

## Commentary

# A case of *Pyrola* plantlets with picky palates leads to new insights on mycoheterotrophic seedlings and the fungi that feed them

### The life of a seedling

Plants have evolved many strategies to ensure seed germination and seedling survival. One of the most intriguing strategies utilized by a subset of dust-seed producing plants is initial mycoheterotrophy. Rather than relying solely on a seed's own reserves, initially mycoheterotrophic plants employ fungi to provide much or all of the carbohydrates necessary to feed a developing seedling (Leake, 1994). This strategy has allowed plants to circumvent the tradeoff of producing numerous small seeds capable of long distance dispersal that consequently possess limited carbon (C) reserves to feed a growing seedling. However, initial mycoheterotrophy has its own limitations. For example, often it involves very specific pairings among plants and particular lineages of fungi (Leake *et al.*, 2004). Thus, a plant dependent on specific fungi for germination or seedling development must first come into physical contact with a compatible fungal partner. The paper in this issue of *New Phytologist* by Hashimoto *et al.* (pp. 620–630) is the first to reveal the identity of the fungi responsible for the mycoheterotrophic germination and seedling development of *Pyrola asarifolia* (tribe Pyroleae, family Ericaceae) a fairly widespread evergreen herb found in Japan, North America, and Europe, whose early fungal associates have remained a mystery until now.

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*'... these results provide an example of a truly unique and previously undocumented symbiotic interaction between plants and fungi.'*

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### Carbon supply and demand

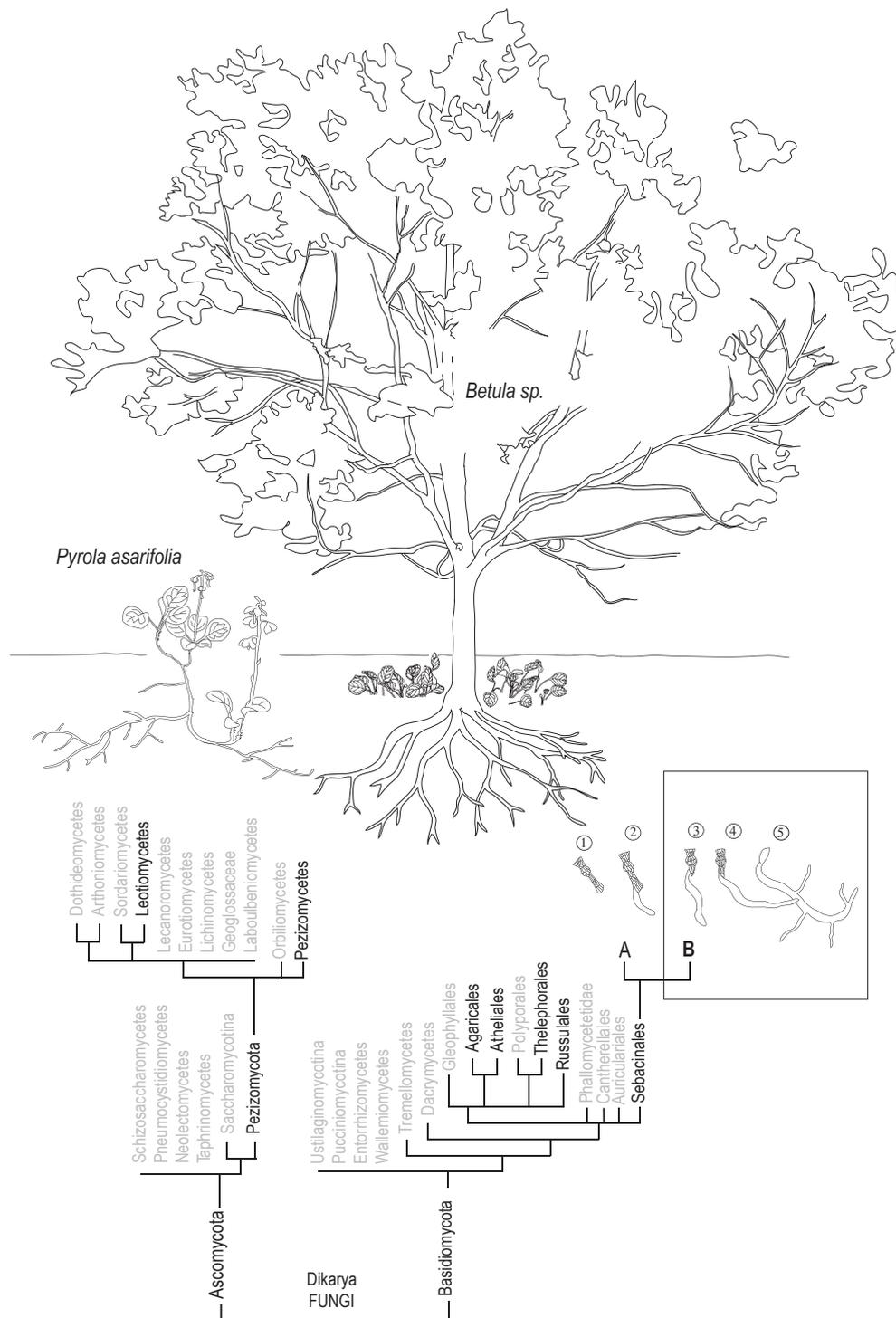
Other species in the family Ericaceae, specifically those in the tribes Monotropeae and Pterosporeae, also require fungi for

germination and seedling development (Bidartondo & Bruns, 2005). However, unlike most Pyroleae species, adult plants in Monotropeae and Pterosporeae are achlorophyllous, lack the ability to photosynthesize, and remain mycoheterotrophic throughout their lifecycles (Leake, 1994). Even past their seedling stages these species retain tight associations with specific fungal partners that continue to provide them with C and other nutrients (Bidartondo, 2005). The partners involved in the mycoheterotrophic lifestyles of Pterosporeae and Monotropeae species all belong to the phylogenetically diverse functional guild known as ectomycorrhizal fungi (Bidartondo, 2005). Ectomycorrhizal fungi are common symbionts of many trees, and receive photosynthetically derived C from their host trees in exchange for providing an increased supply of essential nutrients such as nitrogen and phosphorus (Smith & Read, 2008). Single ectomycorrhizal fungal genets can simultaneously form symbiotic associations with multiple plants, thereby physically connecting unrelated plant taxa (Fig. 1; Simard & Durall, 2004). Thus, the ultimate C sources for fully myco-heterotrophic Pterosporeae and Monotropeae species are surrounding autotrophic trees.

Recent research on the nutritional modes of some adult orchid and Pyroleae species previously thought to be autotrophic has found that they are actually living partially mycoheterotrophic lifestyles (Selosse & Roy, 2009). Partial mycoheterotrophy entails leafy-green plants acquiring C both from photosynthesis and fungi. Similar to many fully mycoheterotrophic plants, they utilize ectomycorrhizal fungi to meet their C demands. However, in the family Orchidaceae partial and full mycoheterotrophy are exceptional nutritional strategies, with only *c.* 200 species discovered thus far. The majority of orchids are only mycoheterotrophic during germination and seedling development. During these phases, most orchids rely on otherwise saprotrophic or plant parasitic fungi rather than ectomycorrhizal fungi for nourishment (Dearnaley, 2007). Additionally, the association of orchids with these fungi can be specialized on specific fungal strains, or broader involving different families within Basidiomycota (Dearnaley, 2007). It is unknown if the majority of Pyroleae species are, like most orchids, only mycoheterotrophic in their earliest developmental stages and reliant on mycobionts other than ectomycorrhizal fungi. The findings of Hashimoto *et al.* provide new and interesting insights into the life history strategy of at least one Pyroleae species.

### Unearthing the secrets to Pyroleae germination

Until now, the fungi responsible for Pyroleae seed germination and early development were largely unknown, but were suspected to be ectomycorrhizal fungi. This suspicion was based on prior findings that the roots of adult Pyroleae species harbor a diversity of ectomycorrhizal fungi (Tedersoo *et al.*, 2007; Zimmer *et al.*,



**Fig. 1** Fungal associates of *Pyrola asarifolia* adult plants, seedlings, and *Betula* spp. as determined by Hashimoto *et al.* (in this issue of *New Phytologist* pp. 620–630). Fungal lineages in bold contain ectomycorrhizal taxa that form symbioses with *Betula* spp., a subset of which also associate with adult *P. asarifolia* plants. Whereas the seedlings of *P. asarifolia* shown in the five developmental stages delineated by Hashimoto *et al.* associate strictly with the nonectomycorrhizal fungal lineage Sebaciales clade B in stages 3–5. The fungi associated with younger seedlings in stages 1–2 are yet to be determined. The fungal phylogeny is adapted from the AFTOL project (Blackwell *et al.*, 2012).

2007; Vincenot *et al.*, 2008; Hynson & Bruns, 2009; Toftegaard *et al.*, 2010). However, the data presented by Hashimoto *et al.* provide a surprising twist to these expectations. The authors were able to bait and recover fungi from *P. asarifolia* seedlings via *in situ* seed burial and germination at 17 sites on the island of Hok-

kaido, Japan, and via *ex situ* germination in mesocosms. Using DNA barcoding methods they were able to identify the fungi responsible for *P. asarifolia* seedling development, as well as those associated with adult plants and surrounding trees (Fig. 1). The fungi they identified from tree, and adult *P. asarifolia* roots were

similar to previous findings; *P. asarifolia* roots were colonized by a diversity of ectomycorrhizal fungi that were a subset of those associated with surrounding trees (Fig. 1). However, and surprisingly, *P. asarifolia* seedlings were not associated with ectomycorrhizal fungi. Rather, they were solely associated with specific and closely related fungi in the nonectomycorrhizal group Sebaciales clade B (Fig. 1; *sensu* Weiss *et al.*, 2004). This suggests that the physiological requirements for initial mycoheterotrophy in Pyroleae may be more similar to some groups of orchids than closely related species in Pterosporeae or Monotropeae. The authors also hint at currently unpublished data that indicates *P. asarifolia* remains partially mycoheterotrophic into adulthood. Combined, these results provide an example of a truly unique and previously undocumented symbiotic interaction between plants and fungi. As the plant develops, it not only gains additional fungal partners, but likely switches from nonectomycorrhizal to ectomycorrhizal sources of C. Whether this change in fungal partners is associated with a change in the type of C compounds received by *P. asarifolia* is not yet known, but is an intriguing avenue for further research.

### Ericaceae and Orchidaceae two seeds in a pod?

Most species of orchids within the subtribe Caladeniinae, and at least a few within the closely related Prasophyllinae and Acianthinae, are associated with Sebaciales clade B fungi as mycoheterotrophic seedlings (Dearnaley, 2007). Some of these orchids, such as species in the genus *Caladenia*, form specific and lifelong symbioses with these fungi. Others, such as some *Microtis* species, partner with Sebaciales clade B fungi in addition to other common orchid symbionts, such as fungi in the order Tulsanellales (Dearnaley, 2007 and references cited therein). With a couple of possible exceptions, there are no clear examples of orchids that begin their lives dependent on Sebaciales clade B fungi and then switch to ectomycorrhizal fungi. However, there are many thousands of species throughout the Orchidaceae for which seedling and/or adult mycobionts have not yet been investigated. It remains to be seen if some orchids, similar to *P. asarifolia*, transition from Sebaciales clade B fungi in their seedling stages to ectomycorrhizal fungi later in life.

### A prix fixe of fungi

The alternation of fungal partners in *P. asarifolia* may have been selected for based on the availability and suitability of fungal hosts. Owing to their complete reliance on fungi early in life, it is not surprising that seedlings of *P. asarifolia* depend on a relatively common and widely distributed group of fungi such as those in Sebaciales clade B. This group, which apparently includes saprotrophs, endophytes, and mycorrhizal mutualists of other Ericaceae species and several liverwort families, is notable for its ecological plasticity (Selosse *et al.*, 2007; Weiss *et al.*, 2011). However, such fungi outside the ecto- and arbuscular mycorrhizal guilds rarely host partially or fully mycoheterotrophic species, suggesting that they are poorly suited to supporting adult plants. This is consistent with the shift in *P. asarifolia* from

Sebaciales clade B to ectomycorrhizal fungi at an unknown ontogenic stage (Fig. 1).

Associating with nonectomycorrhizal fungi during early development may be a strategy evolved by *P. asarifolia*, and possibly other Pyroleae species, that limits seedlings' competition with conspecific adults for C. As Hashimoto *et al.* demonstrate, seedling germination was not enhanced in the presence of adult plants. Interestingly, this lack of 'parental nurture' is not the case among select other ectomycorrhizal plants. For instance, the seedlings of some ectomycorrhizal tree species have increased survival rates when living close to their parent trees (van der Heijden & Horton, 2009). This increase in conspecific seedling survival is attributed to shared ectomycorrhizal fungi through which a parent tree can pass C fixed in the overstory to seedlings growing in the light-depleted understory. Because Pyroleae species spend their entire lives in the understory, competition for light remains a selective pressure throughout their adult stages. Partial mycoheterotrophy allows some Pyroleae species to skirt their dependency on light for survival, but this lifestyle may come at a cost to their fungal partners. Therefore, to diffuse competition for fungal C among seedlings and adults, and to short circuit the arms race between plants exploiting fungal C and fungi evolving resistance, increased seed germination away from parent plants and with a nonectomycorrhizal fungus would certainly be beneficial. What remains to be determined is the developmental stage at which the shift in fungal symbionts takes place, and if failure to make this switch is a bottleneck to survival.

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