

Stealing sugar from the honey fungus

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Over 95% of all terrestrial plant species form an intimate structural and functional interaction with hyphae of soil fungi—the mycorrhiza (Smith & Read, 2008). The nature of this interaction is widely seen as mutualistic, with plants providing the fungi with fixed carbon and fungi delivering mineral nutrients to their plant partners. This is, however, not the complete picture. Almost 1,000 plant species from at least 17 families and more than 100 genera have clearly subverted this mutualistic interaction (Leake, 1994; Merckx et al., 2013). These plants are achlorophyllous and rely completely on their fungal partners for carbon and mineral nutrient supply. Their type of nutrition is classified as *full mycoheterotrophy* (Merckx, 2013) and may arguably be referred to as parasitism. One of these species is *Gastrodia elata* (Figure 1), the object of an important study by Ho et al. (2020) elucidating the physiology of a fungus-to-plant sugar transport and published in this issue of *Plant, Cell & Environment*. *G. elata* belongs to the almost 250 fully mycoheterotrophic orchids currently known (Merckx et al., 2013).

Growing evidence suggests that among the orchids several types of nutrition exist, positioned somewhere between mutualism and full parasitism as two endpoints of a spectrum. The orchid mycorrhiza is a highly intimate interaction between plants and fungi for two reasons: (a) All orchids form an endomycorrhiza, with fungal hyphae growing into the symplast of the root cortex cells, forming dense hyphae coils (pelotons) and providing a high surface area for matter exchange (Smith & Read, 2008). (b) Orchids produce millions of tiny dust-like seeds without endosperm and thus, are completely dependent on carbon and mineral nutrient supply by their mycorrhizal fungi for their germination and early seedling development (protocorm stage) (Smith & Read, 2008). This heterotrophic start into orchid life is classified as *initial mycoheterotrophy* (Merckx, 2013). Later on, the vast majority of orchid species develop chlorophyllous leaves for photosynthesis, but remain mycorrhized throughout their entire life cycle. The most frequent fungal partners of initially mycoheterotrophic

orchids are easily cultivable, ubiquitous saprotrophic basidiomycetae of the rhizoctonia group (Bernard, 1909). However, saprotrophic and pathogenic wood or litter decomposing fungi as well as fungi simultaneously forming ectomycorrhizas (ECM) with neighbouring trees have been identified as partners of initially mycoheterotrophic orchid protocorms, too. These fungi are almost exclusively partners of the fully mycoheterotrophic adult orchids.

A hallmark of ECM and of wood or litter decomposing fungi is their enrichment in heavy stable carbon (^{13}C) and nitrogen (^{15}N) isotopes in comparison to neighbouring autotrophic plants. As a consequence, mycoheterotrophic orchids associated with these two functional groups of fungi become also significantly enriched in ^{13}C and ^{15}N relative to neighbouring plants (Gebauer & Meyer, 2003; Ogura-Tsujita, Gebauer, Hashimoto, Umata, & Yukawa, 2009). The documented ^{13}C and ^{15}N enrichment in fully mycoheterotrophic ECM-associated orchids was instrumental to identify another type of mycoheterotrophic nutrition among the chlorophyllous orchids, which were found to be isotopically positioned between achlorophyllous fully mycoheterotrophic orchids and neighbouring autotrophic non-orchids (Gebauer & Meyer, 2003). This means that chlorophyllous ECM-associated orchids gain carbon simultaneously from two sources, namely their own photosynthesis and fungal partners. This type of nutrition is now classified as *partial mycoheterotrophy* (Merckx, 2013). Interestingly, all fully mycoheterotrophic orchids and the ECM-associated, partially mycoheterotrophic orchids share deeply shaded forest grounds as habitats. Thus, a predisposition due to an initially mycoheterotrophic start into the life of all orchids, together with light limitation for photosynthesis are likely drivers towards partially and fully mycoheterotrophic nutrition (Preiss, Adam, & Gebauer, 2010). What remains open is the question where the adult chlorophyllous rhizoctonia-mycorrhized orchids stand. Recent analyses showed that they are significantly enriched in ^2H (Gebauer, Preiss, & Gebauer, 2016), which is indicative of heterotrophic

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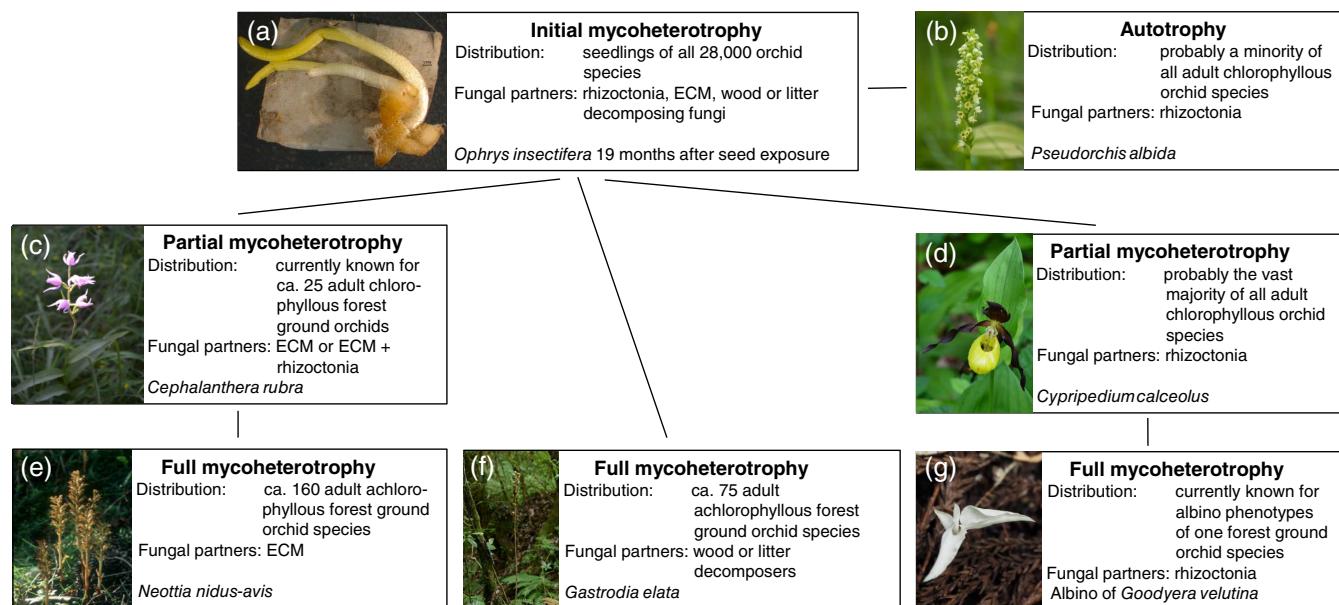


FIGURE 1 Types of carbon gains by orchids based on development stages and mycorrhizal association with different functional groups of fungal partners as well as their frequency distribution within the orchid family. Photographs show examples for each type. Until now only one adult fully mycoheterotrophic orchid species associated with fungi of the rhizoctonia group, *Goodyera velutina* as albedo phenotype, has been identified (Suetsugu, Yamato, Matsubayashi, & Tayasu, 2019). Images courtesy of Julienne Schweiger (a,b), Sheng-Kun Yu (f) and Shitaro Takuto (g). Images (c, d and e) by Gerhard Gebauer

nutrition (Cormier, Werner, Leuenberger, & Kahmen, 2019). Only one chlorophyllous rhizoctonia-mycorrhizal orchid species, *Pseudorchis albida*, turned recently out as neither significantly enriched in ^{13}C , ^{15}N or ^2H (Schiebold, Bidartondo, Lenhard, Makiola, & Gebauer, 2018) and therefore, has to be considered as probably autotrophic (Figure 1). Thus, the vast majority of all adult chlorophyllous rhizoctonia-mycorrhizal orchid species is probably partially mycoheterotrophic, though apparently less successful in gaining carbon from fungal partners than ECM-associated partial mycoheterotrophs (Schiebold et al., 2018). Apparently, truly autotrophic orchids are only a minority. A fungus-to-plant organic carbon transport is, thus, not limited to a few unique orchid species, but rather is a widespread phenomenon within the entire orchid family.

Unfortunately, knowledge about the type of carbon compounds transferred from fungi to plants and the underlying transport mechanisms is widely missing. The same applies to the other direction of carbon transfer in mycorrhizal associations, that is, the supply of fixed carbon from plants to their fungal partners. Even in the most intensively studied plant-fungal symbioses, arbuscular mycorrhiza and ectomycorrhiza, major questions remain unanswered. Carbon compounds have to pass at least two membranes and the extracellular space between the membranes on their way from one partner to the other. Proteins mediating these membrane passages have not been unequivocally identified (Roy, Reinders, Ward, & McDonald, 2020). The study by Ho et al. (2020), therefore, represents an important advance for the understanding of plant-fungal associations in general. The authors analysed carbon acquisition of the fully mycoheterotrophic achlorophyllous orchid *G. elata*, a popular species in Chinese traditional

medicine. The genome of *G. elata* shows hallmarks of heterotrophy such as an overall reduced number of protein-coding genes and specifically the loss of genes essential for photosynthesis (Yuan et al., 2018). *G. elata* spends much of its lifecycle as a tuber underground and in association with the basidiomycete *Armillaria mellea*, also known as honey mushroom, a common root pathogen with a wide host range. Ho et al. (2020) queried transcriptome data for *G. elata* tubers to identify carbon transfer candidate genes. This strategy is widely used to address the challenging questions as to which nutrients are transferred between plants and fungi, and which transporters are involved in the nutrient exchange (Roy et al., 2020). The two genes GeSUT3 and GeSUT4 encoding putative sucrose transport (SUT/SUC) proteins were singled out, because the authors identified sucrose as the dominant sugar in *G. elata* tubers. SUTs are H^+ /sucrose symporters involved in phloem loading and in sucrose uptake into sink cells (Julius, Leach, Tran, Mertz, & Braun, 2017). Sequence similarity to known sucrose transporters from *Arabidopsis thaliana* and various crop species was high enough to assign GeSUT3 and 4 to the well-defined SUT/SUC clades SUT2-IIIB and SUT4, respectively. Transport studies with yeast cells expressing GeSUT4, indeed, established proton coupling as well as a high specificity and affinity for sucrose. Strong expression in *Armillaria*-infected tuber cells was confirmed by *in situ* hybridization.

SUTs fall into different clades with typical subcellular localization, that is, either in the plasma membrane or the tonoplast. Sequence similarities place GeSUT4 in a group with vacuolar transporters. Surprisingly, the authors found evidence for dual targeting to plasma membrane and the tonoplast. Correspondingly, the proposed model implicates GeSUT4 in several processes. Firstly, GeSUT4 is postulated

to mediate the uptake of sucrose into cells infected by the fungus and in the uptake into neighbouring large cells, which appear to be symptomatically separated from the infected cells by a thick cell wall without plasmodesmata. Secondly, GeSUT4 may function in the export of stored sucrose from the vacuoles of infected and large cells. The sucrose uptake activity is further supported by observations in *A. thaliana* plants overexpressing GeSUT4. High external sucrose was toxic and, more importantly, root colonization by mutualistic bacteria was impaired, presumably because uptake mediated by GeSUT4 lowered the rhizosphere sucrose concentration the bacteria can feed on. Interestingly, this is consistent with the findings of perhaps the only study published to date reporting genetic evidence for the physiological role of a plant sugar transporter in a mycorrhizal interaction (Bitterlich, Krügel, Boldt-Burisch, Franken, & Kühn, 2014). Tomato plants impaired in the function of SISUT2, a transporter localized in the periarbuscular membrane, showed more efficient establishment of arbuscular mycorrhiza. This was interpreted as an inhibiting effect of sucrose uptake back into root cells, which limits the carbon availability for the fungus. Thus, sugar uptake from the extracellular space between fungal hyphae and invaded plant cells appears to be a common feature of plant-fungal associations both in autotrophic plants and fully mycoheterotrophic orchids regardless of the direction of the net carbon transfer. This, indeed, illustrates the evolutionary importance of sucrose transporters in plant-environment interactions. What remains to be determined in the specific case of *G. elata* is the metabolic origin of the sucrose in the extracellular space, the molecular identity of the sucrose efflux system and the physiological function of GeSUT3, for which no transport activity could be directly demonstrated.

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