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FROM COMPLETE AUTOTROPHY TO HOLOMYCOHETEROTROPHY: THE AMAZING JOURNEY OF ORCHID DIVERSIFICATION

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Abstract

Orchids are diverse members of one of the largest plant families, the Orchidaceae. These plants are extremely diverse and species rich and apparently the major causes of their diversification can be attributed to their pollination mechanism, symbiotic relationship, and shift in their trophic modes. Starting from fully autotrophic to holomycoheterotrophic (MH) forms, these plants show a clear transition, with evolutionary gene loss, mainly in photosynthetic genes (ndh-family) and subsequent a few housekeeping genes. On the other hand, fungal partners have also lost a few essential genes for their pathogenicity and became completely adapted to symbiosis with, often, reversal of nutrient flow. The physiological impact of gene loss is enumerated to be quite profound on hosts, which is yet to be learnt. During the course of evolution of MH, orchids have also evolved many pseudogenes, which probably helped in their diversification. The present communication attempts to compile the fungal-mediated diversification of orchids with a view to understanding the process.

Introduction

WITH AROUND 29,481 species (WFO, 2023) encompassing 693 genera (POWO, 2025), the family Orchidaceae is one of the most fascinating plant families. Their evolutionary success is often correlated with i), mostly epiphytic habitat; ii), highly specialised pollination strategies (like deception and rewardless pollination, food mimicry, sexual mimicry, brood-site mimicry or territorial antagonism, *etc*.); iii), close association with fungal partners (either in the form of endophytes or mycorrhizae); iv), nutritional modes (complete photoautotrophy, mycoheterotrophy, and mixotrophy) (Leng *et al*., 2024); and v), seed germination process (Cozzolino and Widmer, 2005). Besides these, the zygomorphy in the flower might have also played a pivotal role in the overall diversification of orchids in the modern neotropical and tropical world. It is appropriate to mention that orchids are the perfect candidates to understand the evolutionary ecology from above the ground (pollination ecology) to underground (fungal symbiosis).

Terrestrialization and subsequent diversification of land plants, in the evolutionary history of angiosperms is, no doubt, partially related to the closely knitted associationship with their fungal partners. Their nutritional support might have triggered the rootless aquatic plants to colonize on land and diversify, providing a perfect example of coevolution (Hoysted *et al*., 2018; Pirozynski and Malloch, 1975). Orchid Mycorrhizae (ORM) are one of the four major types of mycorrhizae (Leng *et al*., 2024). According to them, ORM typically sets a particular symbiotic relationship with orchids, starting early during seed germination to maturity and generating pelotons belonging to root endophytic fungi. Nearly 20% of the photosynthetically fixed carbon is channelised to the fungal partner in fungal symbiosis, which, in return, provides nearly 80% of nitrogen and 100% phosphorous to the host plant that are essential for their seed germination, proper growth, and proliferation (Hoysted *et al*., 2018; Leng *et al*., 2024).

It is not surprising that there are tremendous trophic variations amongst the orchids. While most of them are autotrophic, some forms are completely mycoheterotrophic, often leafless. Non-photosynthetic mycoheterotrophic forms are estimated to encompass nearly 43 genera, belonging to three out of five subfamilies of Orchidaceae, *i.e*. Vanilloideae, Orchidoideae, and Epidendroideae (Kim *et al*., 2020). The present review tries to enumerate the vast diversity and evolution of this specialized plant group, perceived from the fungal association point of view. The main questions are i), what was the main reason for trophic change in orchids or is this change beneficial for orchid diversification? ii), What are the major evolutionary pathways the orchids might have adopted to bring about these changes? iii), How fungal partners assisted this evolution, if they might have? And finally iv), Can we say that such a change is actually mediated by fungal association?

Possible Origin and Evolution of Mycoheterotrophism in Orchids

Mycoheterotrophy (MH) in the extant land plants might have evolved several times in the angiosperm lineage

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during the course of evolution, independently. However, how this has evolved in the autotrophic lineage is yet to be elucidated (Li *et al*., 2022). In Orchidaceae, on the contrary, there is evidence of the complete transition from autotrophic to fully mycoheterotrophic mode (Li *et al*., 2022). Besides these, partially mycoheterotrophic orchids, which use their fungal partners for an 'additional' carbon course, even after performing photosynthesis, also helps understanding the evolution of this concept to a large extent (Li *et al*., 2022). In fact, it would not be surprising to believe that fungal parasitism, mutualistic mycorrhizal interactions, and holomycoheterotrophism are common and continuous intimate ecological interactions between fungal partners and their host plants (Merckx, 2013) (Fig. 1). While there is least exploitation of fungus by their hosts in case of autotrophs, fully heterotrophs have i), lost complete ability to rely on photosynthesis for carbon source and ii), exploit their fungal partners to the maximum carbon source (Merckx, 2013).

Recent comparative genomic analysis revealed that there is loss of photosynthetic genes in both nuclear and chloroplast genomes of fully MH orchids. To site an example, it was shown that holomycoheterotrophic, *Platanthera guangdongensis* has a chloroplast genome of 88,060 bp with 60 genes, considerably smaller than that of partially mycoheterotrophic *P. zijinensis* (plastid genome size of 151,858 bp and containing 128 genes) (Li *et al*., 2022) and that of another MH orchid, *Gastrodia elata* is only 35,304 bp with 28 genes (Li *et al*., 2022). Similarly, these two MH species (*P. guangdongensis* and *G. elata*) have only zero and six antenna-protein genes, which, in normal photosynthetic forms are as many as 9 in number and are needed in the light harvesting complex during photosynthesis (Li *et al*., 2022). The number of photosynthetic pathway genes have also drastically reduced from 50 (in fully autotrophic forms) to 27 (in *P. guangdongensis*) and 4 (in *G. elata*). These findings are suggestive that loss of photosynthesis ability might have been one of the most important triggering factors to adopt complete mycoheterotrophism. It is documented that more than 500 plant species have lost their ability to photosynthesize and entirely rely on their fungal partners for mineral and organic nutrients (Jakalski *et al*., 2021), and the orchids might have experienced as many as (or more than) 30 independent losses of photosynthesis (Timilsena *et al*., 2023).

Interestingly, partial forms have sometimes elaborated their genomes. For example, the partial MH, *P. zijinensis* has 54 photosynthetic pathway genes (more than fully autotrophic forms) (Li *et al*., 2022). Yuan *et al*. (2018) has also supported the loss of genes associated with photosynthesis and reduction of gene families involved in resistance to pathogens in *G. elata* when compared to the photosynthetic orchids. Additionally, an expansion of gene families that are putatively involved in fungal interaction was also suggested by Yuan *et al.* (2018). The generally accepted pattern in this case is considered as i) reduction of plastome with the functional and physical loss of *ndh*-family genes; ii), subsequent loss of photosynthesis genes like *paf1*, *paf2* (Photosystem Assembly Factors), cytochromes (*pet* genes) and others like *cemA*, *etc*.; iii), the loss of ribulose-1,5-bisphosphate carboxylase oxygenase (RuBisCO) large subunit gene, which may be retained in some nonphotosynthetic forms, along with; iv), the loss of *rpo* genes (plastid encoded RNA polymerase genes), which are essential for the transcription of photosynthetic genes in chloroplast; v), loss of *atp* genes (members of thylakoid ATP synthase family), which maintains proton gradients in the thylakoid functioning (Timilsena *et al*., 2023).

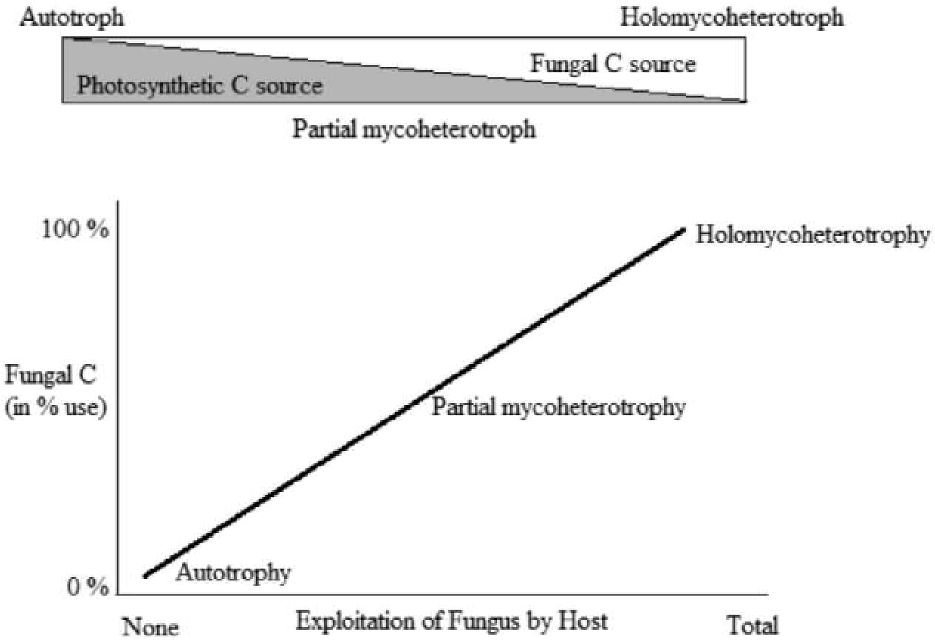

Fig. 1. Trophic change pattern in autotrophic, partial mycoheterophic (MH), and MH orchids.

In a similar pattern of experiment, it was shown that the size of plastid genome is significantly smaller in MH *Galeola lindleyana* (100749 bp with 32 genes) as compared to autotrophic *Vanilla aphylla* (150165 bp with 65 genes) or *Vanilla madagascariensis* (151552 bp with 71 genes) or *Vanilla planifolia* (148011 bp with 72 genes) (Zhou *et al*., 2023). One striking feature appeared is the complete or partial loss of *ndh*-gene family (NADH dehydrogenase-like) in Vanilloideae subfamily plastome (plastid genome) or in the Orchidaceae family (Kim *et al*., 2020), like (*ndhA*, *ndhB*, *ndhC*, *ndhH*, *etc*.), which is documented to have direct evolutionary correlation with trophic changes in several plants, including orchids, independently (Mohanta *et al*., 2020; Zhou *et al*., 2023). The frequency of *ndh*-gene loss in terrestrial form is more as compared to epiphytic forms. The loss of this class of platome gene is taken as an important step towards the evolution of mycoheterotrophy, albeit the facts that i), photosynthetic ability may still be retained in some species, which is owing to the function of this gene may be affected by nuclear (or even mitochondrial) genome (Kim *et al*., 2020); ii), the function of this gene may be maintained due to "*.... RNA editing after pseudogenization*" (Kim *et al*., 2020). It is thus suggested that loss of most photosynthesis genes is also supported by the partial retention of some genes, which are often reprogrammed and are involved in the fatty acid and amino acid biosynthesis (Jakalski *et al*., 2021; Kim *et al*., 2020; Timilsena *et al*., 2023). Ecologically, it is quite easy to perceive that loss of *ndh*-family of genes from orchids might also have increased their tendency to grow in low light, as epiphyte (possibly) or even in dark, shaded *etc.* (Barrett *et al*., 2019). Since the function of these family of genes is not essential in afore stated habitats, evolutionary loss and subsequent niche adaptation might have also enabled the fungal partners to rely less on the orchids for carbon source and thus, the fungal carbon source became inevitable for the host as well (Fig. 1). This situation is observed in green, leafless orchid *Corallorhiza trifida*, which has lost most *ndh* genes, retaining their photosynthetic ability to a least extent (inefficiently) and thus relies completely on the mycotrophic carbon (Barrett *et al*., 2019; Cameron *et al*., 2009).

At the population level, the impact of this loss of genes might have been very serious and profound. If we conceive the relaxed selection and genetic drift, immediately after the gene loss, we might understand why such MH orchids have small, patchy, and narrow distribution, autogamous or rely on vegetative propagation (Barrett *et al*., 2019; Merckx *et al*., 2013). On the other hand, it is also possible that gene loss might have disrupted more than one pathway in such a way that each of these 'nearly broken' pathways behave strangely in the population, which "*... is the result of chance*" (Barrett *et al*., 2019). However, these domains remain speculative and need immediate attention with proper understanding in order to comment on the evolution of orchids.

The plastome size of all MH orchids were less than 150 kb while that for fully autotrophic forms were at least 140 kb. The obligate and partial mycoheterotrophs could be broadly categorised into three main clusters, depending on the gene-loss pattern analysis (Kim *et al*., 2020), *i.e*. i), first with about 100 functional genes present; ii), second with nearly 55-90 functional genes present, and; iii), third with nearly only 25-30 functional genes present. This clearly indicates that the gene loss, in order to shift from one trophic level to another, did not happen as a continuous process. Instead, it might have happened in a stepwise manner, in three stages (Kim *et al*., 2020). Some of the species, like *Cymbidium macrorhizon*, *Eulophia zollingeri*, *Dipodium roseum*, and *Limodorum abortivum* even after losing *ndh*-gene, bear chlorophyll in their stem and flower stalk and carry out low level photosynthesis only during certain phase in their life cycle. This might be considered as an intermediate during the transition from autotroph to obligate mycoheterotroph (Kim *et al*., 2020).

In the second stage of evolution, it is hypothesized that genes related to photosynthesis ability, like *pet*, *psa, psb,* and *rpo* (which is a housekeeping gene) are lost in orchids (Kim *et al*., 2020). These losses were independent in the lineage and account for nearly 55 gene loss in the second stage. Loss of these genes, namely, *psa* and *psb* in different lineages of plants, including orchids, depicts a), their minimal role in the process of photosynthesis; or b), the probable compensation of these genes by the nuclear genome (Mohanta *et al*., 2020). Subsequently, the third stage accounted for fewer than 33 gene loss. However, during the third stage, all photosynthetic genes were lost and several (= most) housekeeping genes like *rps*, *rpl*, *trn* were lost. Additionally, there were no pseudogenes existing at this stage of trophic change evolution in orchids (Kim *et al*., 2020). It is documented that during this third phase of evolutionary gene loss, some 20 genes were conserved in the highly contracted plastid genome. This may suggest that those 20 genes might perform some minimum functions needed for the integrity of the plastid genome (Kim *et al*., 2020). The main question in this regard can be raised as to how many genes from a genome can be lost eventually during the process of evolution, as we can observe that there is nearly 50% loss in photosynthetic pathway genes in some members of the *Platanthera* genus (Li *et al*., 2022). If these genes, (which are being lost) are of less importance to the survival of the organism, or are of to some degree dispensable, or may pose a slight negative impact on the fitness (reproductive) under certain circumstances, they may be lost during the course of evolution (Albalat and Canestro, 2016). It is well documented that gene loss, during the process of evolution plays a pivotal role in enhancing the adaptation of the organisms to the newer environment (Sharma *et al*., 2018). It is often argued that gene loss during evolution leads to adaptation is "*.....likely the result of relaxed selection to maintain a gene whose function became obsolete*", a mechanism termed as 'use it or lose it', and that gene loss typically provide a pavement to "*.... an evolutionary mechanism for phenotypic adaptations*", termed as 'less is more' principle (Sharma *et al*., 2018). The second hypothesis is more prevalent and of importance, as it is observed "*.... when populations are exposed to changes in the patterns of selective pressures owing to drastic shifts*" (Albalat and Canestro, 2016). One of the proposed mechanisms for the stabilizing population after the gene loss could be termed as *Environment-dependent conditional dispensability* (Albalat and Canestro, 2016) indicating thereby that it may probably be due to that only those genes are lost during the course of evolution, that appear to be dispensable and are involved in processes that are only required under certain environmental conditions. Another possible explanation of gene loss is assuming that genes from certain categories, called GO (Gene Ontology: A system for classification of genes in terms of their associated biological processes, cellular components, and molecular functions in a species-independent manner) are more prone to be lost in certain species, as compared to others, which is a function of both biological and environmental constraints. On the other hand, if the species suffers relaxation for a given biological or environmental constraints, there is co-elimination of some other genes which are i) either functionally linked in some distinct pathway, (ii) or complexes associated with the relaxed constraint (Albalat and Canestro, 2016). The progressive non-functionalization of the plastome genes, in relation to the loss of photosynthetic genes during evolution in many plants (members of Orobanchaceae) lead to the accumulation of several mutagenic factors, like microsatellites, long homopolymer stretches, forward or palindromic repeats of variable lengths, and a much lower GC content, which eventually increased intramolecular (and illegitimate) recombination among genes, which in turn might have stimulated the deletion of few more dispensable plastome fragments (Wicke *et al*., 2013). This scenario, which is yet to be elucidated, might have followed a similar pattern in orchids.

Eventually, the question that remained to be answered is what fraction of the population shows such loss-of-function variants? How do species with fewer genes cope with the changing environment? Can photosynthetic and holomycoheterotrophic forms coexist (as found at least in *Corallorhiza* or *Neottia*) more often, in the same ecological conditions? Precisely, what triggered the functionality loss or gene loss and pseudo-organisation?

A few researchers are of the opinion that mycoheterotrophy, specifically complete dependence and total absence of autotrophy, as found in *P. guangdongensis* and *G. elata* might have shown parallel evolution, independently from their putative ancestral lineages. Additionally, the loss of major photosynthetic genes and to adopt complete mycoheterotrophism has also been correlated with the current ecological distribution patterns of extant genera. For example, *G. elata* have lost more nuclear and plastid genes as compared to *P. guangdongensis*, and adopted mycoheterotrophism more "completely" during the course of evolution, Nearly 90 species of the *Gastrodia* genus are holomycoheterotrophic with widespread distribution, on the contrary to species of *Platanthera*, which have only three complete mycoheterotrophic forms with narrow range of distribution (Li *et al*., 2022).

The question arises that whether the fungal partner also lost some genes during the course of evolution, while becoming symbiotic to orchids? The evidence came from the phylogenetic analysis of loss of enzymes in fungal class, which eventually decreased their ability to decompose lignocellulose of the host and cleave sucrose, thereby exerting a selection pressure on the fungi to have become highly reliant on their hosts photoassimilates, while preserving plant cell integrity, which is found in few members of Ascomycota (Martin *et al*., 2016; Strullu-Derrien *et al*., 2018). The genes that were prominent in the gene loss process included class II peroxidase and cellulases, whereas genes needed for scavenging soil organic content for nitrogen and phosphorus were retained by these fungi. Unlike ECM, the so called *decay apparatus* in OM is much more profound and well preserved over the course of evolution (Strullu-Derrien *et al*., 2018), which might have been "*... exploited indirectly by the plant for N and P supply* (Kohler *et al*., 2015; Martino *et al*., 2018)*, and even C supply in orchids*" (Selosse and Martos, 2014; Strullu-Derrien *et al*., 2018). Additionally, it is well documented that the change in the reserve of hydrolyzing enzymes like CAZyme (Carbohydrate-Active enzymes) is often associated with the lifestyle change in plant-associated fungi (Almario *et al*., 2017). This enzyme is needed for the penetration into host tissue and has been shown to have evolutionary significance and early expansion during 299 mya during the earliest diversification of AM fungi (Strullu-Derrien *et al.*, 2018). Such an expansion might have triggered the evolution of pathogenic fungal ancestors towards mycorrhizal forms through speculative intermediate beneficial endophytic forms (Almario *et al.*, 2017; Strullu-Derrien *et al.*, 2018). Further, it may be asked that how can the reduction of gene (gene loss) in some mycorrhizae form be explained? It is shown that in certain EM (not strictly in OM), conversely, there is significant gene loss, especially genes which are coded for plant cell-wall degrading enzymes (Almario *et al.*, 2017). It is possible to believe that these mycorrhizae parallely evolved to survive on the debris of the host cells and eventually became less dependent on their hosts for photosynthetically derived carbon (Almario *et al.*, 2017).

Pseudogenes are typically mutations accumulated in the DNA sequences which resemble the functional genes, but are non-functional and do not code for proteins (Xie *et al.*, 2019). These are suggested to have originated neutrally during evolution. These have been shown to play an important role in the diversification of plants (Xie *et al.*, 2019). Table 1 represents the number of pseudogenes in common mycoheterotrophic orchids (Kim *et al.*, 2020). Orchids have eventually lost most of the photosynthetic genes (like *ndh*-family and others) during the course of evolution from fully autotrophic to holomycoheterotrophic form. It is often argued that genes were pseudogenized before they were fully deleted from the genome in orchids, considering the plastid genome degrades in a gradual manner (Zhou *et al.*, 2023). The probable origin of pseudogenes could have been either by frameshift mutation or by premature codon termination (Balakirev and Ayala, 2003). Indeed, shortening in the intron region of *ndh*B gene in the plastome of *Galeola lindleyana* orchid is indicative of the pseudogene development of the original functional gene (Zhou *et al.*, 2023). Thus, it may be suggested that the non-functionalization of the chloroplast genome in orchids might have started "*... with the pseudogenization and loss of the ndh gene family*" (Zhou *et al.*, 2023). Studies on selected orchids have also confirmed that the photosynthetic gene is first pseudogenized and then lost completely, thereby producing one or more pseudogenes during trophic change (Zhou *et al.*, 2023). It is also documented that degree of loss of genes, pseudogenization in photosynthetic genes, thereby producing pseudogenes, display a very evolutionary progress, which depicts the reduction in the overall genome size in the MH orchids (Barrett and Davis, 2012). For example, the plastid-encoded RNA polymerase (PEP-gene) "*.... have experienced deletions and evolved premature stop codons*", which might have rendered them nonfunctional and evolved as a pseudogene in mycoheterotrophic *Corallorhiza* (Barrett and Davis, 2012). On several occasions, it was observed that losses and subsequent pseudogenes among members of *ndh*-complex directly coincided with the heterotrophic nutrition in orchids and other monocots (Barrett and Davis, 2012).

Table 1. Number of pseudogenes in mycoheterotrophic orchids.

Orchid species	Number of pseudogenes
Cyrtosia septentrionalis, Epipogium aphyllum, E. roseum, Limodorum abortivum	0
Gastrodia elata, Lecanorchis kiusiana	2
Dipodium roseum, Lecanorchis japonica	3
Rhizanthella gardneri	4
Eulophia zollingeri	6
Corallorhiza bulbosa	7
Corallorhiza maculata var. *mexicana, C. odontorhiza, C. wisteriana, Cymbidium macrorhizon, Neottia nidus-avis*	8
Corallorhiza macrantha	9
Hetaeria shikokiana	19
Neottia acuminata	20
Corallorhiza mertensiana, C. maculata var. *occidentalis, C. maculata* var. *maculata*	22
Neottia listeroides	25
Aphyllorchis montana, Hexalectris warnockii	26
Neottia camtschatea	29
Corallorhiza striata var. *vreelandii*	30

Source: Kim *et al.* (2020)

The possible rate at which the gene loss is incorporated or compensated in the population. Are these degradation gradual or abrupt? And can we really observe the insertion or deletion rates in the taxa? (Barrett *et al.*, 2018). In recent Bayesian coevolutionary analyses, it was found that in mycoheterotrophic *Corallorhiza striata*, there were elevated rates of dN and dS (dN: Non-synonymous substitutions, are mutations that change the amino acid sequence of a protein, and can be caused by point mutations or indels; dS: Synonymous substitutions: are mutations that do not change the amino acid sequence and can be caused by point mutations or indels) as compared to autotrophic taxa (Barrett *et al.*, 2018). A similar trend was observed in *Hexalectris*, where the overall substitution rate associated with both photosynthetic and housekeeping genes was elevated (Barrett *et al.*, 2019). This is suggestive that there were overall elevated mutations in the mycoheterotrophic forms. However, some scholars are of opinion that the shift from autotrophic to heterotrophic mode and the loss of major functional gene groups often disturbs and disrupts the normal evolutionary equilibrium, and eventually results in the phases of alternating acceleration and deceleration, described as *ratcheting effect* (Wicke *et al*., 2016). At the same time, it is also hypothesized that rapid changes due to "... *loss of function occurs as selection pressure weakens on photosynthesis*" (Barrett *et al*., 2018). According to Wicke *et al*. (2016). these alternating phases, first where there is phase of acceleration and a more relaxed selection coincides with a burst of gene loss, followed by phase of deceleration and stringent and intensified selection to retain the functional complexes for the essential genes. Such a pattern, according to these authors, suggests that "*... plastomes of parasites have evolved towards new rate equilibrium*". The probable cause for this elevated rate is attributed to "*.... transcript and protein turnover rate-dependent substitution rate shifts*".

If we consider the loss of photosynthesis genes (as shown in many holomycoheterotrophic orchids) and retention of some essential genes in the plastome, we can perceive a condition called "*.... 'punctuational' stages of gene loss, with each stage of stasis representing a new, temporary equilibrium with higher mutation rates* (Barrett *et al*., 2018). It is documented that codon use and substitution rates decreased in heterotrophic plants, owing to the fact that they are more relaxed as they obtain a major part of their organic sources from the microbial partner (Wicke *et al*., 2016). The reduced need to assemble the thylakoid machinery, selection pressures exerted on the mycoheterotrophs might be more relaxed, not only for photosynthetic genes, also for genes encoding transcriptional and translational machinery. This allows efficient and enhanced accumulation of indel (insertion and deletion) mutations, thereby altering and increasing dN and dS (Wicke *et al*., 2016). It is also argued that elevated substitutional rates are directly linked to mode of nutritions in orchids (Barrett *et al*., 2019). These mycoheterotrophs tend to compensate for the loss of carbon (due to eventual loss of photosynthesis) and sharply increase their metabolic rates. An increase in the metabolic rate causes higher levels of oxidative stress and further DNA damage (Barrett *et al*., 2019). In fact, in mycoheterotrophic *Epipactis helleborine* albino plants, there are upregulations in the genes involved in oxidative stress, which is correlated with the higher dependence on fungal carbon (Barrett *et al*., 2019; Suetsugu *et al*., 2017). Further studies in this direction to find out the association of metabolic rate, substitution rate, and phylogenetic relationships in autotrophic, partial mycoheterotrophic and holomycoheterotrophic orchids would open new window and better understanding of orchid diversification mediated by fungal association.

Impact of Photosynthetic Genes on Plant Metabolism

Nearly more than 500 species, including many from the family Orchidaceae, are holomycoheterotrophic. These have lost during the course of evolution, their photosynthetic abilities and solely rely on their fungal partners for minerals (like N and P) and organic nutrients, technically reversing the net carbon flow (Ho *et al*., 2021; Jakalski *et al*., 2021). Such a metabolic evolution might have occurred at least 50 times in other plant families and as many as (or more than) 30 independent times amongst orchids (Barrett *et al*., 2019; Merckx and Freudenstein, 2010).

Since the three probable stages of trophic changes are prominent in many genera of orchids, the multidimensional interaction or the metabolic evolution can be best studied on orchids (Jakalski *et al*., 2021). However, researches in this area are yet to be done and elucidated.

There is, no doubt, a great reduction of gene content in the MH forms, when compared to fully autotrophic orchids, which included not only photosynthetic genes, but gene families involved in resistance to pathogens (Yuan *et al*., 2018). Conversely, there are expansions of genes which are primarily concerned with the interaction with fungal partners (Yuan *et al*., 2018). Thus, the major question remains is i), is this only gene loss or sometimes it is gene expansion with neofunctionalization of retained genes? (Jakalski *et al*., 2021) and ii), how much effect does the gene loss impose on the overall metabolism of the orchid?

In a recent trial, Jakalski *et al*. (2021) analyzed the transcriptomes (gene expression level) in various parts, mycorrhizae of mycoheterotrophic two orchids, *Epipactis aphyllum* and *Neottia nidus-avis*, and found that gene expression differed considerably, like out of 9109 genes analysed in stem versus flowers of *N. nidus-avis*, 4644 are downregulated and 4465 upregulated; while those for 5315 total genes in stem versus flowers in *E. aphyllum*, 2123 are downregulated and 3192 upregulated. It is also documented that the genes involved in the antioxidant metabolism are eventually upregulated in mycoheterotrophic orchids, as compared to their autotrophic counterparts, as evident from gene expression analysis in albino variety of *Epipactis helleborine* (Suetsugu *et al*., 2017). This increased stress, which is resulting in the eventual upregulation of the stress-related genes, might be due to increased lysis of intracellular pelotons in heterotrophic forms (Suetsugu *et al.*, 2017). One of the most important photosynthetic genes (*ndh*-family) is known to be associated with the response to several abiotic stresses (Sabater, 2021). Conversely, plants lacking ndh-genes often face environmental difficulties in adapting to changing environments, thus they tend to retain themselves in 'mild' environmental conditions and often switch to heterotrophy (Sabater, 2021), which could be a possible explanation for why orchids, especially mycoheterotrophic forms, show narrow range of distribution. There is evidence which depicts that there is a bidirectional carbon flow in the mycoheterotrophic symbiosis, which is rather unusual and unique (Suetsugu *et al.*, 2017). A similar result based on isotope mediated analysis on *Cephalanthera longifolia* showed that mycoheterotrophic forms are dependent on the fungal carbon, while autotrophs recovered 33% of their carbon from fungi, thus showing mixotrophy (Abadie *et al.*, 2006). Additionally, there is growing evidence which suggests that albino orchids (as shown in *Cephalanthera damasonium*), suffers with fitness loss, water retention in the organs and low seed output (Barrett *et al.*, 2018; Julou *et al.*, 2005). Investigations made by Julou *et al.* (2005) have supported the following findings: i), mycoheterotrophs (orchids) are often less developed, less fertile (Julou *et al.*, 2005); ii), their fruit and seed content do not differ significantly from their autotrophic counterparts, as shown in the low vegetative success in *E. helleborine* (Julou *et al.*, 2005; Salmia, 1989); iii), these lack invading capacities in the surrounding where they generally grow, owing to their reduced reproductive fitness (Julou *et al.*, 2005); iv), MH orchids are often extremely specific for a given genus of fungal partner, like *E. microphylla* show specificity for *Tuber* spp., which might be necessary to limit the fungal carbon source (Julou *et al.*, 2005; Selosse *et al.*, 2004); v), The MH orchids often have a shorter lifespan than those autotrophic forms, produce shoots later (possibly only after the carbon is available from the fungi), dry early after flowering (probably due to higher transpiration) (Julou *et al.*, 2005); vi), contrary to the higher transpiration, it is also documented that in myco-heterotroph *Monotropa hypopitys* stomata are mostly lost during evolution (Julou *et al.*, 2005; Leake *et al.*, 2004). However, a similar pattern is not documented in MH orchids.

Thus, overall, it may be inferred that retention of photosynthetic features might be limiting factor for the success of MH forms and that the loss of photosynthesis (due to gene loss) may be a discontinuous step, which is not successful in mixotrophic species (Julou *et al.*, 2005). However, this domain of physiological impacts on the photosynthetic gene loss is yet juvenile in orchids, and a new arena can open up if they are systematically elucidated in near future. Loss of photosynthesis in normal green plants impacts their metabolism (Jakalski *et al.*, 2021). In different organs, Jakalski *et al.* (2021) tried to analyze the physiology through the gene expression and found that different genes expressed differently reflection partition of metabolic functions between organs of the plants. For instance, In flower, higher activity of cell division, primary cell wall and signaling pathways attributing floral development.

Conclusion

Mycoheterotrophy (MH) is common in orchids, with a few forms that are partial MH. The majority of orchids show an evolutionary gene loss during trophic change, especially photosynthetic genes in the beginning, followed by few housekeeping genes from their plastomes. The loss of housekeeping genes might have been compensated by the nuclear genome. Thus, these plants became more dependent on the fungal C, N and P sources. Additionally, the pseudogenization in the plastome, following the gene loss, might have aided in the trophic evolution. However, the major questions like i), how the gene loss is stabilised in the dynamic population at a given ecological conditions; ii), how fungal specificity (as found in some orchids) are developed in a given environmental condition, following trophic evolution; iii), how much the fungal partner contributed in the post gene-loss genomic evolution in orchids, *etc.* remains partially unanswered, due to lack of intermediates and mechanistic studies. Future elucidation in these regards would certainly open a new path to understand diversification and evolution of the members of the family Orchidaceae.

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