Naturalis Repository

The role of mycorrhizal fungi in driving ecotype formation in mycoheterotrophic plants

Hans Jacquemyn, Kenji Suetsugu and Vincent Merckx

DOI
https://doi.org/10.1111/njb.04083

Downloaded from
Naturalis Repository

Article 25fa Dutch Copyright Act (DCA) - End User Rights
This publication is distributed under the terms of Article 25fa of the Dutch Copyright Act (Auteurswet) with consent from the author. Dutch law entitles the maker of a short scientific work funded either wholly or partially by Dutch public funds to make that work publicly available following a reasonable period after the work was first published, provided that reference is made to the source of the first publication of the work.

This publication is distributed under the Naturalis Biodiversity Center 'Taverne implementation' programme. In this programme, research output of Naturalis researchers and collection managers that complies with the legal requirements of Article 25fa of the Dutch Copyright Act is distributed online and free of barriers in the Naturalis institutional repository. Research output is distributed six months after its first online publication in the original published version and with proper attribution to the source of the original publication.

You are permitted to download and use the publication for personal purposes. All rights remain with the author(s) and copyrights owner(s) of this work. Any use of the publication other than authorized under this license or copyright law is prohibited.

If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the department of Collection Information know, stating your reasons. In case of a legitimate complaint, Collection Information will make the material inaccessible. Please contact us through email: collectie.informatie@naturalis.nl. We will contact you as soon as possible.

NORDIC JOURNAL OF
BOTANY

Review

The role of mycorrhizal fungi in driving ecotype formation in mycoheterotrophic plants

Hans Jacquemyn[1], **Kenji Suetsugu**[2] **and Vincent Merckx**[3,4]

[1]Department of Biology, KU Leuven, Heverlee, Belgium
[2]Department of Biology, Graduate School of Science Kobe University, Hyogo, Kobe, Japan
[3]Understanding Evolution, Naturalis Biodiversity Center, Leiden, the Netherlands
[4]Department of Evolutionary and Population Biology, Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, Amsterdam, the Netherlands

Correspondence: Hans Jacquemyn (hans.jacquemyn@kuleuven.be)

Nordic Journal of Botany
2023: e04083
doi: 10.1111/njb.04083

Subject Editor: Pål Axel Olsson
Editor-in-Chief: Torbjörn Tyler
Accepted 18 August 2023
Published 6 October 2023

In his seminal paper, Göte Turesson proposed the term ecotype as the 'ecological unit to cover the product arising as a result of the genotypical response of an ecospecies to a particular habitat'. Turesson further outlined that to fully understand the ecology of a species, a knowledge of its most important ecotypes is needed. Whereas Turesson's original idea of an ecotype mainly referred to the response of a species to abiotic conditions, there is mounting evidence that ecotypes can also originate as a response to biotic conditions. Hence, to understand the ecology of a species, one should also understand the distribution and variation in biotic interactions. For plants, one such biotic interaction involves mycorrhizal fungi. Particularly in mycoheterotrophic plants, i.e. plants that rely on mycorrhizal fungi for their carbon supply, adaptation to and specialization on mycorrhizal fungi have the potential to drive ecotype formation and speciation. In this paper, we provide evidence that populations of mycoheterotrophic plants inhabiting contrasting habitats commonly encounter divergent mycorrhizal fungal communities leading to geographic mosaics of mycorrhizal interactions. Adaptation to local fungal communities, in turn, can induce (partial) reproductive isolation and contribute to ecotype and ultimately species formation as a result of decreased fitness of immigrant genotypes (immigrant inviability). In the most extreme case, loss of photosynthesis and mycorrhizal switching have led to reproductive isolation and the development of novel species that have become fully mycoheterotrophic. Such shifts have occurred repeatedly during the evolutionary history of land plants. We conclude that mycorrhizal fungi not only contribute to the early divergence of populations of mycoheterotrophic plants, but also play a crucial role in the further diversification and origination of this unique set of species that rely on mycorrhizal fungi for obtaining carbon and completing their life cycle.

Keywords: diversification, Göte Turesson, immigrant inviability, speciation

NORDIC SOCIETY OIKOS

www.nordicjbotany.org

Introduction

One of the main insights of the 19th century was that species are not static entities, but evolve and can give rise to new species as the result of a complex process called natural selection (Darwin and Wallace 1858, Darwin 1859). In most cases, the evolution of new species is a very long process that may take hundreds to thousands of generations (Coyne and Orr 2004). Because of this long time, it is tempting to search for stages during the speciation process (Wallace 1865, Turesson 1922a, Clausen 1951, Grant 1981, Wu 2001, Lowry 2012). One of the first to develop the idea that the evolution of new species goes through a series of successive stages was the Swedish botanist Göte Turesson (1892–1970). Using transplantation and crossing experiments mostly with species from the genera *Atriplex* and *Hieracium* as a study system, Turesson (1922a) showed that species often consist of varieties that differ in many ways from each other dependent on the habitat where they occur. Turesson (1922a) called these varieties ecotypes: 'The term ecotype is proposed here as ecological unit to cover the product arising as a result of the genotypical response of an ecospecies to a particular habitat.' In later writings, Turesson (1922b, 1925, 1929, 1931) indicated that ecotypes have genetically adapted to their particular environments and should not be seen as rigid abstract entities, but are constantly evolving groups that themselves are the product of natural selection.

Building on Turesson's work and his own experiments, Clausen (1951) identified similar groups of locally adapted populations, but he called these no longer ecotypes, but ecological races. Just as in Turesson's work, these ecological races are considered the product of local adaptation processes and have become partially reproductively isolated from each other. As such, ecological races can be considered as one of the earliest stages in the speciation process (Lowry 2012). Although the definition of these stages is clearly arbitrary and other authors have criticized the validity of the terms 'ecotype' and 'ecological race', the recognition that speciation is a slow process that includes the formation of ecologically specialized populations or ecotypes, has significantly advanced the field (Doebeli and Dieckmann 2003, Coyne and Orr 2004, Nosil 2007, Lowry et al. 2008, Nakazato et al. 2008, Schluter 2009). Moreover, the recent development of new molecular tools including high-throughput sequencing and genomics has caused a renewed interest in the topic (Lowry et al. 2008, Nosil et al. 2009) and has brought the work of Turesson back to the forefront (Lowry 2012).

Notwithstanding Tureson (1922b) acknowledged there is a genetic basis to the differentiation of plant populations, the factors driving this differentiation were not always clearly articulated. Since Turesson conducted most of his research along the coast and the inland of Sweden, this led him to believe that abiotic factors were the main drivers of ecotype formation. In his seminal paper, Turesson (1922a) writes: 'As a natural consequence we are led to the inference that a change in the inorganic world must bring about a corresponding change in the organic, inducing a recombination of Mendelian factors now distributed in living organisms and resulting in the formation of new genotype compounds or species (= evolution)'. Hence, we get the notion that new genotypes or species arise solely from changes in the abiotic environment. However, Turesson's successors increasingly stressed the importance of reproductive isolating barriers that limit gene flow in the process of ecotype formation (Gregor 1931, 1938, Gregor et al. 1936, Clausen et al. 1939). Clausen (1951), for example, recognized the importance of many ecological barriers including temporal flowering isolation and pollinator isolation in contributing to reproductive isolation. Clearly, biotic factors that limit gene flow among populations can be expected to be important drivers of ecotype formation. There is mounting evidence that pollinators have the potential to drive speciation at several different stages of the evolutionary process. For example, various studies in the Cape Region of South Africa have shown strong spatial variation in flower traits such as color, shape and odor, and strong matches between pollinator and flower dimensions (Anderson and Johnson 2009, Newman et al. 2014, Van der Niet et al. 2014a). In addition, reciprocal translocation experiments or experiments using artificial flowers have indicated that these flower types are adapted to local pollinator communities, demonstrating that pollinators can be a driving force of ecotype formation in plants. Similar results were found for several other plant species growing in the Cape Region and elsewhere in the world (van der Niet et al. 2014b), indicating that pollinator-driven selection can be an important process driving differentiation in flower morphology among local populations and contributing to ecotype and ultimately species formation (Johnson 2006).

Pollinators are only one of several biotic factors that have the potential to drive divergence between populations. Another biotic factor that can potentially drive population divergence is mycorrhizal fungi. These fungi aid plants with the acquisition of nutrients and water in exchange for photosynthetically derived carbon. It has been estimated that about 90% of all land plants form associations with mycorrhizal fungi under natural conditions (van der Heijden et al. 2015, Brundrett and Tedersoo 2018). However, in some plants, the mutualistic association between plants and fungi has been disrupted and the plants obtain carbon from fungi (Leake 1994). These plants are called mycoheterotrophic plants (Leake 1994, Merckx 2013). Because mycoheterotrophic plants crucially rely on fungi for carbon, it can be expected that they have strongly adapted to fungi and that therefore differences in mycorrhizal fungi can be a determining force driving the divergence of populations of mycoheterotrophic plants (Taylor et al. 2004). However, there is still little information about the role of mycorrhizal fungi in driving ecotype formation and speciation in mycoheterotrophic plants.

The overall aim of this paper is to review the available knowledge about the potential role of mycorrhizal fungi in driving ecotype formation and ultimately speciation in mycoheterotrophic plants. We start our review with a definition of mycoheterotrophy, we then assess the geographical context in which adaptation to fungi can occur, highlight studies that have shown evidence of mycorrhizal fungi driving ecotype formation and finally we put our results in a broader phylogenetic perspective. We conclude this review with future perspectives that may stimulate further research on the role of mycorrhizal fungi in driving ecotype formation and speciation in mycoheterotrophic plants.

Mycoheterotrophic plants

About 90% of vascular plants associate with mycorrhizal fungi to obtain essential minerals (N, P) and water from the soil in exchange for photosynthetically derived carbon (Bonfante and Genre 2010, van der Heijden et al. 2015, Brundrett and Tedersoo 2018) and as such the interaction between plants and mycorrhizal fungi is arguably one of the most important interactions on Earth (Tedersoo et al. 2020). Based on several criteria of morphological differentiation of root tissues and host plant lineages, different types of mycorrhizal fungi can be discerned (Brundrett and Tedersoo 2018) and these fungi associate with different types of plants. Whereas arbuscular mycorrhizal fungi associate with a wide variety of plants, ectomycorrhizal fungi and ericoid mycorrhizal fungi associate primarily with trees and plants from the Ericaceae, respectively. Orchids form a separate group of plant species and commonly associate with a distinct set of saprotrophic fungi, so-called rhizoctonia, although some orchids also associate with ectomycorrhizal (Taylor and Bruns 1997) and wood and litter decaying fungi (Ogura-Tsujita et al. 2009, Suetsugu et al. 2020). In most plant species the relationship between plants and fungi is a mutualistic interaction, i.e. both partners benefit from the association. However, some plants have broken down this mutualistic relationship and partly or completely rely on fungi to obtain their carbon (Leake 1994). These plants are commonly known as 'mycoheterotrophic' plants (Merckx 2013).

Within mycoheterotrophic plants, a distinction can be made between initially, partially and fully mycoheterotrophic plants (Leake 1994, Gebauer and Meyer 2003, Merckx 2013, Jacquemyn and Merckx 2019). Initially mycoheterotrophic plants often produce very small seeds that are devoid of nutritional resources (endosperm) and therefore depend on mycorrhizal fungi for their nutrition during germination and early development (e.g. many species of orchids) or during their gametophyte phase (several species of ferns and lycophytes) and later become putatively autotrophic as adults or sporophytes, respectively. Partially mycoheterotrophic plants retain the ability to obtain carbon from fungi throughout their entire life in combination with photosynthesis (Gebauer and Meyer 2003). The extent of their dependency on fungal carbon varies depending on local ecological factors such as light availability (Preiss et al. 2010) or the physiological properties of the plant (Stöckel et al. 2011, Jacquemyn et al. 2021, Suetsugu and Matsubayashi 2021, 2022). Finally, fully mycoheterotrophic plants are non-photosynthetic throughout their entire life cycle and completely depend on fungal carbon. This mode of life occurs in a small fraction of land plants: currently, about 580 leafless achlorophyllous species have been described that are potentially fully mycoheterotrophic, all within angiosperms except for a single liverwort species (Jacquemyn and Merckx 2019). Detailed phylogenetic analyses have suggested that the evolution of full mycoheterotrophy from autotrophy has occurred at least 40 times during plant diversification and most likely occurs through intermediate initial and partial mycoheterotrophic stages (Motomura et al. 2010, Merckx et al. 2013, Těšitelová et al. 2015, Lallemand et al. 2016, Wang et al. 2021, Suetsugu et al. 2022). Fully mycoheterotrophic plants are often associated with a narrower phylogenetic range of fungi compared to their autotrophic relatives, and these fungi are either a subset or distinct from those of autotrophic plants, suggesting that host switching and local adaptation to mycorrhizal fungi have been important drivers of speciation associated with increased dependence on mycoheterotrophy.

Geographic variation in fungal availability

For fungi to drive ecotype formation and speciation, at least two conditions have to be met. First, plant populations occupying different habitats or occurring in geographically distinct areas are associated with different sets of mycorrhizal fungi and second plants have adapted to their local fungal communities in such a way that the fitness of immigrant seeds is consistently lower than that of native seeds, initiating reproductive isolation and thus the possibility of ecotype formation. However, at present, we don't know precisely how mycorrhizal communities vary in space (Tedersoo 2017) and whether this variation is sufficient to drive ecotype formation. Xing et al. (2020) investigated variation in mycorrhizal communities associated with two widespread terrestrial orchids (*Epipactis helleborine* and *Gymnadenia conopsea*) across a large geographic (7000 km) gradient in Eurasia and showed strong turnover in mycorrhizal communities (Fig. 1). In both species, most of the multiple-site dissimilarity was the result of taxon replacement and not caused by loss of taxa. Similarly, Duffy et al. (2019) showed strong turnover in the mycorrhizal communities associated with *Spiranthes spiralis* in Europe. Renny et al. (2017) surveyed arbuscular mycorrhizal communities associated with the fully mycoheterotrophic species *Arachnitis uniflora* from Bolivia to Patagonia and found that environmental and climatic factors were strongly associated with the distribution of arbuscular mycorrhizal fungal diversity. Environmental and host factors also explained most of the variation in ectomycorrhizal diversity in the soil of forest habitats in Europe (van der Linde et al. 2018). Overall, these results indicate that mycorrhizal communities vary substantially across large geographic gradients and that local habitat conditions can strongly determine fungal availability in the soil and the resulting community structure in the roots.

At smaller geographic scales, similar variations can be observed between populations occupying different environments. Jacquemyn et al. (2016), for example, showed that the mycorrhizal communities associating with adult plants

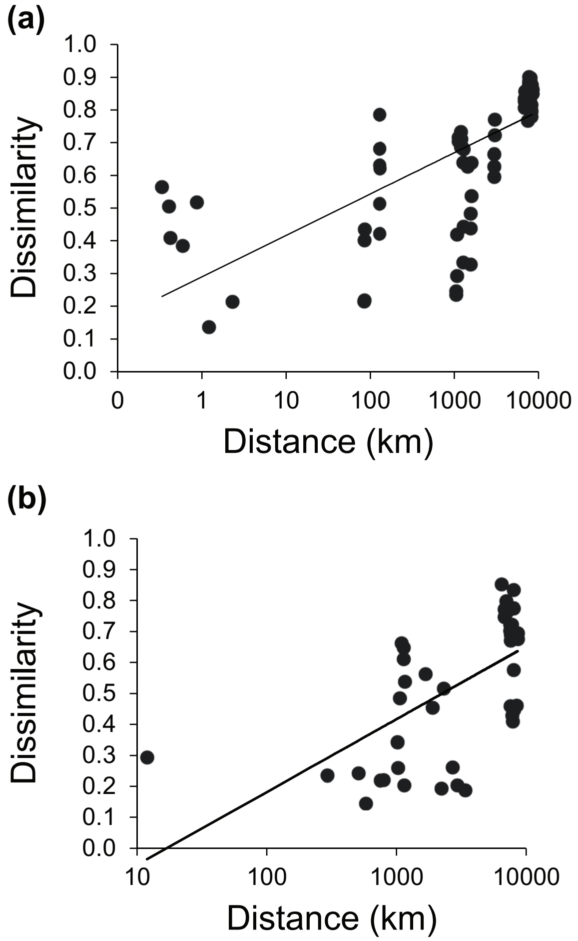

Figure 1. Large-scale variation in mycorrhizal community composition in two widespread orchid species. Relationships between mycorrhizal dissimilarity and geographic distances for populations of (a) *Epipactis helleborine* and (b) *Gymnadenia conopsea* (adapted from Xing et al. 2020).

of *E. helleborine* differed significantly between plants growing in forests and dunes. Out of a total of 90 putative fungal OTUs (operational taxonomic units) that were found in both habitats, only ten were shared between them and these shared fungi were only sporadically observed and probably did not contribute much to seed germination or growth. Mycorrhizal communities of the dune populations were mainly dominated by members of the fungal genera *Inocybe*, *Cortinarius* and *Thelephora*, whereas communities in the forest habitat were mainly dominated by members of the genus *Hebeloma* (Jacquemyn et al. 2016). Strong mycorrhizal divergence has also been reported between coastal and inland populations of *E. helleborine* in Japan (Ogura-Tsujita and Yukawa 2008), with individuals from coastal dunes being exclusively associated with the pezizalean fungus *Wilcoxina*, which is ectomycorrhizal with pine trees. This fungus was not found in inland populations, which are associated with several other ascomycetous ectomycorrhizal taxa of Pezizales, including *Wilcoxina*, *Tuber* and *Hydnotrya*. Similarly, plants in dune and forest populations of the partially mycoheterotrophic *Pyrola rotundifolia* associated with different mycorrhizal communities, most likely related to the different tree species that can be found in both habitats (*Betula pendula* in forest populations, *Salix repens* in dune populations) (Jacquemyn et al. 2018b).

Similarly, Oja et al. (2015) and Wang et al. (2023) showed significant differences in community composition between fungal communities associated with grassland and forest populations of the terrestrial orchid *Neottia ovata*. Whereas plants from grasslands predominantly associated with rhizoctonia fungi of the family Serendipitaceae, plants in forests also recruited several ectomycorrhizal fungi. More evidence of geographic mosaics of mycorrhizal specificity can be found in the fully mycoheterotrophic *Monotropa uniflora*, which associates with different members of the Russulaceae at different populations throughout its geographic range (Bidartondo 2005). In this particular case, individual plants were never found to form mycorrhizas with more than one fungal lineage at the same time, even when neighboring *M. uniflora* were mycorrhizal with other Russulaceae or if mushrooms and tree ectomycorrhizas of other Russulaceae were growing in the immediate neighborhood (Bidartondo 2005). Overall, these results indicate that at various spatial scales environments not only differ in abiotic factors, but also in mycorrhizal communities and that this may lead to geographic mosaics of mycorrhizal interactions (Thompson 1994, Bidartondo 2005). However, whether these differences in mycorrhizal communities are strong enough to induce local adaptation and reproductive isolation remains less understood.

Local adaptation and the evolution of reproductive isolation

When species occur over large geographical ranges or grow in strongly contrasting habitats, it can be expected that populations adapt to both the abiotic environment (e.g. precipitation regime, geological substrate) and other organisms (pollinators, fungi) (Nosil 2007). Ecological adaptation, in turn, can be expected to contribute to species formation in several ways. First, populations or ecotypes adapted to different habitats often evolve differences in flowering time that may hamper the exchange of pollen between populations (i.e. temporal isolation) (Grant 1981, Lowry et al. 2008, Melo et al. 2014). Second, a fitness advantage of locally adapted ecotypes relative to introduced or immigrant ecotypes can counterbalance the process of gene flow through seed dispersal and contribute to reproductive isolation among ecotypes (i.e. immigrant inviability) (Nosil et al. 2005, Lowry et al. 2008, Melo et al. 2014, Richards and Ortiz-Barrientos 2016). Third, when two ecotypes can hybridize through pollen flow, reduced fitness of the resulting hybrids can occur because only one-half of the locally adapted alleles can come to expression, a phenomenon known as extrinsic hybrid inviability (Schluter 2000, Rundle and Whitlock 2001, Baack et al. 2015). Although it can be expected that in species that critically rely on mycorrhizal fungi for the completion of their life cycle, differences in soil mycorrhizal partners act as a source of divergent selection and therefore contribute to ecological speciation, their actual role remains relatively unexplored.

Osborne et al. (2017) provided compelling evidence that arbuscular mycorrhizal fungi (AMF) promoted coexistence and niche divergence of the sympatric palm species *Howea belmoreana* and *H. forsteriana* on Lord Howe Island, a minute and isolated island located in the Tasman Sea between Australia and New Zealand. In this case, speciation most likely involved adaptation to AMF communities occurring in different soils (older volcanic rocks and Pleistocene calcareous deposits) (Osborne et al. 2017), which in turn triggered differences in flowering time and hence the evolution of reproductive isolation (Savolainen et al. 2006). The diversity of AMF was significantly lower in *H. forsteriana* occurring on volcanic soils compared to *H. belmoreana* growing on volcanic soil and *H. forsteriana* on calcareous soil. Survival experiments further showed that sterilization of natural soil reduced *Howea* fitness in every soil–species combination except *H. forsteriana* on volcanic soil. Moreover, there was also evidence that AMF-associated genes exhibited evidence of divergent selection between *Howea* species, indicating that the ability to form AMF associations was one of the main factors driving speciation in these two sister species.

Further evidence for local adaptation to mycorrhizal partners and hence mycorrhiza contributing to reproductive isolation is best provided by using an experimental approach. One of the strongest approaches for demonstrating adaptation to local environmental conditions is performing common garden or reciprocal translocation experiments (Cheplick 2015). In reciprocal translocation experiments, the performance of home individuals is compared with that of individuals from other populations or environments. If ecotypes are truly adapted to their respective environments, the expectation is that ecotypes will be fittest in their home environment and should be evidenced by a significant interaction effect of ecotype × environment. These experiments have been commonly used to show evidence of pollinator ecotypes (Clausen et al. 1940, Robertson and Wyatt 1990, Ågren and Schemske 2012, Boberg et al. 2014), but are less available for mycorrhizal fungi (Johnson et al. 2010).

Bidartondo and Bruns (2005) performed seed germination experiments in the field with several fully mycoheterotrophic species of the Monotropoideae (Ericaceae). Germination was only induced by fungi that associate with the roots of mature plants or by closely related congeners. However, seedlings developed best when associating with fungi present on adult roots, indicating a performance tradeoff between the breadth of host range and rate of development. Similar performance tradeoffs were shown by Jacquemyn et al. (2018a), who used reciprocal seed germination experiments to assess whether dune and forest populations of the partially mycoheterotrophic orchid *E. helleborine* showed higher germination and protocorm formation in their home environments. Both genetic and morphological analyses indicated that plants from both habitats were genetically and morphologically different from each other and therefore most likely had undergone specific adaptations to their respective environments (Jacquemyn et al. 2018a). Moreover, the mycorrhizal fungi associated with plants growing in dune and forest habitats differed substantially from each other (Jacquemyn et al. 2016). Results of the seed germination experiments showed that the proportion of germinating seeds depended significantly on the habitat where the seeds were buried, ecotype and the interaction between habitat and ecotype (Fig. 2a). Seeds from the forest habitat that were sown in dune habitat showed the lowest proportion of germinating seeds (5.7%), while 43.8% of these seeds germinated in forest habitat. No such effects were found for the dune ecotype. In this case, 56.0 and 35.9% of all seeds germinated in dunes and forests, respectively. The average number of protocorms observed within a single seed package also depended significantly on the habitat where the seed packages were buried and on the interaction between habitat and ecotype (Fig. 2b). However, in this case

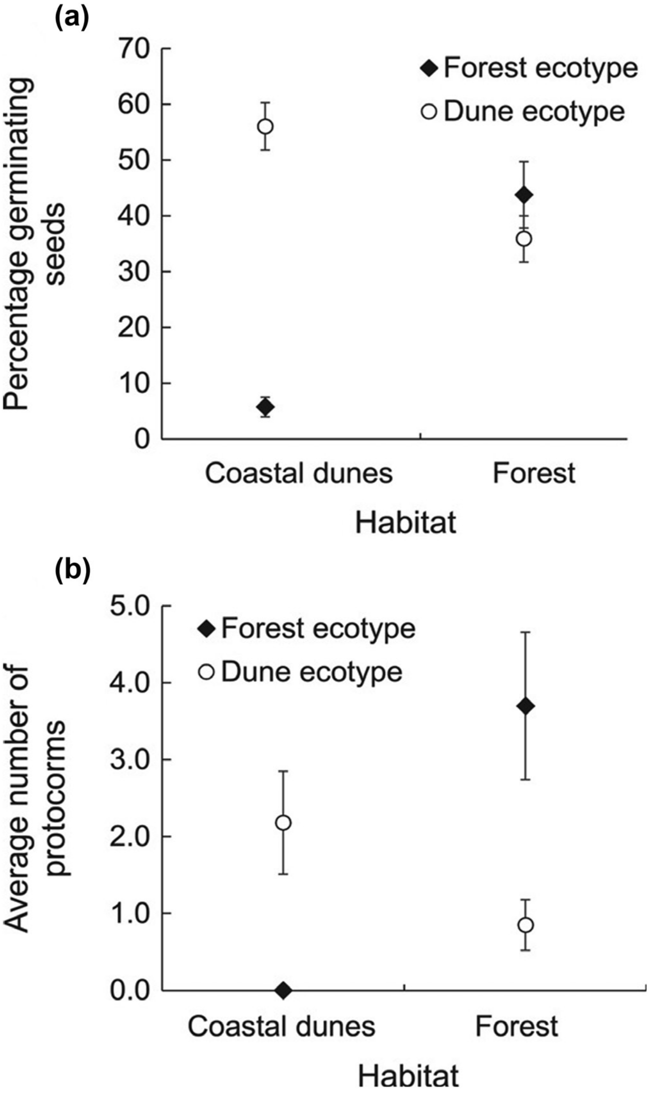

Figure 2. Differences in seed germination (a) and protocorm development (b) of seeds collected from dune and forest individuals of *E. helleborine* between native and non-native habitat (adapted from Jacquemyn et al. 2018a).

no significant effect of ecotype was observed. Overall, these results indicate that the two ecotypes were fittest in their own environment. The associated strength of reproductive isolation due to selection against immigrants can be calculated as:

$$\mathrm{RI} = 1 - w_i / w_n \tag{1}$$

with w_i the total number of protocorms originating from immigrant seeds and w_n the total number of protocorms originating from native seeds. Selection against immigrants was complete (RI = 1.00) for seeds of the forest ecotype migrating into dune habitats and 0.6 for seeds of the dune ecotype dispersing into forest populations.

Similar experiments were performed for the partially mycoheterotrophic *Pyrola rotundifolia* (Jacquemyn et al. 2018b). This species can also be found in a number of different habitats, including broad-leaved forests and heathland, rich swampy forests, fens, mountain birch woodland and tundra (Johansson et al. 2014). Molecular analyses have shown that populations occurring in dune and forest habitats are associated with different mycorrhizal communities. In both habitats, mycorrhizal communities were dominated by members of the Thelephoraceae, but dune populations showed a higher incidence of members of the Inocybaceae, whereas plants from forest populations showed more associations with members of the Russulaceae. Similar to the results for *Epipactis*, there was a strong effect of ecotype (forest versus dune) and the interaction between environment and ecotype on seed germination success. The number of seedlings within a seed package was always higher for native seeds than for immigrant seeds and this difference was again most pronounced in the dune populations. In the forest populations, the difference in germination success between native and immigrant seeds was substantially lower (Jacquemyn et al. 2018b). Overall, the results of these experiments indicate that plants of mycoheterotrophic species growing in different environments often associate with different mycorrhizal communities and that non-native seeds show lower germination than native seeds. As a result, pronounced differences in mycorrhizal community composition and selection against immigrants may constitute an important reproductive barrier at the early stages of the speciation process and hence initiate ecotype formation.

Figure 3. Examples of mycoheterotrophic plant species that occupy different habitats and in which host switching and local adaptation to mycorrhizal fungi may have driven ecotype formation and speciation. (a) *Monotropastrum kirishimense* is a fully mycoheterotrophic species that grows exclusively in *P. densiflora* forests and consistently associates with a single *Russula* OTU, whereas (b) *M. humile* occurs in more diverse ectomycorrhizal forests and is associated with several other members of the Russulaceae. (c) *Cremastra appendiculata* is a photosynthetic orchid species that inhabits somewhat open habitats and is mainly associated with rhizoctonias, while (d) the fully mycoheterotrophic *C. aphylla* inhabits dark shaded understories and is consistently associated with wood-decaying fungi of the Psathyrellaceae.

Host switching and local adaptation to mycorrhizal fungi may not only drive ecotype formation but also contribute to speciation. Disruptive selection on host specificity, characterized by tradeoffs in performance between different hosts, can lead to further specialization and eventually promote the formation of two daughter species. Suetsugu et al. (2023) recently described a new fully mycoheterotrophic plant, *Monotropastrum kirishimense* (Ericaceae) (Fig. 3a), based on not only morphology but also fungal usage. *M. kirishimense* has a consistent and specialized association with a particular *Russula* OTU, whereas *M. humile* (Fig. 3b) is associated with several other members of the Russulaceae. Interestingly, *M. kirishimense* is found exclusively in *Pinus densiflora* forests, whereas *M. humile* occurs in more diverse ectomycorrhizal forests, mainly fagaceous forests. Therefore, the differentiation of *M. kirishimense* from *M. humile* may have been caused by host switching and local adaptation. Furthermore, it is highly probable that host switching and local adaptation to mycorrhizal fungi have played a critical role in driving speciation associated with increased dependence on mycoheterotrophy. Recent studies have explored the physiological ecology of *Cremastra aphylla* (Fig. 3d), a fully mycoheterotrophic species, and its photosynthetic sister species, *Cremastra appendiculata* (Fig. 3c), within the *C. appendiculata* species complex (Yagame et al. 2021, Suetsugu et al. 2022, Zahn et al. 2022). It has been observed that *C. aphylla*, which inhabits dark shaded understory, is consistently associated with wood-decaying Psathyrellaceae, whereas *C. appendiculata*, which inhabits somewhat more open habitats, is mainly associated with rhizoctonias. The abundance of fallen logs and trunks in cool-temperate areas may represent more stable and efficient nutrient resources in shaded forests, whereas they may be less available in more open habitats. The utilization of wood-decaying fungi, coupled with the invasion of shaded forest understory, could provide access to larger and older carbon pools, thereby facilitating speciation along with mycoheterotrophic evolution.

A broader phylogenetic perspective

The examples provided above suggest that shifts in mycorrhizal fungi have the potential to drive plant speciation, but supportive evidence is still limited. A more compelling overview can be obtained when using a broader phylogenetic perspective. Wang et al. (2021) recently built a comprehensive dataset of fungal symbionts associated with a large number of orchid species and used a dated plant molecular phylogeny to test the hypothesis that the formation of new species was accompanied by switches in mycorrhizal partners and associated changes in trophic mode. Shifts from autotrophy towards full mycoheterotrophy occurred at least 17 times within the orchid family, and these shifts mostly went through an intermediate state of partial mycoheterotrophy. Phylogenetic hypothesis tests further showed that these shifts towards full mycoheterotrophy were accompanied by shifts in mycorrhizal partners. Whereas the common ancestor of the family most likely associated with a wide variety of fungal groups, including 'rhizoctonias', ectomycorrhizal, and wood- or litter-decaying saprotrophic fungi, associations with ectomycorrhizal or saprotrophic fungi were most likely a prerequisite for evolutionary shifts towards full mycoheterotrophy. Most of these fungi were most likely already present as endophytes in the roots of the ancestors (Selosse et al. 2022), but they only became mycorrhizal when plants lost their capacity to perform photosynthesis. In the arbuscular mycorrhizal plant genus *Burmannia* (Burmanniaceae), which contains both autotrophic, partially mycoheterotrophic and fully mycoheterotrophic species, a similar pattern has been observed: plant phylogenetic relationships suggest that mycoheterotrophy evolved in a stepwise manner from autotrophy over partial mycoheterotrophy to full mycoheterotrophy (Leake 1994, Merckx et al. 2008, Zhao et al. 2021). During this process, which coincides with a niche shift from open grasslands to closed forests (Maas et al. 1986), there was a gradual loss of non-Glomeraceae fungi (Zhao et al. 2021). Moreover, mycorrhizal interactions in *Burmannia* tend to be species-specific and closely related species share more mycorrhizal fungi than distantly related species, suggesting a link between speciation and mycorrhizal interactions (Zhao et al. 2021).

Further evidence for speciation driven by shifts in mycorrhizal partners includes the mycoheterotrophic plant subfamily Monotropoideae (Ericaceae), in which diversification has resulted in five mycoheterotrophic plant lineages, each of which is associated with one of five distantly related basidiomycete fungal lineages (Bidartondo and Bruns 2002, Bidartondo 2005). In *Afrothismia* (Thismiaceae), the specialization of five closely related plant species to five closely related lineages of arbuscular mycorrhizal fungi resulted in a delayed co-speciation pattern (i.e. fungal hosts diverged in advance of their plant parasites) (Merckx and Bidartondo 2008) (Fig. 4). In this case, the photosynthetic ancestor of *Afrothismia* most likely began specializing on particular fungal lineages and this initiated or followed a shift from mutualism to parasitism. However, when this occurred, most extant lineages had already diverged, suggesting that once plants had adapted to a group of related arbuscular mycorrhizal fungi, subsequent specialization and a lack of host-switching forced the plants to track the fungal phylogeny, resulting in the observed delayed

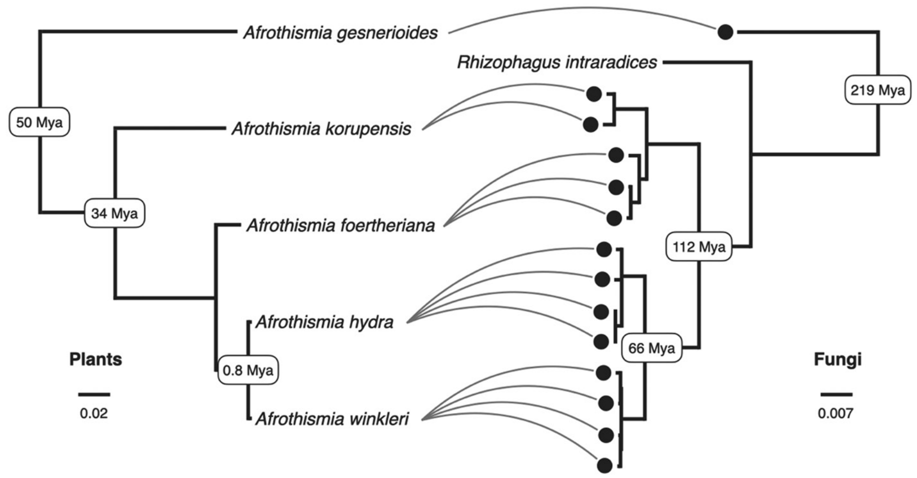

Figure 4. Comparison of multi-gene phylogenies for fully mycoheterotrophic *Afrothismia* plants (left) and arbuscular mycorrhiza fungi (right). Branch lengths represent the number of substitutions per site. Divergence time estimates in million years ago (Mya) are indicated in boxes. Adapted from Merckx and Bidartondo (2008), with nomenclature updated following Schüßler and Walker (2010).

co-speciation process (Fig. 4). Although these patterns point towards a possible involvement of mycorrhizal interactions in plant speciation, research on recently diverged species of fully mycoheterotrophic plants in the genus *Thismia* indicates that in this system mycorrhizal associations are mostly evolutionary conserved, although broad 'host' switches may still lead to lineage-specific mycorrhizal interactions (Merckx et al. 2017).

Future perspectives

Although it is generally acknowledged that ecological differentiation is often the underlying basis for the formation of new taxa (Coyne and Orr 2004, Rundle and Nosil 2005, Nosil 2007, Lowry et al. 2008), the extent to which ecotypes are formed by variation in the environment and how adaptive divergence leads to the formation of plant species are not well understood (Nosil et al. 2009). Moreover, the possibility that mycorrhizal fungi constitute an important reproductive barrier and therefore contribute to taxon formation, has only rarely been considered. Here, we have given an overview of studies showing that mycorrhizal communities of mycoheterotrophic plants vary across space and large environmental gradients and that this variation can induce reproductive isolation. However, there are still major knowledge gaps that limit our understanding of how mycorrhiza-driven speciation. Below we describe three major topics for future research.

The geographical mosaic of mycorrhiza-mediated selection

One of the biggest challenges for understanding the evolution of ecotypes in mycoheterotrophic plants is to determine the ultimate drivers of shifts in mycorrhizal communities. While the above-mentioned examples have indicated that mycorrhizal communities can be highly variable and differ between contrasting habitats (Ogura-Tsujita and Yukawa 2008, Jacquemyn et al. 2016, 2018a, b), the number of studies is still limited and it remains to be seen whether these results can be generalized to other systems. Moreover, it remains unclear what drives the observed variation in mycorrhizal communities. Previous studies have already indicated that fungal communities are often patchily distributed and can differ between sites with different soil chemistry, climatic conditions, and the presence of ectomycorrhizal hosts (McCormick et al. 2012, Renny et al. 2017, Duffy et al. 2019, Ferlian et al. 2021). From the examples highlighted above, it seems that the dominant tree/shrub species and pronounced differences in environmental conditions may be the driving forces leading to mycorrhizal divergence. However, to what extent differences in mycorrhizal communities are caused by other factors such as competition between fungal lineages and priority effects, or result from dispersal limitation needs further research. In addition, most studies have assumed that differences in mycorrhizal fungi are the driving force affecting seed germination and plant performance, but it is possible that abiotic factors such as soil moisture content, pH or organic content also affect germination patterns and subsequent growth. Therefore, in vitro seed germination experiments with fungi retrieved from seed packages and protocorms should be conducted to unambiguously show the role of mycorrhizal fungi in determining immigrant inviability. Bruns and Read (2008), for example, isolated different *Rhizopogon* species to assess the various factors stimulating seed germination in the fully mycoheterotrophic *Sarcodes sanguinea* and *Pterospora andromedea* (Ericaceae, Monotropoideae) under *i*n vitro conditions. Interestingly, their results showed that seeds responded to a slightly broader range of *Rhizopogon* species than what had been observed to be associated with the adult plants, suggesting that results from in vitro germination experiments cannot be readily extrapolated to field conditions and that other factors may be involved under natural conditions. Besides, it is not always possible to conduct in vitro seed germination experiments as several fungi may be very hard to culture under lab conditions.

Mechanisms inducing mycorrhizal switching

Despite limited knowledge of the genetic underpinnings of mycorrhizal associations in mycoheterotrophic plants, the exceptionally high level of mycorrhizal specificity in several mycoheterotrophic plant species is believed to result from fine-tuning their physiology to adapt to specific fungi. Consequently, the disruption of co-adapted gene complexes that regulate host specificity may lead to reduced hybrid fitness and postzygotic isolation. Jacquemyn et al. (2011a, b, 2012), for example, showed that seed germination percentages of hybrid seeds were always lower than those of seeds originating from pure crosses, but this never resulted in complete postzygotic reproductive isolation. Suetsugu et al. (2017) further observed only minor changes in gene expression in the roots and fungi of photosynthetic and albino plants of *E. helleborine*. Similarly, Lallemand et al. (2019) could not find critical metabolic innovations in albino plants to overcome the loss of photosynthesis, suggesting that the evolution towards mycoheterotrophy is more likely to be reliant on the versatility of the metabolism of the plants and the ability to exploit fungal organic resources, especially amino acids, to replace missing photosynthates. However, it is important to note that both green and albino phenotypes of these species typically associate with the same set of fungi (Julou et al. 2005, Suetsugu et al. 2017). These findings suggest that a transition to fungal partners that provide more carbon has not yet occurred in these albino mutants, which therefore may explain the observed minor change in gene expression of green and albino plants. Roy et al. (2013) have also shown that the albino phenotype of partially mycoheterotrophic plants is not entirely adapted to the mycoheterotrophic lifestyle and exhibits some maladaptive traits. To obtain a comprehensive understanding of the genetic basis of mycorrhizal associations during mycoheterotrophic evolution, it is critical to investigate mycoheterotrophic evolution during actual speciation, in addition to studying mutants (i.e. albinos).

Quantifying total reproductive isolation

Local adaptation to mycorrhizal fungi and associated inviability of immigrant seeds are only one of many factors that may induce reproductive isolation (Ramsey et al. 2003, Coyne and Orr 2004, Baack et al. 2015). Moreover, several studies have shown that fungal partners are conserved between closely related species (Jacquemyn et al. 2011a, b, Waterman et al. 2011) and that plant species may recruit the same fungal species even when transplanted to different areas (Waterman et al. 2011). However, these examples involve initially mycoheterotrophic plant species, which for their growth and survival are less dependent on fungi than partially and fully mycoheterotrophic species. In these cases, shifts in pollination traits and associated pollinator species may have been more important in driving speciation than shifts in mycorrhizal fungi. These results indicate that the importance of ecological factors that induce local adaptation may differ between species with different trophic modes, and that diversification of mycorrhizal associations has mainly contributed to the radiation of species that mostly rely on mycorrhizal fungi, namely partially and fully mycoheterotrophic species. This is exemplified by a study of Taylor et al. (2004), who showed that different genotypes of the fully mycoheterotrophic orchid *Corallorhiza maculata* associated with distinct subclades of fungi of the Russulaceae. When these different genotypes were found growing in close proximity, they also maintained their distinct fungal associations, suggesting a very tight relationship between orchid and fungus and divergent selection on resource acquisition mediated by fungi. Nonetheless, to fully understand the absolute and relative contribution of mycorrhizal fungi to ecological speciation in mycoheterotrophic plants, the strength of other reproductive barriers should be quantified (Klooster and Culley 2009). This can be done by carrying out detailed pollinator observations and performing crossing experiments to assess pollinator sharing and the fitness of hybrid plants (Ramsey et al. 2003, Brys et al. 2014, Baack et al. 2015).

Conclusion

Since the seminal work of Göte Turesson on local adaptation and ecotype formation, much progress has been made. Recent work has shown that plants do not only adapt to abiotic conditions, but also to biotic factors and that mycorrhizal fungi are one of the many biological factors that can be expected to contribute to ecotype formation and speciation, especially in mycoheterotrophic plants that strongly rely on these fungi for completion of their life cycle. They do not only contribute to the early divergence of populations inhabiting contrasting habitats, they also play a pivotal role in the further diversification and origination of a completely unique set of species that entirely rely on mycorrhizal fungi for obtaining carbon and completing their life cycle.

Acknowledgements – We thank the scientific committee that organized the symposium on the ecotype concept in Lund (Sweden) on 19 November, 2022 for inviting us to the meeting and allowing us to submit a paper to the special issue.

Funding – VM thanks the European Union (ERC Consolidator grant no. 101045057 – MIXOTROPH) for supporting this work. This work was also supported by the Flemish Fund for Scientific Research (FWO) (grant no.: G093019N, HJ) and PRESTO (grant no.: JPMJPR21D6, KS).

Author contributions

Hans Jacquemyn: Conceptualization (lead); Supervision (lead); Visualization (equal); Writing – original draft (lead); Writing – review and editing (lead). **Kenji Suetsugu**: Conceptualization (supporting); Visualization (equal); Writing – original draft (supporting); Writing – review and editing (supporting). **Vincent Merckx**: Conceptualization (supporting); Visualization (equal); Writing – original draft (supporting); Writing – review and editing (supporting).

Data availability statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

References

Ågren, J. and Schemske, D. W. 2012. Reciprocal transplants demonstrate strong adaptive differentiation of the model organism *Arabidopsis thaliana* in its native range. – New Phytol. 194: 1112–1122.

Anderson, B. and Johnson, S. D. 2009. Geographical covariation and local convergence of flower depth in a guild of fly-pollinated plants. – New Phytol. 182: 533–540.

Baack, E., Melo, M. C., Rieseberg, L. H. and Ortiz-Barrientos, D. 2015. The origins of reproductive isolation in plants. – New Phytol. 207: 968–984.

Bidartondo, M. I. 2005. The evolutionary ecology of myco-heterotrophy. – New Phytol. 167: 335–352.

Bidartondo, M. I. and Bruns, T. D. 2002. Fine-level mycorrhizal specificity in the Monotropoideae (Ericaceae): specificity for fungal species groups. – Mol. Ecol. 11: 557–569.

Bidartondo, M. I. and Bruns, T. D. 2005. On the origins of extreme mycorrhizal specificity in the Monotropoideae (Ericaceae): performance trade-offs during seed germination and seedling development. – Mol. Ecol. 14: 1549–1560.

Boberg, E., Alexandersson, R., Johnsson, M., Maad, J., Ågren, J. and Nilsson, L. A. 2014. Pollinator shifts and the evolution of spur length in the moth-pollinated orchid *Platanthera bifolia*. – Ann. Bot. 113: 267–275.

Bonfante, P. and Genre, A. 2010. Mechanisms underlying beneficial plant–fungus interactions in mycorrhizal symbiosis. – Nat. Commun. 1: 48.

Brundrett, M. C. and Tedersoo, L. 2018. Evolutionary history of mycorrhizal symbioses and global host plant diversity. – New Phytol. 220: 1108–1115.

Bruns, T. D. and Read, D. J. 2008. In vitro germination of nonphotosynthetic, myco-heterotrophic plants stimulated by fungi isolated from the adult plants. – New Phytol. 148: 335–342.

Brys, R., Vanden Broeck, A., Mergeay, J. and Jacquemyn, H. 2014. The contribution of mating system variation to reproductive isolation in two closely related *Centaurium* species (Gentianaceae) with a generalized flower morphology. – Evolution 68: 1281–1293.

Cheplick, G. P. 2015. Approaches to plant evolutionary ecology. – Oxford Univ. Press.

Clausen, J. 1951. Stages in the evolution of plant species. – Cornell Univ. Press.

Clausen, J., Keck, D. D. and Hiesey, W. M. 1939. The concepts ofspecies based on experimentation. – Am. J. Bot. 26: 103–106.

Clausen, J., Keck, D. D. and Hiesey, W. M. 1940. Experimental studies on the nature of species. I. Effect of varied environments on Western North American plants. – Carnegie Institution.

Coyne, J. A. and Orr, H. A. 2004. Speciation. – Sinauer Assoc.

Darwin, C. R. 1859. On the origin of species by means of natural selection. – John Murray.

Darwin, C. R. and Wallace, A. R. 1858. On the tendency of species to form varieties; and on the perpetuation of varieties and species by natural means of selection. – J. Proc. Linn. Soc. 3: 45–62.

Doebeli, M. and Dieckmann, U. 2003. Speciation along environmental gradients. – Nature 423: 259–264.

Duffy, K. J., Waud, M., Schatz, B., Petanidou, T. and Jacquemyn, H. 2019. Latitudinal variation in mycorrhizal diversity associated with a European orchid. – J. Biogeogr. 46: 968–980.

Ferlian, O., Goldmann, K., Eisenhauer, N., Tarkka, M. T., Buscot, F. and Heintz-Buschart, A. 2021. Distinct effects of host and neighbour tree identity on arbuscular and ectomycorrhizal fungi along a tree diversity gradient. – ISME Commun. 1: 40.

Gebauer, G. and Meyer, M. 2003. ^{15}N and ^{13}C natural abundance of autotrophic and myco-heterotrophic orchids provides insight into nitrogen and carbon gain from fungal association. – New Phytol. 160: 209–233.

Grant, V. 1981. The genetic goal of speciation. – Biol. Zentralblatt 100: 473–482.

Gregor, J. W. 1931. Experimental delimitation of species. – New Phytol. 30: 204–217.

Gregor, J. W. 1938. Experimental taxonomy II: initial population differentiation in *Plantago maritima* L. of Britain. – New Phytol. 37: 15–49.

Gregor, J. W., Davey, V. M. and Lang, J. M. S. 1936. Experimental taxonomy I. Experimental garden technique in relation to the recognition of the small taxonomic units. – New Phytol. 35: 323–350.

Jacquemyn, H. and Merckx, V. S. F. T. 2019. Mycorrhizal symbioses and the evolution of trophic modes in plants. – J. Ecol. 107: 1567–1581.

Jacquemyn, H., Brys, R., Cammue, B. P. A., Honnay, O. and Lievens, B. 2011a. Mycorrhizal associations and reproductive isolation in three closely related *Orchis* species. – Ann. Bot. 107: 347–356.

Jacquemyn, H., Merckx, V., Brys, R., Tyteca, D., Cammue, B. P. A., Honnay, O. and Lievens B. 2011b. Analysis of network architecture reveals phylogenetic constraints on mycorrhizal specificity in the genus *Orchis* (Orchidaceae). – New Phytol. 192: 518–528.

Jacquemyn, H., Brys, R., Honnay, O., Roldán-Ruiz, I., Lievens, B. and Wiegand, T. 2012. Non-random spatial structuring of orchids in a hybrid zone of three *Orchis* species. – New Phytol. 193: 454–464.

Jacquemyn, H., Waud, M., Lievens, B. and Jacquemyn, H. 2016. Differences in mycorrhizal communities between *Epipactis palustris*, *E. helleborine* and its presumed sister species *E. neerlandica*. – Ann. Bot. 118: 105–114.

Jacquemyn, H., De Kort, H., Vanden Broeck, A. and Brys, R. 2018a. Immigrant and extrinsic hybrid seed inviability contribute to reproductive isolation between forest and dune ecotypes of *Epipactis helleborine* (Orchidaceae). – Oikos 127: 73–84.

Jacquemyn, H., Waud, M. and Brys, R. 2018b. Mycorrhizal divergence and selection against immigrant seeds in forest and dune populations of the partially mycoheterotrophic *Pyrola rotundifolia*. – Mol. Ecol. 27: 5228–5237.

Jacquemyn, H., Brys, R., Waud, M., Evans, A., Figura, T., Selosse, M.-A. 2021. Mycorrhizal communities and isotope signatures in two partially mycoheterotrophic orchids. – Front. Plant Sci. 12: 618140.

Johansson, V. A., Mueller, G. and Eriksson, O. 2014. Dust seed production and dispersal in Swedish Pyroleae species. – Nord. J. Bot. 32: 209–214.

Johnson, S. D. 2006. Pollinator-driven speciation in plants. – In: Harder, L. D. and Barrett, S. C. H. (eds.), The ecology and evolution of flowers. Oxford Univ. Press, pp. 295–310.

Johnson, N. C., Wilson, G. W. T., Bowker, M. A., Wilson, J. A. and Miller, R. M. 2010. Resource limitation is a driver of local adaptation in mycorrhizal symbioses. – Proc. Natl Acad. Sci. USA 107: 2093–2098.

Julou, T., Burghardt, B., Gebauer, G., Berveiller, D., Damsein, C. and Selosse, M.-A. 2005. Mixotrophy in orchids: insights from a comparative study of green individuals and nonphotosynthetic individuals of *Cephalanthera damasonium*. – New Phytol. 166: 639–653.

Klooster, M. R. and Culley, T. M. 2009. Comparative analysis of the reproductive ecology of *Monotropa* and *Monotropsis*: two mycoheterotrophic genera in the Monotropoideae (Ericaceae). – Am. J. Bot. 96: 1337–1347.

Lallemand, F., Gaudeul, M., Lambourdière, J., Matsuda, Y., Hashimoto, Y. and Selosse, M.-A. 2016. The elusive predisposition to mycoheterotrophy in Ericaceae. – New Phytol. 212: 314–319.

Lallemand, F., Martin-Magniette, M.-L., Gilard, F., Gakière, B., Launay-Avon, A., Delannoy, É. and Selosse, M.-A. 2019. In situ transcriptomic and metabolomic study of the loss of photosynthesis in the leaves of mixotrophic plants exploiting fungi. – Plant J. 98: 826–841.

Leake, J. R. 1994. The biology of myco-heterotrophic ('saprotrophytic') plants. – New Phytol. 127: 171–216.

Lowry, D. B. 2012. Ecotypes and the controversy over stages in the formation of new species. – Bot. J. Linn. Soc. 106: 241–257.

Lowry, D. B., Rockwood, R. C. and Willis, J. H. 2008. Ecological reproductive isolation of coast and inland races of *Mimulus guttatus*. – Evolution 62: 2196–2214.

Maas, P. J. M., Maas-van de Kamer, H., Van Benthem, J., Snelders, H. C. M. and Rübsamen, T. 1986. Burmanniaceae. – Flora Neotrop. 42: 1–189.

McCormick, M. K., Taylor, D. L., Juhaszova, K., Burnett, R. K. Jr., Whigham, D. F. and O'Neill, J. P. 2012. Limitations on orchid recruitment: not a simple picture. – Mol. Ecol. 21: 1511–1523.

Melo, M. C., Grealy, A., Brittain, B., Walter, G. M. and Ortiz-Barrientos, D. 2014. Strong extrinsic reproductive isolation between parapatric populations of an Australian groundsel. – New Phytol. 203: 323–334.

Merckx, V. 2013. Mycoheterotrophy: the biology of plants living on fungi. – Springer.

Merckx, V. and Bidartondo, M. I. 2008. Breakdown and delayed cospeciation in the arbuscular mycorrhizal mutualism. – Proc. R. Soc. B 275: 1029–1035.

Merckx, V., Chatrou, L. W., Lemaire, B., Sainge, M. N., Huysmans, S. and Smets, E. F. 2008. Diversification of myco-heterotrophic angiosperms: evidence from Burmanniaceae. – BMC Ecol. Evol. 8: 178.

Merckx, V. S. F. T., Freudenstein, J. V., Kissling, J., Christenhusz, M. J. M., Stotler, R. E., Crandall-Stotler, B., Wickett, N., Rudall, P. J., Maas-van de Kamer, H. and Maas, P. J. M. 2013. Taxonomy and classification. – In: Merckx, V. (ed.), Mycoheterotrophy: the biology of plants living on fungi. Springer, pp. 19–101.

Merckx, V. S. F. T., Gomes, S. I. F., Wapstra, M., Hunt, C., Steenbeeke, G., Mennes, C. B., Walsh, N., Smissen, R., Hsieh, T.-H., Smets, E. and Bidartondo, M. I. 2017. The biogeographical history of the interaction between mycoheterotrophic *Thismia* (Thismiaceae) plants and mycorrhizal Rhizophagus (Glomeraceae) fungi. – J. Biogeogr. 44: 1869–1879.

Motomura, H., Selosse, M.-A., Martos, F., Kagawa, A. and Yukawa, T. 2010. Mycoheterotrophy evolved from mixotrophic ancestors: evidence in *Cymbidium* (Orchidaceae). – Ann. Bot. 106: 573–581.

Nakazato, T., Bogonovich, M. and Moyle, L. C. 2008. Environmental factors predict adaptive phenotypic differentiation within and between two wild Andean tomatoes. – Evolution 62: 774–792.

Newman, E., Manning, J. C. and Anderson, B. 2014. Matching floral and pollinator traits through guild convergence and pollinator ecotype formation. – Ann. Bot. 113: 373–384.

Nosil, P. 2007. Ecological speciation. – Oxford Univ. Press.

Nosil, P. et al. 2005. Perspective: reproductive isolation caused by natural selection against immigrants from divergent habitats. – Evolution 59: 705–719.

Nosil, P., Funk, D. J. and Ortiz-Barrientos, D. 2009. Divergent selection and heterogeneous genomic divergence. – Mol. Ecol. 18: 375–402.

Ogura-Tsujita, Y. and Yukawa, T. 2008. *Epipactis helleborine* shows strong preference towards ectomycorrhizal fungi with contrasting geographic distributions in Japan. – Mycorrhiza 18: 331–338.

Ogura-Tsujita, Y., Gebauer, G., Hashimoto, T., Umata, H. and Yukawa, T. 2009. Evidence for novel and specialized mycorrhizal parasitism: the orchid *Gastrodia confusa* gains carbon from saprotrophic *Mycena*. – Proc. R. Soc. B 276: 761–767.

Oja, J., Kohout, P., Tedersoo, L., Kull, T. and Kõljalg, U. 2015. Temporal patterns of orchid mycorrhizal fungi in meadows and forests as revealed by 454 pyrosequencing. – New Phytol. 205: 1608–1618.

Osborne, O. G., De-Kayne, R., Bidartondo, M. I., Hutton, I., Baker, W. J., Turnbull, C. G. N. and Savolainen, V. 2017. Arbuscular mycorrhizal fungi promote coexistence and niche divergence of sympatric palm species on a remote oceanic island. – New Phytol. 217: 1254–1266.

Preiss, K., Adam, I. K. U., and Gebauer, G. 2010. Irradiance governs exploitation of fungi: fine-tuning of carbon gain by two partially mycoheterotrophic orchids. – Proc. R. Soc. B 277: 1333–1336.

Ramsey, J., Bradshaw, H. D. and Schemske, D. W. 2003. Components of reproductive isolation between the monkeyflowers *Mimulus lewisii* and *M. cardinalis* (Phrymaceae). – Evolution 57: 1520–1534.

Renny, M., Acosta, M. C., Cofré, N., Domínguez, L. S., Bidartondo, M. I. and Sérsic, A. N. 2017. Genetic diversity patterns of arbuscular mycorrhizal fungi associated with the mycoheterotroph *Arachnitis uniflora* Phil. (Corsiaceae). – Ann. Bot. 119: 1279–1294.

Richards, T. J. and Ortiz-Barrientos, D. 2016. Immigrant inviability produces a strong barrier to gene flow between parapatric ecotypes of *Senecio lautus*. – Evolution 70: 1239–1248.

Robertson, J. L. and Wyatt, R. 1990. Evidence for pollination ecotypes in the yellow-fringed orchid, *Platanthera ciliaris*. – Evolution 44: 121–133.

Roy, M., Gonneau, C., Rocheteau, A., Berveiller, D., Thomas, J. C., Damesin, C. and Selosse, M.-A. 2013. Why do mixotrophic plants stay green? A comparison between green and achlorophyllous orchid individuals in situ. – Ecol. Monogr. 83: 95–117.

Rundle, H. D. and Whitlock, M. C. 2001. A genetic interpretation of ecologically dependent isolation. – Evolution 55: 198–201.

Rundle, H. D. and Nosil, P. 2005. Ecological speciation. – Ecol. Lett. 8: 336–352.

Savolainen, V., Anstett, M.-C., Lexer, C., Hutton, I., Clarkson, J. J., Norup, M. V., Powell, M. P., Springate, D., Salamin, N. and Baker, W. J. 2006. Sympatric speciation in palms on an oceanic island. – Nature 441: 210–213.

Schluter, D. 2000. Ecology of adaptive radiation. – Oxford Univ. Press.

Schluter, D. 2009. Evidence for ecological speciation and its alternative. – Science 323: 737–741.

Schüßler, A. and Walker, C. 2010. The Glomeromycota. A species list with new families and new genera. – Schüßler and Walker.

Selosse, M.-A., Petrolli, R., Mujica, M. I., Laurent, L., Perez-Lamarque, B., Figura, T., Bourceret, A., Jacquemyn, H., Li, T., Gao, J., Minasiewicz, J. and Martos, F. 2022. The waiting room hypothesis revisited by orchids: were orchid mycorrhizal fungi recruited among root endophytes? – Ann. Bot. 129: 259–270.

Stöckel, M., Meyer, C. and Gebauer, G. 2011. The degree of mycoheterotrophic carbon gain in green, variegated and vegetative albino individuals of *Cephalanthera damasonium* is related to leaf chlorophyll concentrations. – New Phytol. 189: 790–796.

Suetsugu, K. and Matsubayashi, J. 2021. Subterranean morphology modulates the degree of mycoheterotrophy in a green orchid *Calypso bulbosa* exploiting wood-decaying fungi. – Funct. Ecol. 35: 2305–2315.

Suetsugu, K., Yamato, M., Miura, C., Yamaguchi, K., Takahashi, K., Ida, Y., Shigenobu, S. and Kaminaka, H. 2017. Comparison of green and albino individuals of the partially mycoheterotrophic orchid *Epipactis helleborine* on molecular identities of mycorrhizal fungi, nutritional modes and gene expression in mycorrhizal roots. – Mol. Ecol. 26: 1652–1669.

Suetsugu, K., Matsubayashi, J. and Tayasu, I. 2020. Some mycoheterotrophic orchids depend on carbon from dead wood: novel evidence from a radiocarbon approach. – New Phytol. 227: 1519–1529.

Suetsugu, K. and Matsubayashi, J. 2022. Foliar chlorophyll concentration modulates the degree of fungal exploitation in a rhizoctonia-associated orchid. – J. Exp. Bot. 73: 4204–4213.

Suetsugu, K., Haraguchi, T. and Tayasu, I. 2022. Novel mycorrhizal cheating in a green orchid: *Cremastra appendiculata* depends on carbon from deadwood through fungal associations. – New Phytol. 235: 333–343.

Suetsugu, K., Hirota, K. S., Hsu, T. C., Kurogi, S., Imamura, A. and Suyama, Y. 2023. *Monotropastrum kirishimense* (Ericaceae), a new mycoheterotrophic plant from Japan based on multifaceted evidence. – J. Plant Res. 136: 3–18.

Taylor, D. L. and Bruns, T. D. 1997. Independent, specialized invasions of ectomycorrhizal mutualism by two nonphotosynthetic orchids. – Proc. Natl Acad. Sci. USA 94: 4510–4515.

Taylor, D. L., Bruns, T. D. and Hodges, S. A. 2004. Evidence for mycorrhizal races in a cheating orchid. – Proc. R. Soc. B 271: 35–43.

Tedersoo, L. 2017. Biogeography of mycorrhizal symbiosis. – Springer.

Tedersoo, L., Bahram, M. and Zobel, M. 2020. How mycorrhizal associations drive plant population and community biology. – Science 367: eaba1223.

Těšitelová, T., Kotilínek, M., Jersáková, J., Joly, F.-X., Košnar, J., Tatarenko, I. and Selosse, M.-A. 2015. Two widespread green *Neottia* species (Orchidaceae) show mycorrhizal preference for Sebacinales in various habitats and ontogenetic stages. – Mol. Ecol. 24: 1122–1134.

Thompson, J. N. 1994. the coevolutionary process. – Univ. of Chicago Press.

Turesson, G. 1922a. The species and the variety as ecological units. – Hereditas 3: 100–113.

Turesson, G. 1922b. The genotypic response of the plant species to habitat. – Hereditas 3: 211–350.

Turesson, G. 1923. The scope and import of genecology. – Hereditas 4: 171–176.

Turesson, G. 1925. The plant species in relation to habitat and climate. – Hereditas 6: 147–236.

Turesson, G. 1929. Ecotypical selection in siberian *Dactylis Glomerata* L. – Hereditas 12: 335–351.

Turesson, G. 1931. The geographical distribution of the alpine ecotype of some Eurasiatic plants. – Hereditas 15: 329–346.

van der Heijden, M. G., Martin, F. M., Selosse, M.-A. and Sanders, I. R. 2015. Mycorrhizal ecology and evolution: the past, the present, and the future. – New Phytol. 205: 1406–1423.

van der Linde, S. et al. 2018. Environment and host as large-scale controls of ectomycorrhizal fungi. – Nature 558: 243–248.

Van der Niet, T., Pirie, M. D., Shuttleworth, A., Johnson, S. D. and Midgley, J. J. 2014a. Do pollinator distributions underlie the evolution of pollination ecotypes in the Cape shrub *Erica plukenetii*? – Ann. Bot. 113: 301–315.

Van der Niet, T., Peakall, R. and Johnson, S. D. 2014b. Pollinator-driven ecological speciation in plants: new evidence and future perspectives. – Ann. Bot. 113: 199–211.

Wallace, A. R. 1865. On the phenomena of variation and geographic distribution as illustrated by the Papilonidae of Malayan region. – Trans. Linn. Soc. Lond. 25: 1–71.

Wang, D., Jacquemyn, H., Gomes, S. I. F., Vos, R. A. and Merckx, V. S. F. T. 2021. Symbiont switching and trophic mode shifts in Orchidaceae. – New Phytol. 231: 791–800.

Wang, D., Gebauer, G., Jacquemyn, H., Zahn, F. E., Lorenz, J., Gomes, S. I. F., van der Hagen, H., Schilthuizen, M. and Merckx, V. S. F. T. 2023. Variation in mycorrhizal communities and the level of mycoheterotrophy in grassland and forest populations of *Neottia ovata* (Orchidaceae). – Funct. Ecol. 37: 1948–1961.

Waterman, R. J., Bidartondo, M. I., Stofberg, J., Combs, J. K., Gebauer, G., Savolainen, V., Barraclough, T. G. and Pauw, A. 2011. The effects of above- and belowground mutualisms on orchid speciation and coexistence. – Am. Nat. 177: E54–E68.

Wu, C.-I. 2001. The genic view of the process of speciation. – J. Evol. Biol. 14: 851–865.

Xing, X., Gao, Y., Zhao, Z., Waud, M., Duffy, K. J., Selosse, M.-A., Jakalski, M., Liu, N., Jacquemyn, H. and Guo, S. 2020. Similarity in mycorrhizal communities associating with two widespread terrestrial orchids decays with distance. – J. Biogeogr. 47: 421–433.

Yagame, T., Lallemand, F., Selosse, M. A., Funabiki, E. and Yukawa, T. 2021. Mycobiont diversity and first evidence of mixotrophy associated with Psathyrellaceae fungi in the chlorophyllous orchid *Cremastra variabilis*. – J. Plant Res. 134: 1213–1224.

Zahn, F. E., Lee, Y. I. and Gebauer, G. 2022. Fungal association and root morphology shift stepwise during ontogenesis of orchid *Cremastra appendiculata* towards autotrophic nutrition. – AoB Plants 14: plac021.

Zhao, Z., Li, X., Liu, M. F., Merckx, V. S. F. T., Saunders, R. M. K and Zhang, D. 2021. Specificity of assemblage, not fungal partner species, explains mycorrhizal partnerships of mycoheterotrophic *Burmannia* plants. – ISME J. 15: 1614–1627.