we know little of long-term forest dynamics and disturbance history.'

In this issue of *New Phytologist*, Rozendaal *et al.* (pp. 759–769) directly address this knowledge gap. They ask the question: is the early growth of trees that have recently established in a Neotropical forest different from the early growth of trees that established decades or centuries earlier in the same forest? They build on recent work by Landis & Peart (2005) in the northern temperate zone, in which observational studies of current tree growth were combined with a retrospective tree-ring analysis approach to compare early growth rates between extant seedlings and adult trees for species of differing shade tolerance. Landis & Peart hypothesized that early growth rates in shade-intolerant species would be higher in the adults because only the fastest growing seedlings and saplings would survive the early per-

iod of stand development (i.e. the juvenile selection effect). And, this is precisely what they found. By contrast, they expected that the early growth of adults of shade-tolerant species would not differ from the growth of the current seedling and sapling crop, as fast growth was not a prerequisite for success among species that could survive in shady conditions. However, to their surprise, they found that the slow-growing, shade-tolerant species also showed a strong juvenile selection effect.

Rozendaal *et al.* have modified Landis & Peart's (2005) approach and applied it to a suite of tropical tree species growing in Bolivia. This is not a trivial undertaking. In temperate forests, there is a long history of using tree-rings to reconstruct stand development patterns and assess the impacts of past disturbances (Oliver & Larson, 1996). In tropical forests, where few tree species have annual growth rings, we know little of long-term forest dynamics and disturbance history. That is beginning to change, however. Several recent studies have demonstrated the potential for successful dendroecological reconstructions in tropical forests in Asia (Baker *et al.*, 2005; Buckley *et al.*, 2007; Sano *et al.*, 2009), Africa (Worbes *et al.*, 2003) and the Americas (Brienen & Zuidema, 2006). While these studies have all been conducted in seasonally dry forests with many fewer species than their hyper-diverse aseasonal analogues, they provide new – and important – insights into the temporal modes of change in species-rich tropical forest communities. They have, however, focused primarily on adult trees. Rozendaal *et al.* have lowered their gaze to the small trees and examined growth patterns during the critical period of establishment. They took advantage of several planned selective logging coupes in forests in northeastern Bolivia to collect stem cross-sections from individuals across the full range of size classes for five species differing in shade tolerance. From each section they calculated the total cross-sectional area of new wood added each year across a range of size classes. So, for example, they calculated how fast an individual was growing when it was 1–2 cm dbh, 2–3 cm dbh, etc. An important advantage of the approach of Rozendaal *et al.* is that it provides insight into not only growth patterns at the earliest ontogenetic stages of trees of different ages, but across the entire developmental history of these trees. And, this, it turns out, is where things get interesting...

Unlike Landis & Peart (2005), Rozendaal *et al.* found that the juvenile selection effect was not ubiquitous. Some species showed evidence of it, whereas others did not. Surprisingly, the most shade-intolerant species, *Cedrela odorata*, which was expected to exhibit the strongest juvenile selection effect, did not show any evidence of faster early growth in the older trees – its growth seemed independent of tree size and age. By contrast, *Cedrelinga catenaeformis*, the next most shade-intolerant species, showed strong juvenile selection effects across nearly all size classes. The three shade-tolerant species, *Clarisia racemosa*, *Peltogyne* cf. *heterophylla*

and *Pseudolmedia laevis*, showed the most curious pattern, however. In the smallest size class (0–1 cm dbh), early growth rates were higher in older trees (although not significantly so for *Pseudolmedia*). But, from 1 to 10 cm dbh, all three species showed evidence of historical growth increases; that is, saplings and poles were growing faster now than they had in the past.

The results of Rozendaal *et al.* raise two interesting questions. First, why does an extremely shade-intolerant tree species not show evidence of a juvenile selection effect? Second, why would older trees of relatively shade-tolerant species show faster growth (relative to more recent recruits) in the smallest size class and then slower growth for much of the rest of their youth? The first question may simply reflect the extreme high growth of the *Cedrela* – in the 0–1 cm dbh size class, its basal area growth was two (!) orders of magnitude greater than that of the shade-tolerant species. Any juvenile selection effects may have already played out in an earlier ontogenetic stage. However, the second question is the more intriguing. While CO₂ fertilization would be the facile explanation, Rozendaal *et al.* are appropriately cautious in pushing that link. They provide several other suggestions to explain the observed pattern. In particular, they note that there is no evidence of large disturbances at their sites in the tree-ring data. The observed pattern suggests a more subtle change in the underlying disturbance regime, though. All of the significant differences in growth (both positive and negative) have occurred in relatively small size classes (< 10 cm dbh), which is indicative of small-scale, rather than large-scale, disturbances. In the wake of small-scale disturbances (such as treefall gaps), shade-tolerant trees often establish contemporaneously with shade-intolerant trees, growing quickly for a brief period before being overtopped. Oliver Phillips and colleagues have documented greater rates of biomass turnover (and therefore small gap formation) in Neotropical forests in recent decades as a result of higher rates of mortality and recruitment (Phillips & Gentry, 1994; Phillips *et al.*, 1998). In principle, this should lead to more gaps in the forest canopy (relative to the past), but those gaps should be smaller. If the gaps are smaller, then the earliest growth of new recruits should be slower than in the past, when less frequent, but larger, canopy gaps would have formed. However, an increase in biomass turnover and gap formation in modern times would also lead to higher light intensities in the subcanopy, which would favour growth of established saplings and poles (e.g. 1–10 cm dbh). The result would be the observed historical increases in growth.

Like all good research, the study of Rozendaal *et al.* raises more questions than it answers. Two obvious sets of questions stand out. First, are historical growth changes nonlinear? Is there evidence of threshold events or baseline shifts in growth? Second, are these historical growth changes

occurring elsewhere? In a recent study of adult individuals of eight tree species from the eastern USA, Johnson & Abrams (2009) showed that younger trees are growing faster at a given size than older trees did at the same size (i.e. historical growth increase) and that this was consistent across all levels of shade tolerance. These results provide an interesting counterpoint to Rozendaal *et al.*'s study by showing that larger trees are capable of benefitting from changes to growing conditions. Clearly, there is a need for a broader survey of comparative dendroecological studies in which past growth patterns are examined in the light of current growth dynamics. A key point from this study is that placing current processes into their historical context is necessary for understanding the organisms and the communities in which they grow. Such studies provide useful insights into long-term variability in tree growth, the relative contributions of disturbance regimes and climate variability on moderating this variability, and potential avenues for future growth.

Patrick J. Baker

Australian Centre for Biodiversity & School of Biological Sciences, Monash University, Vic. 3800, Clayton, Australia
(tel +61 3 9905 0508;
email patrick.baker@sci.monash.edu.au)

References

- Baker PJ, Bunyavechewin S, Oliver CD, Ashton PS. 2005. Disturbance history and historical stand dynamics of a seasonal tropical forest in western Thailand. *Ecological Monographs* 75: 317–343.
- Brienen RJW, Zuidema PA. 2006. Lifetime growth patterns and ages of Bolivian rain forest trees obtained by tree-ring analysis. *Journal of Ecology* 94: 481–493.
- Buckley BM, Palakit K, Duangsathaporn K, Sanguantham P, Prasomsin P. 2007. Decadal scale droughts over northwestern Thailand over the past 448 years: links to the tropical Pacific and Indian Ocean sectors. *Climate Dynamics* 29: 63–71.
- Johnson SE, Abrams MD. 2009. Age class, longevity, and growth rate relationships: protracted growth increases in old trees in the eastern United States. *Tree Physiology* 29: 1317–1328.
- Landis RM, Peart DR. 2005. Early performance predicts canopy attainment across life histories in subalpine forest trees. *Ecology* 86: 63–72.
- Oliver CD, Larson BC. 1996. *Forest stand dynamics*. New York, NY, USA: John Wiley and Sons, Inc.
- Phillips OL, Gentry AH. 1994. Increasing turnover through time in tropical forests. *Science* 263: 954–958.
- Phillips OL, Malhi Y, Higuchi N, Laurance WF, Nunez PV, Vasquez RM, Laurance SG, Ferreira LV, Stern M, Brown S *et al.* 1998. Changes in the carbon balance of tropical forests: evidence from long-term plots. *Science* 282: 439–442.
- Rozendaal DMA, Brienen RJW, Soliz-Gamboa CC, Zuidema PA. 2010. Tropical tree-rings reveal preferential survival of fast-growing juveniles and increased juvenile growth rates over time. *New Phytologist* 185: 759–769.
- Sano M, Buckley BM, Sweda T. 2009. Tree-ring based hydroclimate reconstruction over northern Vietnam from *Fokienia hoginsii*: eighteenth century mega-drought and tropical Pacific influence. *Climate Dynamics* 33: 331–340.

Worbes M, Staschel R, Roloff A, Junk WJ. 2003. Tree ring analysis reveals age structure, dynamics and wood production of a natural forest stand in Cameroon. *Forest Ecology and Management* 173: 105–123.

Key words: CO₂ fertilization, recruitment, stand dynamics, tree-rings, tropical forests.

Letters

Fungal hosts for mycoheterotrophic plants: a nonexclusive, but highly selective club

In nature there are numerous examples of cheaters that subvert a normally mutualistic interaction with a symbiotic partner (Bronstein, 2001). The majority of mycoheterotrophic (MH) plants studied thus far cheat one of the most widespread mutualisms on earth – the mycorrhizal symbiosis. Because the mycorrhizal symbiosis has evolved in three major lineages of the fungal kingdom (Fig. 1), it is no surprise that each of these diverse groups of fungi has been infiltrated by MH plants (Fig. 1). In fact, there are only two orders within the Glomeromycota – a group of obligate plant symbionts and one class of Ascomycota that contains some mycorrhizal fungi – that have not (yet!) been found to associate with mycoheterotrophs (Fig. 1). In addition to MH plants that have cheated the mycorrhizal mutualism, there are also examples of MH plants which gain carbon directly from saprotrophic fungi that break down and assimilate complex organic substrates.

Of the three major fungal lineages that have been exploited by MH plants, the Glomeromycota is the most ancient mycorrhizal group and supports the greatest number of MH species (Leake, 1994). These fungi form arbuscular mycorrhizae (AM), the most common mycorrhizal association of plants in general (Smith & Read, 2008). Interestingly, the oldest group of mycorrhizal fungi also support perhaps some of the oldest lineages of MH plants, such as club mosses, ferns and whisk ferns, many of which have fully MH gametophytes (Winther & Friedman, 2007, 2008, 2009; Leake *et al.*, 2008). Arbuscular mycorrhizal MH plants studied thus far have been found to associate mainly with fungi in the ‘*Glomus* A’ clade. However, it remains unknown if fungi in this group are also dominant mycobionts of autotrophic plants, in which case the apparent preference of MH plants for these fungi would not be unexpected.

Within the Agaricomycetes, all orders that contain mycorrhizal fungi have been exploited by MH plants (Fig. 1). In particular, fungi that normally form mutualistic ectomycorrhizae (EM) with many tree species have been repeatedly taken advantage of by MH species in the family Orchidaceae and in the subfamily Monotropoideae (Ericaceae; Taylor *et al.*, 2002; Bidartondo, 2005; Fig. 1, Supporting Information Table S1). Owing to the diversity of fungal guilds that associate with mycoheterotrophs (Fig. 1, Table S1) it does not appear that any particular lineage of mycorrhizal fungi are more prone to be cheated than any other, and the ability of mycorrhizal fungi to provide sufficient carbon transfer from autotrophic plants to MH plants is phylogenetically widespread (Leake & Cameron, 2010).

The *Hymenoscyphus ericae* complex is perhaps the most widespread example of a mycorrhizal lineage that has not yet been found to host MH plants. This exception is striking, for two reasons. First, fungi within the *H. ericae* complex are abundant mycorrhizal symbionts with members of Ericaceae across huge areas of the globe (Smith & Read, 2008), and there is now mounting evidence that the plant partners of these fungi are not limited to Ericaceae, and can form mycorrhizae with some tree species (Curlevski *et al.*, 2009; Grelet *et al.*, 2009, and references therein). Second, even though none of the ericaceous plants that associate strictly with fungi from the *H. ericae* complex are known to be mycoheterotrophic, the majority of species in the closely related and potentially ancestral group Monotropoideae are fully mycoheterotrophic.

An apparently parallel evolutionary path to mycoheterotrophy has arisen among nonphotosynthetic orchids in the tribes Diseae, Gastrodieae and Vanilleae, which associate strictly with normally saprotrophic fungi (Table S1). To date, MH orchids associated with saprotrophic fungi have been found in multiple countries, including tropical forests of the Caribbean, Taiwan, Myanmar, India and Australia as well as in more temperate regions of Japan and Korea. Similar to MH plants that associate with mycorrhizal fungi, those that depend on saprotrophic fungi usually show a preference for particular fungal hosts (Yamato *et al.*, 2005; Ogura-Tsujita & Yukawa, 2008; Ogura-Tsujita *et al.*, 2008; Martos *et al.*, 2009). So far there is no evidence of

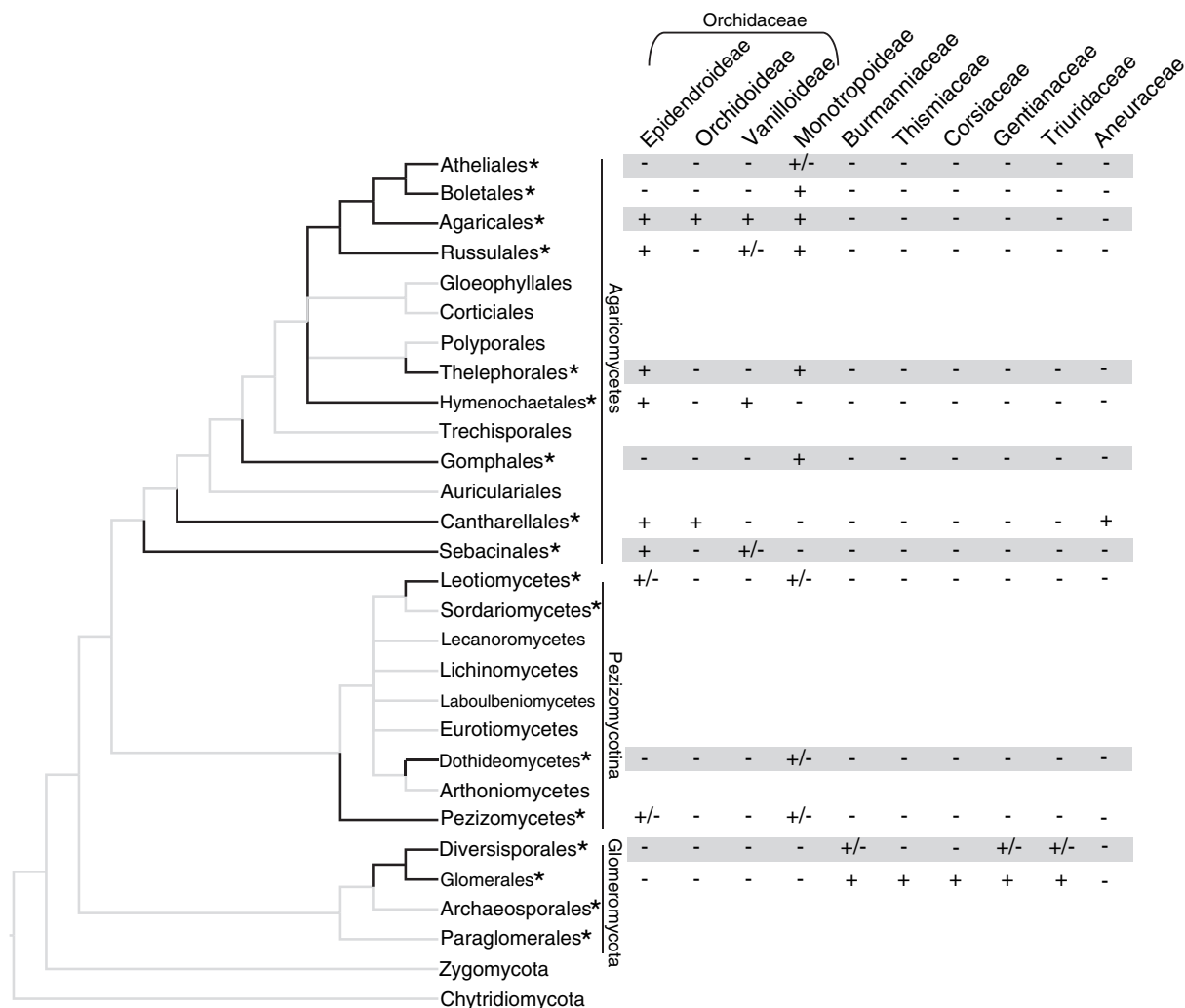


Fig. 1 A trimmed phylogeny of the kingdom Fungi, showing the three main fungal groups that have been found to host mycoheterotrophic plants – the Agaricomycetes, the ascomycetous Pezizomycotina and the Glomeromycota. Black branches represent the specific fungal lineages that associate with one or more groups of mycoheterotrophic plants; asterisks indicate fungal groups known to contain mycorrhizal fungi. Listed across the top are the plant families or subfamilies in the case of the orchids and monotropes (Ericaceae) that have evolved mycoheterotrophy. Each column indicates the fungi that either fungal specialist (+) or nonspecialist (+/-) mycoheterotrophic species within the listed plant groups have, or have not (-) partnered with. Only plant lineages that contain species which are mycoheterotrophic throughout their life cycle (full mycoheterotrophy) have been included in this figure. References for this figure can be found in the Supporting Information Table S1.

MH plants outside Orchidaceae that have exploited saprotrophic fungi in this way; however, there remain many unstudied groups of MH plants, especially in the tropics.

Within this phylogenetically and physiologically widespread set of fungal hosts of MH plants is nested another pattern – specificity; with few exceptions, individual MH plants are specialized on single families, genera, or even species of fungi (Taylor *et al.*, 2002; Bidartondo, 2005). Sometimes what appear to be less specific patterns of fungal preferences are caused by the existence of cryptic species of MH plants that each associate with certain fungal taxa (Taylor *et al.*, 2004). Two recent studies of MH orchids in the tropics appear to be exceptions to the specificity rule. In the study by Roy *et al.* (2009), two species of MH orchids

in the tribe Neottieae were found to associate with multiple EM fungi, and Martos *et al.* (2009) found two species of MH orchids in the tribes Orchidoideae and Epidendroideae that associate with various litter-decaying or wood-decaying basidiomycetes. Another example of a generalist MH species outside the Orchidaceae is *Pyrola aphylla* (Ericaceae), which has recently been found to associate with numerous EM fungi throughout the plant's distribution in western USA (Hynson & Bruns, 2009).

The factors leading to specificity among MH plants remain unclear, but there are emerging data that may shed some light on the role of specificity. For instance, Julou *et al.* (2005) and others have proposed that for a plant to transition from a primarily autotrophic to a MH lifestyle

there must first be a shift in the fungal associates of the plant, then selection of a specific fungal host, followed by the loss of photosynthesis and a transition to full mycoheterotrophy (Bidartondo *et al.*, 2004). In Julou *et al.*'s (2005) study, this first step was exemplified by green orchids that have shifted from associating with *Rhizoctonia* species, a polyphyletic genus of fungi that are common symbionts of most orchids, to a suite of EM fungi. They also found that albino mutants of these normally green orchids associated with a diversity of EM fungi, and, based on the albino orchids' carbon and nitrogen stable isotope values, they appeared to be fully mycoheterotrophic (Leake & Cameron, 2010). This, along with the recent findings of Hynson & Bruns (2009) and Roy *et al.* (2009), indicate that the loss of photosynthesis in mycoheterotrophs is not strictly contingent on fungal specialization, and apparently the ordering of steps towards the evolution of MH plants is not fixed.

Two obvious questions remain: why specialize? And what plant–fungal interactions determine the fungal host of adult mycoheterotrophs? Similar to many parasites, fungal specificity among mycoheterotrophs may be favored so that the plant can fine-tune its physiology to maximize the benefits from its fungal host over the course of its life cycle (Leake & Cameron, 2010). The cost of this fine-tuning to the mycoheterotroph may be that it prevents broad host jumps and severely limits the expansion of its distribution, while the cost to the fungal host remains unknown (Bidartondo, 2005). An alternative and perhaps not mutually exclusive explanation for why most adult MH plants have specific fungal partners may be that the plant has been rejected by the pool of available fungi present at a site and is thus engaged in a co-evolutionary arms race with fungi (Taylor, 2004; Bidartondo, 2005). However, the rarity of MH plants in most settings makes it seem unlikely that they would create much selective pressure on fungi. In addition, if an arms race were the primary factor that determines the fungal host of mycoheterotrophs, then one would expect to find more examples of host switches in distant plant populations or where there are encounters with a 'naïve' fungus, such as after a long-distance dispersal event (Bidartondo & Bruns, 2005). This type of host switching was demonstrated by Bidartondo & Bruns (2005) among *Monotropa uniflora* individuals, but the switches were only to fungal congeners.

An arms race model might also be expected to lead to cladogenesis, where the phylogenies of MH plants and their fungal hosts would become concordant over evolutionary time. Merckx & Bidartondo (2008) documented the best example of such phylogenetic concordance but with an interesting, and incongruent, twist. The phylogeny of MH plants in the genus *Afrothismia* (Thismiaceae) track the phylogeny of their fungal hosts in the *Glomus* A clade fairly well. However, molecular clock estimates show that this tracking could not have occurred as a result of co-speciation, because the diversification in the *Glomus* A clade took place 70 million

yr before the existence of *Afrothismia* (Merckx & Bidartondo, 2008). This means that even though *Afrothismia* speciation tracked the phylogeny of *Glomus* taxa, *Glomus* taxon speciation was unrelated to that of *Afrothismia* species!

Before we bury the arms race model, however, we need to consider how MH plants interact with close relatives of their fungal hosts. Several studies have shown that seeds of MH plants germinate in response to their specific fungal hosts, but they will also germinate in response to closely related fungi (McKendrick *et al.*, 2000; Leake *et al.*, 2004; Bidartondo & Bruns, 2005). These 'mistakes' occur in the field, and in some cases seedling development starts, yet no mature plants associated with the wrong fungus have been found (McKendrick *et al.*, 2000; Leake *et al.*, 2004; Bidartondo & Bruns, 2005). This means that at some stage of development the plants associated with the wrong fungi either die or manage to switch to their correct symbiont. Death seems to be the more likely possibility, but the question remains of whether this is a plant response that is initiated by the fungus, supporting the arms race model, or simply a physiological mismatch. It may soon be possible to differentiate between these two possibilities by examining fungal gene expression when associated with compatible and incompatible MH plants.

There remain numerous plant lineages that contain fully, and potentially facultative, MH species that have yet to be studied in detail, especially in the tropics. Many recent studies have revealed new information on the identities of the fungi capable of hosting mycoheterotrophs, but it remains a mystery how and why particular fungi become targets. Extant populations of MH plants are examples of successful subversions of the fungal community; however, in nature there are settings where the fungal hosts for mycoheterotrophs are present, but the plants are not. Thus, there remain many unseen and unexplained factors that are potentially protecting fungal communities and networks from cheaters. We have revealed many of the players, but who or what are acting as the referees remains a mystery.

Acknowledgements

The authors would like to thank Tom Madsen and Vincent Merckx for their assistance in the creation of Fig. 1 and Table S1. We apologize if any references or studies were overlooked in Table S1.

Nicole A. Hynson^{1*} and Thomas D. Bruns²

¹Department of Environmental Science, Policy & Management, University of California Berkeley, Berkeley, CA, USA;

²Department of Plant & Microbial Biology, University of California Berkeley, Berkeley, CA, USA

(*Author for correspondence: tel +1 510 643 5483; email nhynson@berkeley.edu)

References

- Bidartondo MI. 2005. The evolutionary ecology of myco-heterotrophy. *New Phytologist* 167: 335–352.
- Bidartondo MI, Bruns TD. 2005. On the origins of extreme mycorrhizal specificity in the Monotropoideae (Ericaceae): performance trade-offs during seed germination and seedling development. *Molecular Ecology* 14: 1549–1560.
- 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 of London. Series B, Biological Sciences* 271: 1799–1806.
- Bronstein JL. 2001. The exploitation of mutualisms. *Ecology Letters* 4: 277–287.
- Curlevski NJA, Chambers SM, Anderson IC, Cairney JWG. 2009. Identical genotypes of an ericoid mycorrhiza-forming fungus occur in roots of *Epacris pulchella* (Ericaceae) and *Leptospermum polygalifolium* (Myrtales) in an Australian sclerophyll forest. *FEMS Microbiology Ecology* 67: 411–420.
- Grelet GA, Johnson D, Paterson E, Anderson IC, Alexander IJ. 2009. Reciprocal carbon and nitrogen transfer between an ericaceous dwarf shrub and fungi isolated from *Piceirhiza bicolorata* ectomycorrhizas. *New Phytologist* 182: 359–366.
- Hynson NA, Bruns TD. 2009. Evidence of a myco-heterotroph in the plant family Ericaceae that lacks mycorrhizal specificity. *Proceedings of the Royal Society of London. Series B, Biological Sciences* 276: 4053–4059.
- Julou 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 damasonium*. *New Phytologist* 166: 639–653.
- Leake JR. 1994. Tansley review No. 69. The biology of myco-heterotrophic ('saprophytic') plants. *New Phytologist* 127: 171–216.
- Leake JR, Cameron DD. 2010. Physiological ecology of mycoheterotrophy. *New Phytologist* 185: 601–605.
- Leake JR, McKendrick SL, Bidartondo M, Read DJ. 2004. Symbiotic germination and development of the myco-heterotroph *Monotropa hypopitys* in nature and its requirement for locally distributed *Tricholoma* spp. *New Phytologist* 163: 405–423.
- Leake JR, Cameron DD, Beerling DJ. 2008. Fungal fidelity in the myco-heterotroph to-autotroph life cycle of Lycopodiaceae: a case of parental nurture? *New Phytologist* 177: 572–576.
- Martos F, Dulormne M, Pailler T, Bonfante P, Faccio A, Fournel J, Dubois M-P, Selosse M-A. 2009. Independent recruitment of saprotrophic fungi as mycorrhizal partners by tropical achlorophyllous orchids. *New Phytologist* 184: 668–681.
- McKendrick SL, Leake JR, Taylor DL, Read DJ. 2000. Symbiotic germination and development of myco-heterotrophic plants in nature: ontogeny of *Corallorhiza trifida* and characterization of its mycorrhizal fungi. *New Phytologist* 145: 523–537.
- Merckx V, Bidartondo MI. 2008. Breakdown and delayed cospeciation in the arbuscular mycorrhizal mutualism. *Proceedings of the Royal Society of London. Series B, Biological Sciences* 275: 1029–1035.
- Ogura-Tsujita Y, Yukawa T. 2008. *Epipactis helleborine* shows strong mycorrhizal preference towards ectomycorrhizal fungi with contrasting geographic distributions in Japan. *Mycorrhiza* 18: 331–338.
- Ogura-Tsujita Y, Gebauer G, Hashimoto T, Umata H, Yukawa T. 2008. Evidence for novel and specialized mycorrhizal parasitism: the orchid *Gastrodia confusa* gains carbon from saprotrophic *Mycena*. *Proceedings of the Royal Society of London. Series B, Biological Sciences* 276: 761–767.
- Roy M, Watthana S, Stier A, Richard F, Vessabutr S, Selosse M-A. 2009. Two mycoheterotrophic orchids from Thailand tropical dipterocarpacean forests associate with a broad diversity of ectomycorrhizal fungi. *BMC Biology* 7: 51.
- Smith SE, Read DJ. 2008. *Mycorrhizal symbiosis*, 3rd edn. London, UK: Academic Press.
- Taylor DL. 2004. Myco-heterotroph–fungus marriages – is fidelity over-rated? *New Phytologist* 163: 217–221.
- Taylor DL, Bruns TD, Leake JR, Read DJ. 2002. Mycorrhizal specificity and function in myco-heterotrophic plants. In: Heijden MGA & Sanders IR eds. *Ecological Studies. Mycorrhizal Ecology*, 375–413. Springer, Berlin Heidelberg, New York.
- Taylor DL, Bruns TD, Hodges SA. 2004. Evidence for mycorrhizal races in a cheating orchid. *Proceedings of the Royal Society of London. Series B, Biological Sciences* 271: 35–43.
- Winther JL, Friedman WE. 2007. Arbuscular mycorrhizal symbionts in *Botrychium* (Ophioglossaceae). *American Journal of Botany* 94: 1248–1255.
- Winther JL, Friedman WE. 2008. Arbuscular mycorrhizal associations in Lycopodiaceae. *New Phytologist* 177: 790–801.
- Winther JL, Friedman WE. 2009. Phylogenetic affinity of arbuscular mycorrhizal symbionts in *Ptilotum nudum*. *Journal of Plant Research* 122: 485–496.
- Yamato M, Yagame T, Suzuki A, Iwase K. 2005. Isolation and identification of mycorrhizal fungi associating with an achlorophyllous plant, *Epipogium roseum* (Orchidaceae). *Mycoscience* 46: 73–77.

Key words: mutualism, mycoheterotrophic, mycorrhizal fungi, saprotrophic fungi, symbiosis.

Supporting Information

Additional supporting information may be found in the online version of this article.

Table S1 List of published fungal hosts of fully myco-heterotrophic plants.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting information supplied by the authors. Any queries (other than missing material) should be directed to the *New Phytologist* Central Office.

Physiological ecology of mycoheterotrophy

Mycoheterotrophy (Leake, 1994) is the mode of parasitism in plants that has independently evolved most frequently (Merckx & Freudenstein, 2010) and upon which the largest number of vascular plant species depend (cf. Leake, 2005 and Watson, 2009), but the fundamental mechanisms underpinning this plant exploitation of fungal partners for carbon (C) and nutrients remain largely uncharacterized. In the 15 yr since mycoheterotrophy was defined, substantial progress has been made in our understanding of the ecology of these plants and their fungal partners (Selosse & Cameron, 2010), further highlighting the need for the physiology of

mycoheterotrophy to be investigated in order for the basis and origins of this mode of plant nutrition to be understood.

Mycoheterotrophy: the most common, but least understood, mode of parasitism by plants

Approximately 10% of vascular plants have a juvenile, mycoheterotrophic and subterranean phase of growth from spores or seeds, during which time they are critically dependent on specific fungal partners for C and nutrients for establishment (Leake, 2005). On emergence above ground, over 450 species remain fully mycoheterotrophic throughout their lives and lack green leaves (Merckx & Freudenstein, 2010). Others produce green leaves but remain partially dependent upon mycoheterotrophy, whereas most species develop green leaves and become autotrophic.

Full mycoheterotrophy has more than twice the number of evolutionary origins compared with haustoria-forming holoparasitic plants (Watson, 2009; Merckx & Freudenstein, 2010), and occurs in a much wider phylogenetic range of species, spanning from liverworts and basal ferns through dicotyledons and monocotyledons, culminating in the Orchidaceae, the largest family of flowering plants (Leake, 1994). However, whilst the metabolite fluxes into plant haustorial parasites and the mechanisms driving these fluxes are increasingly well understood (Shen *et al.*, 2006), the metabolic basis of mycoheterotrophy is still unresolved.

The four components of mycoheterotroph physiology

An understanding of the physiology of mycoheterotrophy requires (1) the primary sources and pathways of organic C and nutrient assimilation and transport by the fungal partners to be established; (2) the composition, quantity and chronology of metabolite transfers from fungus-to-plant to be identified and measured; (3) the metabolic pathways involved in storage and allocation by the plant of the C and nutrients received from fungal partners to be characterized; and (4) any metabolite fluxes from plant-to-fungus and their roles in establishing, maintaining or repaying the fungal association to be determined (Fig. 1). Pathways 2 and 4 include any signalling compounds passing between the organisms during establishment of the symbiosis and subsequently.

Knowledge of the first of these four components of mycoheterotroph physiology has advanced most significantly in the past 15 yr. Molecular identification of fungal partners in an increasing number of the plants has revealed that many are parasitic upon either arbuscular or ectomycorrhizal fungal partners of adjacent autotrophic plants – which provide

the primary C sources in these cases (Hynson & Bruns, 2010). Transfer of C from trees to a mycoheterotrophic orchid via an ectomycorrhizal fungus has been demonstrated using a $^{14}\text{CO}_2$ tracer (McKendrick *et al.*, 2000) and mycorrhizal root tips of adjacent autotrophs were shown to host the same fungi as mycoheterotrophs (Selosse *et al.*, 2002). A minority of species studied to date exploit saprotrophic litter or wood decay fungi (Martos *et al.*, 2009; Ogura-Tsujita *et al.*, 2009), a trait that may be confined to orchids. Knowledge of the pathways and mechanisms of C and nutrient assimilation and transport in mycorrhizal fungi, and the key genes involved, has also advanced considerably (Bago *et al.*, 2003; Govindarajulu *et al.*, 2005; Müller *et al.*, 2007; Deveau *et al.*, 2008), although there have been no studies on metabolite transport pathways in mycoheterotrophy. Nonetheless, these studies have revealed a range of potential candidate metabolites that might be involved in fungal-to-plant C and nutrient fluxes in mycoheterotrophs. They have also highlighted major differences between the pathways of assimilation and metabolite transport in the hyphae of Glomeralean AM fungi (Bago *et al.*, 2003; Govindarajulu *et al.*, 2005) and ectomycorrhizal basidiomycetes (Müller *et al.*, 2007; Deveau *et al.*, 2008) that are likely to affect the mechanisms of mycoheterotrophy in the plants that exploit these different groups of fungi.

Whilst these developments in fungal metabolomics should facilitate future studies of mycoheterotroph physiology, almost no progress, as yet, has been made in our understanding of the fungus-to-plant metabolite fluxes, the metabolic pathways in the plants and any metabolite fluxes from plant-to-fungus (Fig. 1, components 2–4). The metabolites that pass from fungi into mycoheterotrophs have not been identified, and the relative importance of active transport processes at the fungal–plant interface vs plant digestion of fungal hyphae still needs to be determined. The latter is unlikely to play a major role in C or nutrient supply, but in contrast to active transport, it is a process clearly visible in light microscopy and electron microscopy analyses of mycoheterotrophs (Leake, 1994), so it apparently makes a contribution to these fluxes, even if this is small.

Also uncharacterized are the biochemical pathways leading from metabolites received from the fungal partners to accumulation in the major C storage polymers in mycoheterotrophs, such as starch, lipids and fructans (Leake, 1994). Moreover, the subsequent pathways of deployment of these reserves for growth and reproduction have not been investigated. As growth of mycoheterotrophs is likely to be strongly C limited, there may be very strong selection for metabolic pathways that minimize respiratory losses, possibly leading to atypical or novel metabolic pathways in these plants. This highlights the need for studies specifically to investigate the C and nutrient physiology of mycoheterotrophs, to parallel the studies already undertaken on haustorial plant parasites (Shen *et al.*, 2006).

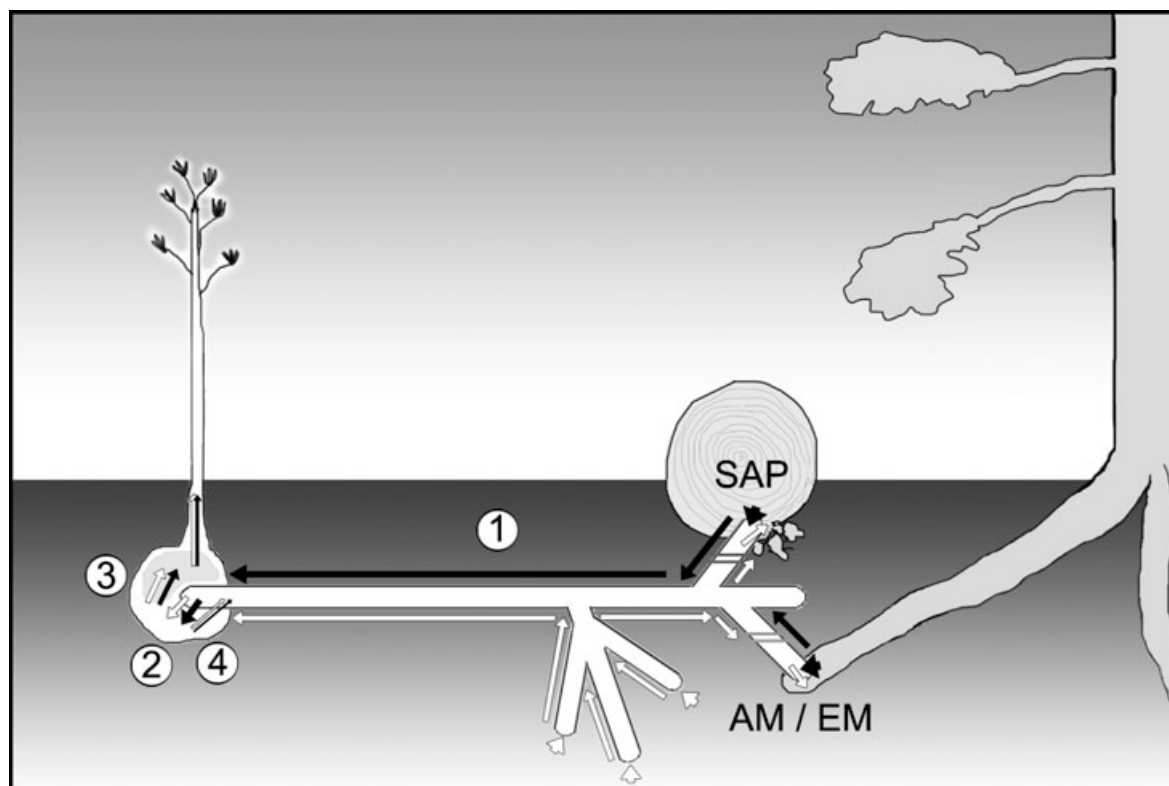


Fig. 1 Schematic diagram of the four components of carbon (black arrows) and nutrient (white arrows) physiology of mycoheterotrophy that affect the metabolism and ^{13}C and ^{15}N enrichment in these plants. (1) Fungal carbon and nutrient assimilation processes, including uptake, metabolism and transport of recent photosynthate of adjacent autotrophic plants through arbuscular mycorrhizal (AM) or ectomycorrhizal (EM) fungal associates, or detrital carbon uptake through saprotrophic fungi (SAP) decomposing wood or litter. The sources and pathways of nutrient uptake and their subsequent transport will also differ between the different groups of fungi. (2) Metabolite and nutrient fluxes from fungal partners into the myco-heterotrophic plants. (3) Metabolic pathways of carbon and nutrient storage and remobilization and allocation in growth and reproduction. (4) Metabolite fluxes from plant-to-fungus, which may be important for stimulating fungal allocation of metabolites to the plant.

Mycoheterotroph carbon and nitrogen isotope signatures

Nitrogen (N) and C isotopes have been widely used to elucidate pathways of nutrient and energy flows in ecosystems, to understand food-webs and trophic hierarchies of organisms. The relative abundance of ^{15}N and ^{13}C systematically increase in abundance up each trophic level of a food-chain as a result of excretion and respiration discriminating in favour of ^{14}N and ^{12}C , enriching the remaining biomass in the heavier isotopes. Gebauer & Meyer (2003) and Trudell *et al.* (2003) were the first to recognize the potential insights to be gained from the analysis of C and N isotope abundance in mycoheterotrophs. They showed that mycoheterotrophs parasitic on ectomycorrhizal fungi are distinctly enriched in ^{13}C and especially in ^{15}N .

The four components of mycoheterotroph physiology (Fig. 1, and outlined above) will contribute to the overall isotopic fractionation seen in the plants, but the lack of characterization of the metabolite pathways into and within these plants constrains our abilities to interpret their isoto-

pic signatures. Nonetheless, stable isotope analysis has now become established as one of the most frequently used tools, together with molecular identification of the fungal partners, to characterize the mycoheterotrophic lifestyle. Isotope analysis has been particularly important in revealing otherwise cryptic partial mycoheterotrophy in species that have green leaves (Gebauer & Meyer, 2003; Julou *et al.*, 2005; Zimmer *et al.*, 2008a).

However, as increasing numbers of studies have reported $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in mycoheterotrophs and adjacent green plants, it has become clear that ^{15}N enrichment is not a universal feature of these plants, enrichment in ^{13}C and ^{15}N is not automatically coupled and the extent of ^{15}N enrichment is not always linearly related to the extent of heterotrophic C gain. These findings highly constrain the valid use of linear two-source isotope-mixing models (Gebauer & Meyer, 2003) for estimation of the extent of mycoheterotrophic N gain in green-leaved species, and additional evidence suggests that the mixing-model approach, based on either ^{13}C or ^{15}N enrichment, can only provide an estimate, and not an absolute mea-

sure, of dependence on mycoheterotrophy. For example, a mixing-model analysis suggested that 23% of the carbon orchid in the *Corallorrhiza trifida* was derived from autotrophy (Zimmer *et al.*, 2008b), whereas direct physiological measurements confirmed the absence of significant photosynthesis in the putatively fully mycoheterotrophic orchid *C. trifida* (Cameron *et al.*, 2009). The ^{15}N enrichment in fungi varies widely between different taxonomic and functional groups. Early studies suggested that ^{15}N enrichment in mycoheterotrophs associated with ectomycorrhizal fungi is proportional to, but enriched by, *c.* 3.6‰ compared with the fruit bodies of the fungal partners (Trudell *et al.*, 2003). This leads to particularly high ^{15}N enrichment in these mycoheterotrophs as their fungal partners are already enriched with ^{15}N as a result of the preferential export of ^{14}N to their autotrophic host plants (see Fig. 1, nutrient pathway into autotroph root). Orchids and pyroloids with green leaves, which are partially mycoheterotrophic, sometimes have higher ^{15}N enrichment than fully mycoheterotrophic species (Gebauer & Meyer, 2003; Hynson *et al.*, 2009). By contrast, recent studies of mycoheterotrophs apparently associated with saprotrophic wood and litter decay fungi in one case found very little enrichment (0.7–1.2‰) compared with saprotrophic fungal fruit bodies (Martos *et al.*, 2009), whilst in another case the plants were 3.2‰ more enriched (Ogura-Tsujita *et al.*, 2009). Enrichment with ^{15}N appears to be associated with the accumulation of high concentrations of N in many mycoheterotrophs, often exceeding the N concentrations in fungal tissues (Julou *et al.*, 2005). However, the mechanism of ^{15}N enrichment in these heterotrophic plants has not been resolved, and is contrary to the pattern seen in autotrophic plants receiving N from mycorrhizal fungal partners where ^{14}N is preferentially passed to the plants and ^{15}N is preferentially retained in the fungi (Trudell *et al.*, 2003). A better understanding of the physiological mechanisms underpinning ^{15}N enrichment in mycoheterotrophic plants is required as this is a major barrier to meaningful interpretation of these isotope signatures.

The patterns of ^{13}C enrichment seen in mycoheterotrophs relative to adjacent green-leaved partial mycoheterotrophs and fully autotrophic plants, fungal fruit bodies and soil organic matter (Gebauer & Meyer, 2003; Trudell *et al.*, 2003; Julou *et al.*, 2005; Hynson *et al.*, 2009) are more consistent with established processes of isotopic discrimination. Fungal assimilation, metabolism and transport of organic C will normally result in preferential losses of $^{12}\text{CO}_2$, and this is likely to be continued in the plant metabolism, further enriching their ^{13}C content relative to their fungal partners. Substantial differences in ^{13}C enrichment has been found in different mycoheterotrophs that can be explained by the C sources used by their fungi (Trudell *et al.*, 2003; Martos *et al.*, 2009). Mycoheterotrophs

parasitic on ectomycorrhizal fungi using photosynthate from adjacent autotrophic plants are much less enriched in ^{13}C than the plants parasitizing saprotrophic fungi that live on lignocellulose and other detrital C sources that are significantly enriched in ^{13}C (Martos *et al.*, 2009).

Anatomical adaptations driving metabolic adaptations?

The remarkable convergent evolution of most mycoheterotrophs to anatomically simplified aboveground and belowground vegetative structures (Leake, 1994; Leake *et al.*, 2008), is consistent with adaptations to conserve C for flowering and seed production. Shoots are typically simplified by the reduction of leaves to scales, stomata are absent or poorly developed and vascular tissues are minimized in structural complexity and cellular volumes (Leake, 1994). The consequences of these anatomical features for internal transport of water and nutrients, and for gas exchange, have not been investigated. In haustorial plant parasites, transpiration rates are exceedingly high and stomata open as a crucial component of drawing a mass flow of water, C and nutrients from their host plants (Shen *et al.*, 2006). Even before developing shoots, many haustorial parasites synthesize sugar alcohols from host carbohydrates to develop high osmotic gradients that establish mass fluxes from the host plants during their subterranean initial stages of development. Intriguingly, there is some evidence of parallel adaptations in some mycoheterotrophs, as gas-exchange measurements of mycoheterotrophic albino mutants of *Cephalanthera* orchids have found transpiration rates that are double those in adjacent green-leaved individuals (Julou *et al.*, 2005), and the depolymerization of stored fructan in *Monotropa hypopitys* may act as an osmoticum at the stage of expansion of the flowering shoots (Leake, 1994).

Evolutionary physiology of mycoheterotrophy

An understanding of the physiology of mycoheterotrophy is required to resolve how plants evolved to parasitize fungi and the mechanistic basis of this mode of nutrition. Has this trophic strategy evolved from plants exploiting the pre-existing fungal-to-plant metabolic pathways of mutualistic mycorrhizal interactions, or has it required the evolution of entirely novel metabolic pathways? Have different groups of mycoheterotrophs evolved to exploit different fungal metabolites, and is this, in turn, controlled by their association with the different major functional groups of fungi (Hynson & Bruns, 2010) involved in mycoheterotrophy?

Where evolution of full mycoheterotrophy is coincident with a switch in fungal functional type, as in orchids that have switched from saprotrophic rhizoctonias to ectomycor-

rhizal basidiomycetes (Hynson & Bruns, 2010), this raises the question as to whether the new fungal partners are selected to provide larger and more sustained supplies, or biochemically more easily exploitable, sources of C and nutrients. Similarly, the extent to which the extreme specificity of many mycoheterotroph fungal associations may be driven by the physiology of the plant–fungal interactions, from putative chemical signalling before infection (Bruns & Read, 2000), through to specific pathways of metabolite transport, remain intriguing but unresolved.

Progress in metabolomic and genomic technologies promise that answers to many of these previously intractable questions will be found in the next 15 yr of research into mycoheterotrophy.

Jonathan R. Leake* and Duncan D. Cameron

Department of Animal & Plant Sciences, Alfred Denny Building, Western Bank, Sheffield S10 2TN, UK

(*Author for correspondence: tel +44 0114 222 0055; email j.r.leake@sheffield.ac.uk)

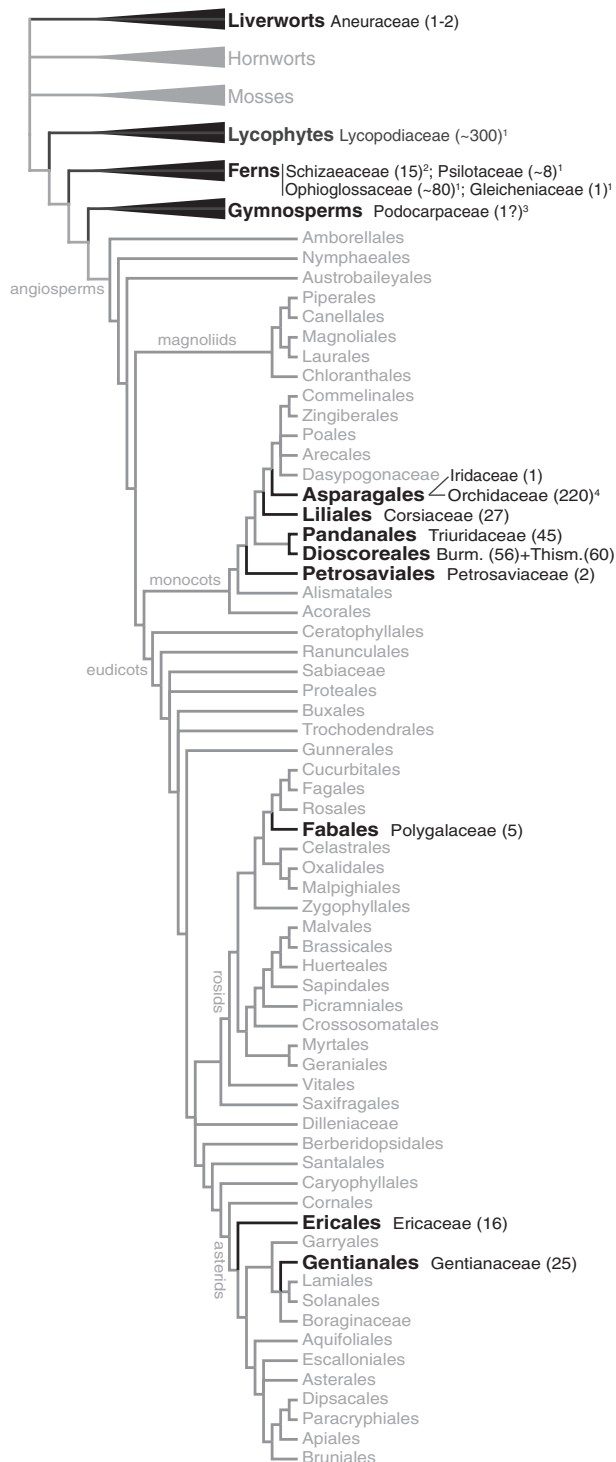
References

- Bago B, Pfeffer PE, Abubaker J, Jun J, Allen JW, Brouillette J, Douds DD, Lammers P, Shachar-Hill Y. 2003. Carbon export from arbuscular mycorrhizal roots involves the translocation of carbohydrates as well as lipids. *Plant Physiology* 131: 1496–1507.
- 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.
- Cameron DD, Preiss K, Gebauer G, Read DJ. 2009. The chlorophyll-containing orchid *Corallorhiza trifida* derives little carbon through photosynthesis. *New Phytologist* 183: 358–364.
- Deveau A, Kohler A, Frey-Klett P, Martin F. 2008. The major pathways of carbohydrate metabolism in the ectomycorrhizal basidiomycete *Laccaria bicolor* S238N. *New Phytologist* 180: 379–390.
- Gebauer G, Meyer M. 2003. ¹⁵N and ¹³C natural abundance of autotrophic and myco-heterotrophic orchids provides insight into nitrogen and carbon gain from fungal association. *New Phytologist* 160: 209–223.
- Govindarajulu M, Pfeffer PE, Jin H, Abubaker J, Douds DD, Allen JW, Bücking H, Lammers PJ, Shachar-Hill Y. 2005. Nitrogen transfer in the arbuscular mycorrhizal symbiosis. *Nature* 435: 819–823.
- Hynson NA, Bruns TD. 2010. Fungal hosts for mycoheterotrophic plants: a non-exclusive, but highly selective club. *New Phytologist* 185: 598–602.
- 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.
- Julou T, Burghardt B, Gebauer G, Berveiller D, Damesin C, Selosse MA. 2005. Mixotrophy in orchids: insights from a comparative study of green individuals and non-photosynthetic individuals of *Cephalanthera damasonium*. *New Phytologist* 166: 639–653.
- Leake JR. 1994. Tansley review: 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.
- Leake JR, Cameron DD, Beerling DJ. 2008. Fungal fidelity in the myco-heterotroph-to-autotroph lifecycle of Lycopodiaceae: a case of parental nurture? *New Phytologist* 177: 572–576.
- Martos F, Dulormne M, Pailler T, Bonfante P, Faccio A, Fournel J, Dubois M-P, Selosse MA. 2009. Independent recruitment of saprotrophic fungi as mycorrhizal partners by tropical achlorophyllous orchids. *New Phytologist* 184: 668–681.
- McKendrick SL, Leake JR, Read DJ. 2000. Symbiotic germination and development of myco-heterotrophic plants in nature: Transfer of carbon from ectomycorrhizal *Salix repens* and *Betula pendula* to the orchid *Corallorhiza trifida* Châtel through shared hyphal connections. *New Phytologist* 145: 539–548.
- Merckx V, Freudenstein JV. 2010. Evolution of myco-heterotrophy in plants: a phylogenetic perspective. *New Phytologist* 185: 607–610.
- Müller T, Avolio M, Olivi M, Benjdia M, Rikirsch E, Kasaras A, Fitz M, Chalot M, Wipf D. 2007. Nitrogen transport in the ectomycorrhiza association: the *Hebeloma cylindrosporum*-*Pinus pinaster* model. *Phytochemistry* 68: 41–51.
- 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 of London. Series B, Biological Sciences* 276: 761–767.
- Selosse MA, Cameron DD. 2010. Introduction to a virtual special issue on mycoheterotrophy: the New Phytologist sheds light on non-green plants. *New Phytologist* 185: 591–593.
- Selosse MA, Weiss M, Jany JL, Tillier A. 2002. Communities and populations of sebacinoid basidiomycetes associated with the achlorophyllous orchid *Neottia nidus-avis* (L.) LCM Rich. and neighbouring tree ectomycorrhizae. *Molecular Ecology* 11: 1831–1844.
- Shen H, Ye W, Hong L, Huang H, Wang Z, Deng X, Yang Q, Xu Z. 2006. Progress in parasitic plant biology: host selection and nutrient transfer. *Plant Biology* 8: 175–185.
- Trudell SA, Rygiel PT, Edmonds 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.
- Watson DM. 2009. Parasitic plants as facilitators: more dryad than Dracula? *Journal of Ecology* 97: 1151–1159.
- Zimmer K, Hynson NA, Gebauer G, Allen EB, Allen MF, Read DJ. 2008a. Wide geographical and ecological distribution of nitrogen and carbon gains from fungi in pyroloids and monotropoids (Ericaceae) and in orchids. *New Phytologist* 175: 166–175.
- Zimmer K, Meyer C, Gebauer G. 2008b. The ectomycorrhizal specialist orchid *Corallorhiza trifida* is a partial myco-heterotroph. *New Phytologist* 178: 395–400.

Key words: evolutionary physiology, fungal metabolites, metabolic pathways, parasitism, stable isotopes.

Evolution of mycoheterotrophy in plants: a phylogenetic perspective

From an evolutionary point of view, mycoheterotrophy represents one extreme end in the mutualism–parasitism continuum of the mycorrhizal symbiosis. Strictly speaking, mycoheterotrophs are fully dependent on fungi for carbon metabolites and have usually lost chlorophyll as a result. Exploitation of fungi by plants has evolved independently many times among Embryophytes. Phylogenetic relation-



ships of mycoheterotrophs provide an insight into the evolution of mycoheterotrophy by comparison of the degree of adaptation to a mycoheterotrophic mode of life between achlorophyllous and related green taxa. This February 2010 Virtual Special Issue of *New Phytologist* contains a comprehensive collection of papers on mycoheterotrophic plants,

Fig. 1 Lineages of plants that have evolved mycoheterotrophy. Land plant phylogeny based on Palmer *et al.* (2004) and APG III (2009) for angiosperm relationships with lineages containing mycoheterotrophic species are indicated in black. All families with mycoheterotrophic species are shown next to the clade to which they belong, with the number of mycoheterotrophic species shown in parenthesis. Burm., Burmanniaceae; Thism., Thismiaceae. ¹In these species the gametophytes are fully mycoheterotrophic, but the sporophytes contain chlorophyll. ²These species (belonging to *Actinostachys* and *Schizaea*) have only mycoheterotrophic gametophytes, but *Schizaea fluminensis* may have a fully mycoheterotrophic life cycle. ³*Parasitaxus usta* is both mycoheterotrophic and directly parasitic according to Feild & Brodribb (2005). ⁴As far as is known, all Orchidaceae are initial mycoheterotrophs.

illustrating the significant progress that has been made in our understanding of their biology (<http://www.newphytologist.com/view/0/virtspecissues.html>; Selosse & Cameron, 2010). However, in many cases the phylogenetic context of the mycoheterotrophic mode of life studied remains poorly known and consequently our understanding of the evolution of mycoheterotrophy is still sparse.

Taxonomic affinities

The vast majority of land plants are mycorrhizal and therefore it may not be surprising that a mycoheterotrophic mode of life has evolved, almost without exception, at least once in all of the major land plant lineages (Fig. 1). Mycoheterotrophs are unknown in the small relictual hornworts (~100 spp.) and in the mosses (~12 000 spp.). The latter can be explained by the fact that all mosses are nonmycorrhizal. Some species of the moss genus *Buxbaumia* are often described as achlorophyllous and thus mycoheterotrophic (Leake, 1994; Bidartondo, 2005). However, despite their reduced habit, all *Buxbaumia* species contain chlorophyll (A. Vanderpoorten & T. Madsen, pers. comm.) and, just like other mosses, *Buxbaumia* is nonmycorrhizal (Duckett *et al.*, 2004). In liverworts at least one species (*Aneura mirabilis*) is fully mycoheterotrophic (Bidartondo *et al.*, 2003). Another closely related species awaits investigation (Crum & Bruce, 1996). The gametophytes of most lycophytes and some fern families are nonphotosynthetic and mycorrhizal, pointing towards a mycoheterotrophic mode at this stage of their life (Winther & Friedman, 2009). For at least one fern species (*Schizaea fluminensis*) it has been suggested that the sporophyte is also mycoheterotrophic but this requires further investigation (Bidartondo, 2005). Some experts consider the parasitic gymnosperm *Parasitaxus usta* as a mycoheterotroph and argue that the plant obtains carbon from fungi but water from a parasitic interaction with a host plant (Feild & Brodribb, 2005). However, the identity and the exact role of the fungi involved in this interaction remain undetermined.

Perhaps best known are angiosperm mycoheterotrophs. Outside of monocots there are at least seven independent origins of mycoheterotrophy: one in Polygalaceae (*Epirixanthes*); two or three in Ericaceae (*Monotropaeae*, *Pterosporeae* and *Pyrola aphylla*); and four in Gentianaceae (*Voyria*, *Voyriella parviflora*, *Cotylanthera* and *Sebaea oligantha*), which account for a total of 46 species. The remaining angiosperm mycoheterotrophs are all monocots. Within monocots at least 43 lineages evolved a fully mycoheterotrophic mode of life independently and there are no less than 411 fully mycoheterotrophic monocots. The Orchidaceae contains the largest number of fully mycoheterotrophic species, at least 210, which represent more than 30 independent occurrences. Significantly, as far as is known, all Orchidaceae (~20 000 spp.) are completely dependent on fungal carbon during the early development of the seedling and are thus 'initial' mycoheterotrophs. In addition, a number of chlorophyll-containing orchids have been shown to be partly dependent on mycorrhizal fungi during their adult stage, a nutrition strategy that is known as partial mycoheterotrophy or mixotrophy. Partially mycoheterotrophic species have also been shown to exist in Ericaceae (Selosse & Roy, 2009).

A phylogenetic context

Recent developments in the field, particularly the discovery of partial mycoheterotrophic species closely related to ecto-mycorrhizal mycoheterotrophs, have shown the need for solid phylogenetic hypotheses to study the evolution of mycoheterotrophy in plants (Selosse & Roy, 2009). Unfortunately, these hypotheses are not available for the majority of mycoheterotrophic clades, and the identification of relatives of mycoheterotrophic plants has proven to be a taxonomic and phylogenetic challenge in many cases. Many mycoheterotrophic plants are rare or difficult to find, and in extreme cases particular species are only known from one or two collections (Maas *et al.*, 1986). Obtaining study material is therefore often the first obstacle to be tackled when trying to unravel the evolutionary history of these intriguing plants. In addition, in parallel to parasitic plants, mycoheterotrophic plants have evolved convergent adaptations in their morphology and anatomy as a result of their peculiar mode of life. In general, mycoheterotrophs are characterized by small 'dust' seeds with undifferentiated embryos. Leaves are typically scale-like or absent entirely, and the vascularization of the stems is often reduced. Stomata are mostly absent from above-ground parts. The subterranean organs of mycoheterotrophic plants are typically highly modified. Structures with absorptive functions may be of relatively minor importance for mycoheterotrophic plants because this function is performed by the fungal symbiont. Root systems often show a trend towards a decrease in surface area: roots are short and thick and lack root hairs. Some

species produce rhizomatous tubers and lack roots entirely (Leake, 1994). As a result of these convergences, unrelated mycoheterotrophs often share a similar habit and consequently many families with mycoheterotrophic members have been thought to be closely related. When morphological characters experience convergent evolution as a result of adaptations to a particular mode of life, molecular data offer a promising solution for inferring phylogenetic affinities. Indeed, the phylogenetic position of many mycoheterotrophic groups has been successfully inferred using DNA data, sometimes with surprising results, which in turn urged a re-evaluation of morphological characters (e.g. the placement of Triuridaceae in Pandanales; Rudall & Bateman, 2006).

However, phylogenetic reconstructions in plants often rely heavily on data from the chloroplast genome. In parallel to what is observed in directly parasitic plants, the chloroplast genome of mycoheterotrophs may be significantly reduced in size. As a result of the relaxation of purifying selection, genes involved in the photosynthetic apparatus may be lacking or are highly divergent (Barrett & Freudenstein, 2008; Freudenstein & Senyo, 2008). However, few studies have examined mycoheterotrophs from a molecular evolution perspective. Whether because of the rarity of the plants or the absence of genetic sequence, mycoheterotrophic species are regularly absent in chloroplast DNA data sets. Moreover, nuclear and mitochondrial data of putative relatives of mycoheterotrophic plants are often not available, and thus a considerable sampling and sequencing effort is needed to infer phylogenies with nonchloroplast DNA data sets. Furthermore, nuclear and mitochondrial substitution rates of mycoheterotrophic plants are often greatly elevated. Whether these rate accelerations are the result of a small effective population size, selective molecular constraints, ecological niche changes, or other causes remains unclear. However, extreme rate heterogeneity may mislead phylogenetic inference methods by producing artificial clades, an error known as 'long-branch attraction' (Merckx *et al.*, 2009). As a result of the problems outlined above, the phylogenetic relationships of many groups of mycoheterotrophic plants are still poorly known. The affinities of Corsiaceae, Thismiaceae and Triuridaceae remain elusive. Many mycoheterotrophic genera are yet to be included in phylogenetic analyses: for example, *Cheilothea* (Ericaceae), *Corsiopsis* (Corsiaceae), *Epirixanthes* (Polygalaceae), *Miersiella* and *Marthella* (Burmanniaceae), *Voyria* (Gentianaceae), *Kihansia*, *Peltophyllum*, *Seychellaria*, *Soridium* and *Triuridopsis* (Triuridaceae), and several genera of Orchidaceae.

The fungal perspective

The advent of molecular biology tools has made the isolation and identification of fungal associates of mycoheterotrophs much more tractable than with morphological

approaches alone (Hynson & Bruns, 2010). It is now possible to reconstruct the phylogenetic patterns of both a group of mycoheterotrophs and their fungal associates to assess relative amounts of specialization, cophylogenetic evolution and host-shifting, making the mycoheterotroph system a useful model in studies of host–parasite relationships. Extreme host specialization towards narrow fungal lineages has been observed in many groups of mycoheterotrophs (Leake, 2004). In a lineage of arbuscular mycorrhizal mycoheterotrophs, this specialization process even resulted in a delayed cospeciation pattern (Merckx & Bidartondo, 2008). Among orchids, studies have revealed a general, although not universal, pattern of shift in fungal utilization from saprophytic/parasitic fungi to ectomycorrhizal species that is correlated with the transition from partial mycoheterotrophy to full mycoheterotrophy (Taylor *et al.*, 2002). However, broad generalization may be premature, because recent studies examining tropical orchid groups have uncovered additional cases of the use of saprophytic fungi (Martos *et al.*, 2009; Ogura-Tsujita *et al.*, 2009). Such studies add to our understanding of the mycoheterotroph phylogeny, as well as expanding our knowledge on the evolutionary context in which diversification has occurred.

Evolution in space and time

Well-supported phylogenetic hypotheses are powerful tools for using to study the evolutionary physiology and ecology of mycoheterotrophy. Phylogenies also give insights into the biogeography and timing of the processes involved. Modern molecular clock techniques now allow us to evaluate the evolution of traits along a geological timescale but have been used only occasionally to study the origin of mycoheterotrophic lineages. In addition to the potential problems with DNA substitution rate heterogeneity, the paucity of fossil material for mycoheterotrophs contributes to the difficulty in reconstructing their evolutionary history. Despite these drawbacks, we now know that there are ancient lineages of mycoheterotrophic plants, and many lineages of mycoheterotrophs have radiated and achieved circumglobal geographic ranges (Bidartondo & Bruns, 2001; Merckx *et al.*, 2008).

Future prospects

As new material and data become available we will be able to identify the relatives of an increasing number of mycoheterotrophic plant lineages. This phylogenetic information will provide a solid framework to map ecological traits and study the distribution, diversification and divergence times of these enigmatic taxa. Combined with new physiological and ecological data on mycoheterotrophs and their relatives, the evolutionary context will allow for the identification of common patterns in the evolution of mycoheterotrophy in

plant lineages (Leake & Cameron, 2010). In addition, efforts to sequence full plastid genomes of partially and fully mycoheterotrophic plants should be undertaken, both to resolve phylogenetic relationships and to obtain insights into the processes of plastid genome evolution of mycoheterotrophs. On a lower taxonomic level, studies on the population genetics of mycoheterotrophs are urgently needed to investigate morphological, phenological and molecular diversity in these groups.

Acknowledgements

The authors thank Tom Madsen, Alan Smith, Nicole Hynson, Marc-André Selosse and Duncan Cameron for insightful discussions. V.M. is supported by the Fund for Scientific Research Flanders (FWO Vlaanderen).

Vincent Merckx^{1,2*} and John V. Freudenstein³

¹Laboratory of Plant Systematics, K.U. Leuven, Kasteelpark Arenberg 31, B-3001 Leuven, Belgium; ²Department of Plant & Microbial Biology, University of California Berkeley, Berkeley, CA 94720, USA;

³Department of Evolution, Ecology, and Organismal Biology, The Ohio State University Herbarium, 1315 Kinnear Road, Columbus, OH 43212, USA

(*Author for correspondence: tel +32 16 32 8637; email vincent.merckx@bio.kuleuven.be)

References

- APG III. 2009. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. *Botanical Journal of the Linnean Society* 161: 105–121.
- Barrett CF, Freudenstein JV. 2008. Molecular evolution of *rbcL* in the mycoheterotrophic coralroot orchids (*Corallorhiza* Gagnebin, Orchidaceae). *Molecular Phylogenetics and Evolution* 47: 665–679.
- Bidartondo MI. 2005. The evolutionary ecology of myco-heterotrophic plants. *New Phytologist* 167: 335–352.
- Bidartondo MI, Bruns TD. 2001. Extreme specificity in epiparasitic Monotropoideae (Ericaceae): widespread phylogenetic and geographic structure. *Molecular Ecology* 10: 2285–2295.
- Bidartondo MI, Bruns TD, Weiss M, Sérgio C, Read D. 2003. Specialized cheating of the ectomycorrhizal symbiosis by an epiparasitic liverwort. *Proceedings of the Royal Society of London. Series B, Biological Sciences* 270: 835–842.
- Crum H, Bruce J. 1996. A new species of *Cryptothallus* from Costa Rica. *The Bryologist* 99: 433–438.
- Duckett JG, Burch J, Fletcher PW, Matcham HW, Read DJ, Russell AJ, Pressel S. 2004. *In vitro* cultivation of bryophytes: a review of practicalities, problems, progress and promise. *Journal of Bryology* 26: 3–20.
- Feild TS, Brodribb TJ. 2005. A unique mode of parasitism in the conifer coral tree *Parasitaxus ustus* (Podocarpaceae). *Plant, Cell & Environment* 28: 1316–1325.
- Freudenstein JV, Senyo DM. 2008. Relationships and evolution of *matK* in a group of leafless orchids (*Corallorhiza* and *Corallorhizinae*; Orchidaceae: Epidendroideae). *American Journal of Botany* 95: 498–505.

- Hynson NA, Bruns TD. 2010. Fungal hosts for mycoheterotrophic plants: a non-exclusive, but slightly selective club. *New Phytologist* 185: 598–601.
- Leake JR. 1994. The biology of mycoheterotrophic ('saprophytic') plants. *New Phytologist* 127: 171–216.
- Leake JR. 2004. Myco-heterotroph/epiparasitic plant interactions with ectomycorrhizal and arbuscular mycorrhizal fungi. *Current Opinion in Plant Biology* 7: 422–428.
- Leake JR, Cameron DD. 2010. Physiological ecology of mycoheterotrophy. *New Phytologist* 185: 601–605.
- Maas PJM, Maas-van de Kamer H, van Benthem J, Snelders HCM, Rübtsamen T. 1986. Burmanniaceae. *Flora Neotropica* 42: 1–189.
- Martos F, Dulormne M, Pailler T, Bonfante P, Faccio A, Fournel J, Dubois M-P, Selosse M-A. 2009. Independent recruitment of saprotrophic fungi as mycorrhizal partners by tropical achlorophyllous orchids. *New Phytologist* 184: 668–681.
- Merckx V, Bidartondo MI. 2008. Breakdown and delayed cospeciation in the arbuscular mycorrhizal mutualism. *Proceedings of the Royal Society of London. Series B, Biological Sciences* 275: 1029–1035.
- Merckx V, Chatrou LW, Lemaire B, Sainge MN, 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. *Cladistics* 25: 64–77.
- 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 of London. Series B, Biological Sciences* 276: 761–767.
- Palmer JD, Soltis DE, Chase MW. 2004. The plant tree of life: an overview and some points of view. *American Journal of Botany* 91: 1437–1445.
- Rudall PJ, Bateman RM. 2006. Morphological phylogenetic analysis of Pandanales: testing contrasting hypotheses of floral evolution. *Systematic Botany* 31: 223–238.
- Selosse MA, Cameron DD. 2010. Introduction to a Virtual Special Issue on mycoheterotrophy: New Phytologist sheds light on non-green plants. *New Phytologist* 185: 591–593.
- Selosse M-A, Roy M. 2009. Green plants that feed on fungi. *Trends in Plant Science* 14: 64–70.
- Taylor DL, Bruns TD, Leake JR, Read DJ. 2002. Mycorrhizal specificity and function in myco-heterotrophic plants. In: van der Heijden MGA, Sanders IR, eds. *The ecology of mycorrhizas (Ecological Studies vol. 157)*. Berlin, Germany: Springer-Verlag, 375–414.
- Winther J, Friedman WE. 2009. Phylogenetic affinity of arbuscular mycorrhizal symbionts in *Ptilotum nudum*. *Journal of Plant Research* 122: 485–496.

Key words: achlorophyllous plants, evolution, mycoheterotrophy, mycorrhizal symbiosis, phylogenetics.



About *New Phytologist*

- *New Phytologist* is owned by a non-profit-making **charitable trust** dedicated to the promotion of plant science, facilitating projects from symposia to open access for our Tansley reviews. Complete information is available at www.newphytologist.org.
- Regular papers, Letters, Research reviews, Rapid reports and both Modelling/Theory and Methods papers are encouraged. We are committed to rapid processing, from online submission through to publication 'as-ready' via *Early View* – our average submission to decision time is just 29 days. Online-only colour is **free**, and essential print colour costs will be met if necessary. We also provide 25 offprints as well as a PDF for each article.
- For online summaries and ToC alerts, go to the website and click on 'Journal online'. You can take out a **personal subscription** to the journal for a fraction of the institutional price. Rates start at £151 in Europe/\$279 in the USA & Canada for the online edition (click on 'Subscribe' at the website).
- If you have any questions, do get in touch with Central Office (newphytol@lancaster.ac.uk; tel +44 1524 594691) or, for a local contact in North America, the US Office (newphytol@ornl.gov; tel +1 865 576 5261).