

Forum

Mixotrophy in Land Plants: Why To Stay Green?

Jakub Těšitel,¹
 Tamara Těšitelová,²
 Julita Minasiewicz,³ and
 Marc-André Selosse^{3,4,*}

Mixotrophic plants combine photosynthesis and heterotrophic nutrition. Recent research suggests mechanisms explaining why mixotrophy is so common in terrestrial ecosystems. First, mixotrophy overcomes nutrient limitation and/or seedling establishment constraints. Second, although genetic drift may push mixotrophs to full heterotrophy, the role of photosynthesis in reproduction stabilizes mixotrophy.

Evolution of Plant Nutrition

Land plants are commonly considered to be **autotrophic** (see [Glossary](#)) organisms that require light, water, and mineral nutrients. These resources are available in limited supply, entailing competition between co-occurring individuals. Plants have evolved strategies to facilitate acquisition of these resources. For example, most plants form associations with microorganisms, such as **mycorrhizal** or nitrogen-fixing symbioses, which provide mineral nutrients gained by microorganisms in exchange for organic carbon. Other plants developed heterotrophic nutrition by exploiting more or less directly surrounding plants by two alternative pathways: **mycoheterotrophy** and plant parasitism. Mycoheterotrophic plants revert the usual carbon flow in plant–fungus mycorrhizal mutualism and extract carbon from fungi, which themselves receive carbon from neighboring plants

or organic substrates [1]. Mycoheterotrophy evolved more than 18 times and encompasses ~30 800 species (updated from [1]). Plant parasitism is a direct parasitic interaction with neighboring plants, which provides the parasite with mineral nutrients, water, and a variable amount of organic carbon [2]. This strategy evolved at least 12 times and encompasses ~4500 species [2,3].

The great majority of mycoheterotrophic and parasitic plants retain photosynthesis, and thus combine autotrophy and **heterotrophy**. This strategy, called **mixotrophy**, turns out to be common among all life domains in land and aquatic ecosystems [4].

Modes of Mixotrophy in Plants

Mixotrophic parasitic plants, called hemiparasites, are almost completely dependent on the host for water and mineral nutrients, but individual species differ in their host-dependency for carbon ([Figure 1](#)). Most species display rates of photosynthesis comparable to those of non-parasitic plants, but others have rudimentary photosynthetic activity, well below the compensation point [2]. Encompassing >4000 species and several life forms (root hemiparasites, mistletoes, and parasitic vines), hemiparasites account for almost 90% of parasitic plant species richness [2,3] ([Figure 1](#)).

Mixotrophic plants obtaining part of their resources from mycoheterotrophy display two non-exclusive strategies. Some are mixotrophic at adulthood (e.g., some Ericaceae and some orchids) and are simply called partial mycoheterotrophs [5]. Others exclusively use fungal carbon for germination, before turning green (and then being partially or fully autotrophic) at adulthood: the so-called initial mycoheterotrophs include all orchids and horsetails (lycops) [1] ([Box 1](#)). Partial and initial mycoheterotrophs encompass >30 300 species (most of which are orchids), and

Glossary

Autotrophy: the ability of an organism to produce its own organic matter, for example by photosynthesis.

Fitness: the evolutionary value of an individual, which can be approximated by its number of offspring.

Hemiparasitism: mixotrophic nutrition mixing autotrophy and parasitic extraction of carbon and other resources from an autotrophic plant.

Heterotrophy: the ability of an organism to acquire organic resources from another organism without a corresponding payoff.

Holoparasitism: heterotrophic nutrition based on parasitic extraction of carbon from a neighboring autotrophic plant.

Mixotrophy: the ability of an organism to mix heterotrophy and autotrophy.

Mutational drift: the random process of accumulation of gene mutations that lead, in the absence of counter-selection, to their inactivation over time.

Mycoheterotrophy: fully or partially heterotrophic nutrition, based on the extraction of carbon from the fungi that form mycorrhizae on roots.

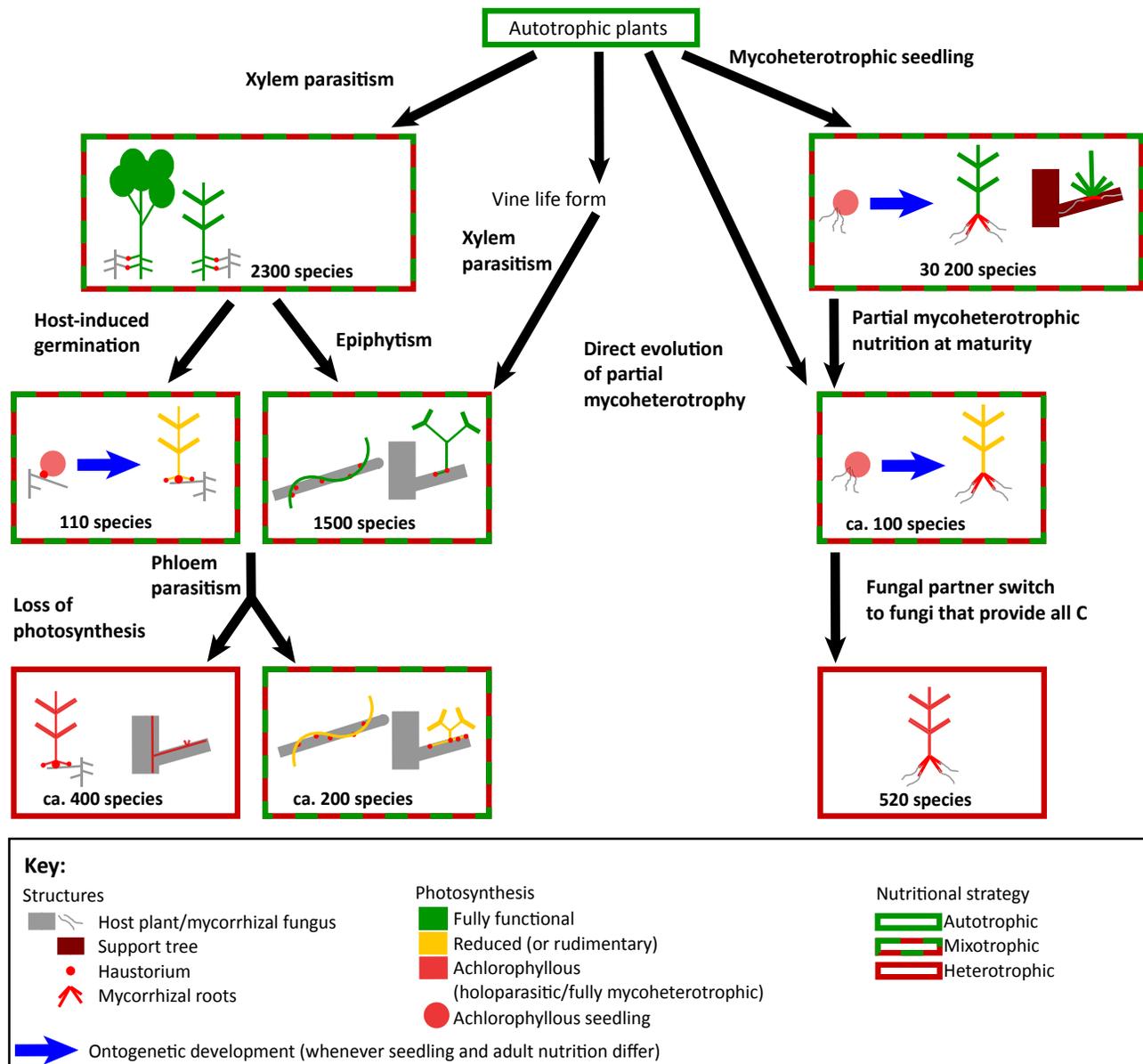
Mycorrhiza: a symbiotic association between soil fungi and the roots of ~90% of land plants.

Phloem: one of two tissues transporting nutrients in vascular plants (together with xylem) that transport soluble organic compounds issuing from photosynthesis (mainly sucrose) to diverse heterotrophic parts of the plant.

Xylem: one of two tissues transporting nutrients in vascular plants (together with phloem) that transport water, mineral nutrients, and a small proportion of organic carbon from roots to aerial parts.

account for 98% of mycoheterotrophic plant species richness [1] ([Figure 1](#)).

Most mixotrophic lineages also encompass fully heterotrophic species [2,5], and mixotrophy is considered to be an evolutionary step from which heterotrophy evolved [4]. Nevertheless, mixotrophic plants out-number purely heterotrophic plants by 10–100-fold. Moreover, phylogenies often show fully heterotrophic groups at the tip of long branches (e.g., [1,3]), which suggests limited diversification of these groups. We analyze here the evolutionary success of mixotrophic species in terms of species diversity over heterotrophic species.



Trends in Plant Science

Figure 1. Evolutionary Pathways to Plant Heterotrophy Based on Plant Parasitic (Left Side) and Mycoheterotrophic (Right Side) Strategies. Major evolutionary innovations facilitating heterotrophic resource acquisition are indicated by arrows. Rectangles indicate the nutritional strategy and encapsulate the principal evolutionary steps in plants with similar physiological functioning and ecological interactions. Abbreviation: C, carbon.

Why Is Mixotrophy Adaptive?

Owing to ecological succession and competition for light, terrestrial ecosystems tend to form canopies that intercept light well above the soil, where mineral resources are stored and where most seeds germinate. This results in spatial

partitioning of resources between the light-limited understorey and the nutrient-limited canopy, and imposes constraints on regeneration from seeds. In this framework, mixotrophy is an adaptive ecological strategy that facilitates mineral and carbon nutrition [4]. In hemiparasites,

the development of a connection to the host **xylem** (Figure 1) facilitates mineral and water supply, providing competitiveness over non-parasitic species. This benefit is often realized through an increase of photosynthetic activity in hemiparasites in virtue of mineral nutrients

Box 1. Germination and Seedling Establishment in Mycoheterotrophic and Parasitic plants

All mycoheterotrophic plants, and many partially mycoheterotrophic plants, produce tiny dust-like seeds (or even spores, in lycopods) with few reserves. Early growth starts only after carbon and nutrients are provided by mycorrhizal fungi. The presence of the necessary fungus is a prerequisite for successful mycoheterotrophic germination [1], and often limits the distribution of these species. In parasitic plants, the evolutionary transition to **hemiparasitism** is not associated with any major modifications of seed structure or germination. The seeds of most root hemiparasites germinate independently of the presence of a host, and the seedling subsequently produces roots to forage for host roots in the soil [2]. Seedlings of stem parasites penetrate the host stems either immediately after germination (mistletoes) or forage for the host by growing their stems (parasitic vines). Host-induced germination comparable to the fungus-assisted seedling growth of mycoheterotrophs represents a further evolutionary step in root parasites: it is present in those root hemiparasites, whose seedlings are fully parasitic, as well as in most **holoparasitic** lineages [2]. It is sometimes also associated with the production of dust-like seeds (e.g., in *Striga*). This later evolutionary innovation is associated with improved carbon nutrition of seedlings, and, exactly as for mycoheterotrophic germination, it releases seedlings from above-ground competition for light with surrounding plants.

obtained from the hosts [6]. A limited amount of carbon is also obtained by hemiparasites from the host xylem, which serves as a 'backup' resource under conditions of stress or competition for light [6]. Partial mycoheterotrophs often adapt to shaded forests by taking advantage of fungal carbon. Balancing the ratio of fungal versus autotrophic nutrition, the level of mycoheterotrophy level can be flexible, which allows adaptation to various (or even annually variable) light levels [7,8]. Initial mycoheterotrophy supplies organic carbon at germination, a critical phase of the plant life cycle [1,4]. This nutrition of seedlings facilitates their establishment and avoids competition for light with adults of the same and other plant species.

Overcoming at least one of the crucial constraints for mineral or carbon nutrition probably opened the way to the evolutionary diversification of mixotrophs. Additional diversifications result from the evolution of mistletoes and parasitic vines, which colonize canopies devoid of nutrients and water by relying on the resources of the host.

Mixotrophy Is Evolutionarily Metastable

One may naively see the two carbon sources of mixotrophs as being redundant, an evolutionarily unstable situation where one or the other source can be lost.

The genetic toolkit for mycoheterotrophy or plant parasitism is poorly understood, although the first interesting patterns are now emerging from genome sequencing efforts [9]. By contrast, the genetic toolkit for photosynthesis involves a very complex set of genes, as exemplified by the fact that ~1% of mutants obtained in a random mutagenesis are non-photosynthetic [5]. Thus, mixotrophic species should be pushed by **mutational drift** to heterotrophy once mixotrophy is achieved, because the persistence of a costly, redundant photosynthetic carbon source is evolutionarily unexpected.

Evolution of full heterotrophy, however, requires special adaptations: in full plant parasites (holoparasites), improved carbon nutrition is mostly associated with the uptake resources from host **phloem** [10]; in full mycoheterotrophs, a switch to different fungal partners providing more carbon is often observed [1]. Recent evidence suggests that such evolutionary steps may be difficult, requiring for example many physiological and developmental adaptations in full mycoheterotrophs [5], or multiple horizontal gene transfer events from the host in the case of holoparasites [9]. Moreover, these adaptations frequently entail higher specificity in heterotrophs compared to relatively generalistic mixotrophic interactions [1,2,11], which imposes stronger ecological constraint on the availability

of partners. This may restrict the evolution of fully heterotrophic plants and thus explain their relatively low species counts.

The importance of the two carbon sources also differs along the life cycle of mixotrophic plant, as highlighted by recent research monitoring natural ^{13}C abundance in these plants. The organic resources derived from a fungal partner or a host with C_4 metabolism naturally differ from the photosynthates of the mixotroph in carbon stable-isotope composition ($^{13}\text{C}/^{12}\text{C}$ ratio). This allows estimation of the carbon contributions of heterotrophic and autotrophic pathways in different organs, based on ^{13}C enrichment [4,6,10]. In orchids that are partially mycoheterotrophic at adulthood, ^{13}C enrichment shows that fungal carbon is used for rhizome survival and initial growth of the inflorescence [6]. Similarly, the hemiparasitic *Striga hermonthica* relies on host-derived carbon during early ontogeny [10]. Conversely, mixotrophs use their own photosynthates after leaf expansion for fruit and seed maturation in both cases [6,10]. Additional observations support this scenario. First, experimental eradication of the fungus at flowering time does not affect fruiting in partial mycoheterotrophs [12]. Second, rare non-chlorophyllous mutants of mixotrophic orchids, which live *in natura* by using exclusively fungal resources, survive well but produce 1000-fold fewer viable seeds [4]. Among parasitic plants, rudimentary photosynthetic activity is also important for seed production and even seedling establishment in mostly heterotrophic dwarf mistletoes (*Arceuthobium*) and dodders (*Cuscuta*) [9] (Box 1). Thus, the importance of photosynthesis for reproduction in mixotrophs may make its evolutionary regression more complex than simple loss of function [4].

To summarize, the transition from mixotrophy to full heterotrophy requires numerous adaptive steps, and the

photosynthetic activity provides mixotrophic plants with the capacity to capitalize on the benefits provided by their heterotrophic resource acquisition pathway (by improved seed production and/or improved germination, better mineral nutrition). Although they are irrelevant for fully heterotrophic plants, these benefits hinder the evolution to heterotrophy. We suggest that these mechanisms explain the abundance of mixotrophs and the metastability of mixotrophy in plant evolution.

Acknowledgments

J.T. and T.T. were supported by the Czech Science Foundation projects nos. 14-36079G (Centre of Excellence PLADIAS) and 16-05677S, respectively. M.-A. Selosse is supported by the Fondation de France and his research together with J.M. is supported by the 2015/18/A/NZ8/00149 grant funded by National Science Centre (Poland).

¹Masaryk University, Department of Botany and Zoology, Kotlarska 267/2, 611 37 Brno, Czech Republic

²University of South Bohemia, Faculty of Science, Branisovska 1760, 370 05 Ceske Budejovice, Czech Republic

³Department of Plant Taxonomy and Nature Conservation, University of Gdansk, Wita Stwosza 59, 80-308 Gdansk, Poland

⁴Institut de Systématique, Évolution, Biodiversité (ISYEB – UMR 7205–CNRS, MNHN, UPMC, EPHE), Muséum national d'Histoire naturelle, Sorbonne Universités, 57 rue Cuvier, CP50, 75005 Paris, France

*Correspondence:

ma.selosse@wanadoo.fr (M.-A. Selosse).

<https://doi.org/10.1016/j.tplants.2018.05.010>

References

1. Merckx, V. (ed.) (2013) *Mycoheterotrophy, the Biology of Plants Living on Fungi*, Springer
2. Těšitel, J. (2016) Functional biology of parasitic plants: a review. *Plant Ecol. Evol.* 149, 5–20
3. Naumann, J. et al. (2013) Single-copy nuclear genes place haustorial Hydnoraceae within piperales and reveal a cretaceous origin of multiple parasitic angiosperm lineages. *PLoS One* 8, e79204
4. Selosse, M.-A. et al. (2017) Mixotrophy everywhere on land and water: the grand écart hypothesis. *Ecol. Lett.* 20, 246–263
5. Roy, M. et al. (2013) Why do mixotrophic plants stay green? A comparison between green orchid and achlorophyllous individuals *in situ*. *Ecol. Monogr.* 83, 95–117
6. Těšitel, J. et al. (2015) Integrating ecology and physiology of root–hemiparasitic interaction: interactive effects of abiotic resources shape the interplay between parasitism and autotrophy. *New Phytol.* 205, 350–360
7. Preiss, K. et al. (2010) Irradiance governs exploitation of fungi: fine-tuning of carbon gain by two partially myco-heterotrophic orchids. *Proc. R. Soc. B* 277, 1333–1336
8. Gonneau, C. et al. (2014) Photosynthesis in perennial mixotrophic *Epipactis* spp. (Orchidaceae) contributes more to shoot and fruit biomass than to hypogeous survival. *J. Ecol.* 102, 1183–1194
9. Yang, Z. et al. (2016) Horizontal gene transfer is more frequent with increased heterotrophy and contributes to parasite adaptation. *Proc. Natl. Acad. Sci. U. S. A.* 113, E7010–E7019
10. Santos-Izquierdo, B. et al. (2008) Targeted distribution of photo-assimilate in *Striga hermonthica* (Del.) Benth parasitic on *Sorghum bicolor* L. *Phytochem. Lett.* 1, 76–80
11. Thorogood, C.J. et al. (2010) Compatibility interactions at the cellular level provide the basis for host specificity in the parasitic plant *Orobanche*. *New Phytol.* 186, 571–575
12. Bellino, A. et al. (2014) Photosynthesis regulation in mixotrophic plants: new insights from *Limodorum abortivum*. *Oecologia* 175, 875–885