Mycorrhizae, trophic modes and rarity in orchids

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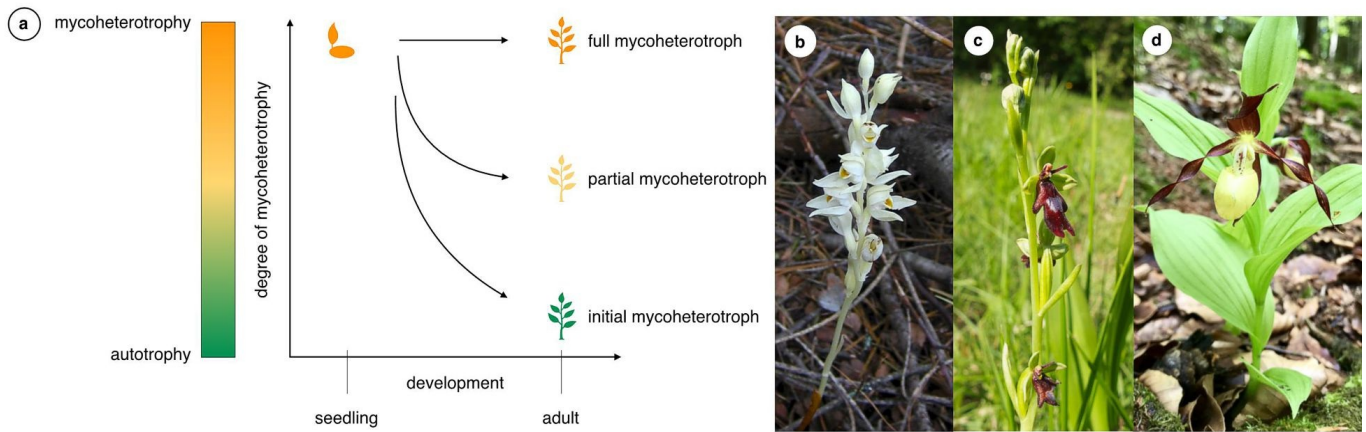[2]Understanding Evolution, Naturalis Biodiversity Center, Leiden, the Netherlands

Figure 1. Different trophic modes of orchids. a. Autotrophy, partial mycoheterotrophy and full mycoheterotrophy should not be seen as three distinct stages in trophic modes, but rather as a gradual transition from one stage to the next. b. *Cephalanthera austiniae,* an example of a fully mycoheterotrophic orchid. c. *Ophrys insectifera,* a partially mycoheterotrophic orchid. d. *Cypripedium calceolus,* an initially mycoheterotrophic species (updated from Jacquemyn and Merckx, 2019).
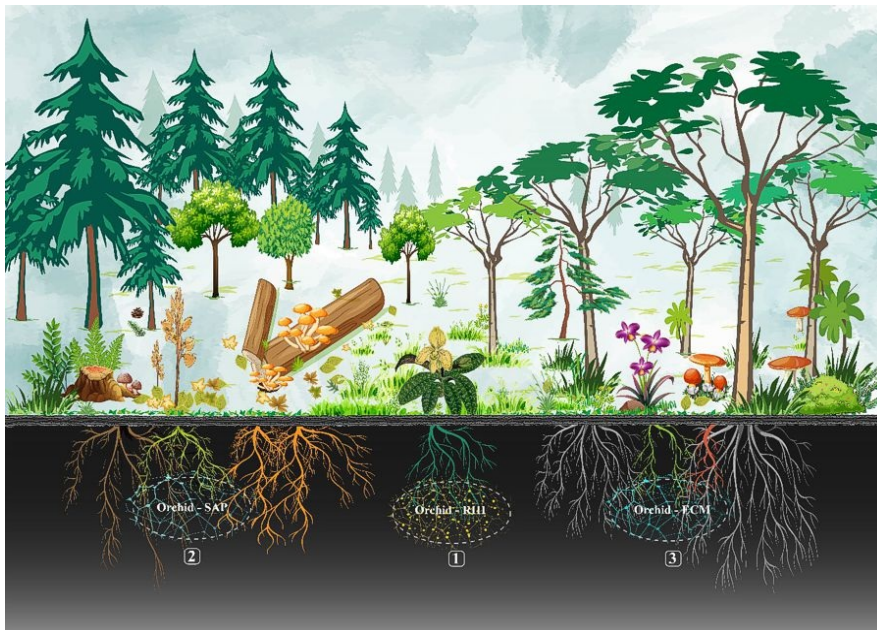

Figure 2. Visual representation of mycorrhizal networks observed in orchids with different trophic modes.

1) Putatively autotrophic orchids mainly associating with rhizoctonia (RHI) fungi.

2) Fully mycoheterotrophic orchids associating with wood- or litter-decaying (SAP) fungi.

3) Partially and fully mycoheterotrophic orchids that associate with ectomycorrhizal (ECM) fungi that link orchids with the roots of living trees.

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Orchid mycorrhizae

Since the early discoveries of the French mycologist Noël Bernard and his German colleague Hans Burgeff that orchids rely on mycorrhizal fungi for seed germination and growth (Bernard, 1899; Burgeff, 1909), there has been a growing interest in the role of mycorrhizal fungi in determining the distribution and local abundance of orchids (McCormick & Jacquemyn 2014; McCormick *et al*. 2018). Because their minute seeds lack an endosperm, orchid seeds need to establish an association with mycorrhizal fungi to obtain the essential resources for germination and subsequent growth to a seedling (Rasmussen *et al*. 1995). Hence, the fungi provide carbon to the developing orchid, a nutritional mode known as mycoheterotrophy. When orchids only rely on mycorrhizal fungi during germination and early development and in later stages become autotrophic, we speak of initial mycoheterotrophy (Merckx *et al*., 2013; Jacquemyn & Merckx, 2019) (Fig. 1). This is most likely the case for the majority of green-leaved orchids, although recent studies have suggested that in many orchid genera green adult plants remain dependent on mycorrhizal fungi for carbon (Gebauer *et al*. 2016). In other orchids, the patterns are clearer and there is strong evidence that adult orchids obtain carbon both through photosynthesis and fungi simultaneously. These orchids are called partially mycoheterotrophic (Figure 1). Typical examples include species from the genera *Epipactis*, *Neottia* and *Cymbidium*. In partially mycoheterotrophic species, a wide variation in dependency on fungi can be found between species, with some species receiving very little carbon from fungi, while others rely very strongly on fungal carbon (Schiebold *et al*., 2017; Jacquemyn *et al*., 2021). Finally, some orchids are non-photosynthetic throughout their entire life and solely depend on fungal carbon. These species are called full mycoheterotrophs (Fig. 1). It has been estimated that about 300 species within the Orchidaceae are fully mycoheterotrophic (Jacquemyn & Merckx, 2019). Classic examples in temperate areas include the phantom orchid (*Cephalanthera austiniae*), ghost orchid (*Epipogium aphyllum*), bird's-nest orchid (*Neottia nidus-avis*), and fall coral-root (*Corallorhiza odontorhiza*). *Gastrodia elata*, *Erythrorchis altissima* and *Aphyllorchis pallida* are typical representatives of tropical systems.

Comparative analyses have shown that autotrophic, partially mycoheterotrophic and fully mycoheterotrophic orchids associate with distinct types of mycorrhizal fungi. Whereas most autotrophic orchids associate with typical 'rhizoctonia' fungi, *i.e.* fungi from the families Tulasnellaceae, Ceratobasidiaceae and Serendipitaceae, partially and fully mycoheterotrophic orchids often associate with fungi that form ectomycorrhizal associations with surrounding tree roots, or with wood- and litter-decaying fungi (Jacquemyn & Merckx, 2019) (Fig. 2). However, with the advent of novel molecular techniques to identify the fungi associating with orchids, it has become clear that most orchids do not associate with a single type of mycorrhizal fungus, but with different types simultaneously. There is increasing evidence that some ectomycorrhizal fungi also occur in rhizoctonia-associated orchids, and *vice-versa* (Jacquemyn *et al*., 2016; Oja *et al*., 2015; Těšitelová *et al*., 2015). For example, adult plants of *Liparis loeselii* have been shown to associate with various fungi of Thelephoraceae, Sebacinaceae, Russulaceae, Tulasnellaceae, Psathyrellaceae and Inocybaceae (Waud *et al*., 2017) and thus combine rhizoctonia and ectomycorrhizal fungi. Roots of *Epipactis palustris* showed associations with a large number of fungal strains of different families, including Tulasnellaceae, Ceratobasidiaceae, Sebacinaceae and Thelephoraceae, and to a lesser extent Inocybaceae, Cortinariaceae, and Herpotrichiellaceae (Jacquemyn *et al*., 2016; Jacquemyn

& Merckx, 2019). Similarly, the partially mycoheterotrophic orchid *Epipactis helleborine* associates with both ectomycorrhizal and rhizoctonia fungi (Jacquemyn *et al*., 2016; Xing *et al*., 2020), and in culture conditions the latter can even be the sole associates (May *et al*., 2020).

The origin of fully mycoheterotrophic orchid species has puzzled researchers for many years (Merckx, 2013). Fully mycoheterotrophic orchid species are mostly restricted to tropical, temperate or boreal forests, suggesting that full mycoheterotrophy is an evolutionary adaptation to the low light conditions characterizing these habitats. Recent phylogenetic analyses have also shown that fully mycoheterotrophic orchids have probably originated from photosynthetic ancestors and that shifts from autotrophy to full mycoheterotrophy often coincided with shifts in fungal partners (Wang et al., 2021). Whereas fully mycoheterotrophic orchids primarily associate with either ectomycorrhizal or wood and litter-decaying saprotrophic fungi, rhizoctonia fungi dominate the associations of their autotrophic ancestors. Because ectomycorrhizal fungi have often been detected alongside rhizoctonia-like fungi in the roots of autotrophic orchids, and because the association with a broad range of fungal partners was most likely the ancestral state in orchids, the symbiotic shift to ectomycorrhizal or wood- and litter-decay saprotrophic fungi is likely an essential predisposition for the evolution of mycoheterotrophy. In at least a few orchid genera (e.g., *Neottia*, *Cymbidium*), a clear correlation between fungal partners and trophic modes has been documented. This was confirmed by a recent overview study that used a dated orchid phylogeny and a comprehensive DNA dataset of orchid fungal symbionts and showed that stepwise shifts from autotrophy over partial mycoheterotrophy to full mycoheterotrophy coincided with shifts in fungal partners (Wang *et al*., 2021). Overall, these results suggest that changes in mycorrhizal associations and ecophysiological traits are tightly correlated in orchids and that the gradual loss of photosynthesis selects for different sets of mycorrhizal partners.

Orchid rarity and mycorrhizal fungi

Many orchids tend to be rare and have small population sizes and it's tempting to think that orchid rarity is in some way related to mycorrhizal partners (Swarts and Dixon, 2009). Orchids that rely on a few mycorrhizal fungi (mycorrhizal specialists) may be more vulnerable to decline and extinction than orchids that are able of relying on a wide range of fungi (mycorrhizal generalists). However, evidence of mycorrhizal specialization affecting orchid decline and rarity is still rare and detailed analyses investigating the relationship between orchid decline and mycorrhizal specificity are largely lacking. In the Australian orchid genus *Caladenia* narrow specificity was related to rarity (Swarts *et al*., 2010). On the other hand, no link between intrinsic rarity and mycorrhizal specificity was observed in the genus *Drakaea* (Phillips *et al*., 2011). Similarly, Bailarote *et al*. (2012) could find no evidence that orchid rarity and persistence were related to fungal diversity. They concluded that next to mycorrhizal specificity other factors (e.g. land use change, pollution, habitat loss) may be more important in determining orchid persistence.

In the same way, it can be expected that partial and fully mycoheterotrophic orchid species that are dependent on a few fungi throughout their entire life are more affected by the association than autotrophic species that only rely on fungi during the early life stages. The fully mycoheterotrophic orchid *Eulophia zollingeri*, for example, relies solely on carbon from the fungus *Psathyrella candolleana* (Coprinaceae), yet the orchid is widespread in tropical Southeast Asia most likely because its host fungus has a widespread distribution as well. Mycorrhizal specificity may have more impact on the occurrence of fully mycoheterotrophic orchids that rely on different partners during different stages of their life. For example, in *Gastrodia* developing seeds and protocorms associate with a single fungus of the genus *Mycena*, while adult plants switch partners and associate with a fungus from the genus *Gymnopus*. Clearly, lack of one of both partners will be detrimental for the species, as it will be no longer capable to complete its life cycle. However, at present there is very little evidence that orchid rarity is linked to variation in orchid mycorrhizal communities. This is partly due to the fact that we don't know which partners orchids associate with and hence we don't know mycorrhizal partner breadth. A comprehensive analysis using orchids of Flanders and the

Netherlands could not find a relationship between trophic mode and probability of extinction. Between 1930 and 2000, eight of 32 studied species (25%) went extinct in Flanders, none of which was partially or fully mycoheterotrophic (Jacquemyn *et al*., 2005). Nonetheless, it needs to be stressed that most fully mycoheterotrophic orchids or mycoheterotrophic plants in general tend to be very rare and often have very small population sizes, which makes them more susceptible to human-induced environmental change.

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How can climate change impact on orchid mycorrhizal association?

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Compounded by habitat fragmentation and the pervasive impact of climate change, biodiversity, and in particular orchids that are among the most threatened plant groups in the world, face an uncertain future. While too little is yet known about the factors limiting the distribution of orchids, the acceleration of climate change has gained scientists' interest since changing conditions will likely alter the dispersal and ecological interactions among organisms (Kelly and Goulden, 2008). It is well known that orchids require symbiotic associations with soil fungi in order to germinate and establish new individuals in nature, but this essential interaction between orchids and their mycorrhizal fungi (OMF) is poorly understood in the context of rapid environmental change. While we know that mycorrhizal fungi improve plant tolerance to abiotic stresses, we know very little regarding the function of individual fungi on orchid fitness and fungal ecological resilience to changing environmental conditions. Shao et al. (2020) first attempted to understand the role of individual fungi by comparing the effects of mycorrhizal monocultures and co-cultures on seed germination and seedling growth of the epiphytic orchid *Dendrobium nobile.* Even if this topic still requires a deeper investigation on more orchid species, this first attempt on an epiphytic orchid opens new questions and perspectives on the synergic role of OMF on orchids that could be more threatened and affected by environmental changes such as terrestrial species.

How are OMF affected by changing climatic conditions? Do ecological dependencies/interactions shift under climate change and how can these be managed to avert species extinctions? Will generalist orchid species be more resilient by shifting the association with resistant fungal taxa compared to specialized orchid species? These globally significant questions will be addressed in my FORECAST Marie Skłodowska-Curie project in collaboration with the Royal Botanic Gardens, Kew (Prof. Michael Fay) and Curtin University (Prof. Kingsley Dixon). Investigating Australian and European terrestrial orchid species from Mediterranean hotspots, we will try to answer these questions and to predict orchids distributions under future climatic scenarios. Hypothetically, these studies will allow us to calibrate conservation efforts by selecting fungal strains able to face global warming.
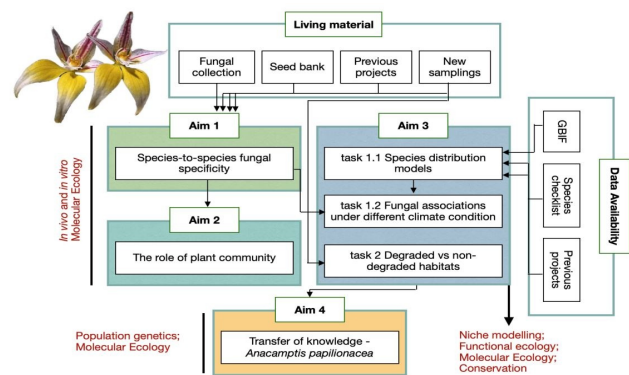
Project Flowchart

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Orchis patens and Orchis canariensis in their natural habitats in Liguria (Italy) and Gran Canaria (Canary Islands), respectively.

Jacopo Calevo, Ph.D.

Thanks to molecular evidence from newly developed nuclear microsatellite markers, *O. patens* subsp. *canariensis* has been finally recognized as a separate (possibly sister) species, *i.e*., *O. canariensis*, and the Italian populations of *O. patens* as a distinct subspecies. Given that the two taxa share the same and apparently wide distributed main orchid mycorrhizal fungus (*Tulasnella helicospora*) the reason for their rarity and narrow distribution should be found in environmental conditions. *Orchis patens'* habitat, indeed, is locally fragmented due to agriculture and (sub)populations persistence is affected by the activity of wild animals (i.e, boars, badgers etc.). For this reason the species is the flagship species in the conservation project LIFEorchids (LIFE17 NAT/IT/000596) co-funded by the European Commission and *in vitro* produced seedlings will be reintroduced in established micro-reserves. Conversely, *Orchis canariensis*, now that has been officially recognized as species, should be assessed for Red Lists and taken into account for conservation efforts. Its populations are indeed declining due to goat extensive grazing and the few surviving individuals grow (in Gran Canaria) on cliffs and rocks far from humans and animals.

Orchis patens

Orchis canariensis growing on cliffs in Gran Canaria together with a succulent member of the Crassulaceae, likely *Aeonium sp*.

Mycorrhizal fungi and root-associated bacteria of *Platanthera*

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Orchids display an obligate requirement for mycorrhizal symbiosis. Mycorrhizal fungi support seed germination and / or subsequent growth of orchids and thus have the potential to affect fitness of individuals and populations. We have recently studied *Platanthera praeclara* and *P. cooperi* (Figs. 1 and 2) to identify linkages between their rarity and mycorrhizal communities, and showed that *Platanthera* sp. exhibit specificity in recruitment of mycorrhizal fungi and these fungi are rare in their respective habitat soils. Specificity of orchids towards mycorrhizal fungi, combined with the lack of suitable mycorrhizal fungus in soil, can clearly limit seed germination or subsequent growth of the host orchids and consequently affect species distributions, rarity and population dynamics. Going beyond mycorrhizal ecology, Dr. Kaur has spearheaded the lab's initiative to assess bacterial communities in orchid roots. Root-associated bacteria can enhance seed germination and seedling growth in orchids though fundamental questions about their ecology remain missing in literature so far. Consequently, we seek to elucidate the functional roles of bacteria in orchid ecology. As a first step, it is necessary to identify if orchids exhibit specificity toward root-associated bacterial communities, and if their recruitment is tied to the soil bacterial communities. Bacterial surveys focused on *P. cooperi* and *P. praeclara* indicated the two host orchids showed differential enrichment of root bacterial communities, and within each orchid the communities showed differences based on the population size and phenological stage of the host orchid. Similar to orchid mycorrhizal fungi, root associated bacteria were rare to non-existent in their respective habitat soils. While the importance of orchid mycorrhizal fungi for orchid conservation is fairly recognized, integration of orchid root associated bacteria in orchid conservation programs needs further attention. For instance, orchid seedlings could be inoculated with species-specific bacteria along with mycorrhizal fungi for orchid reintroduction programs, and soil bacterial surveys should be made an integral part of studies looking for suitable habitats for orchid introductions or translocations. Altogether, mutualistic association with bacteria remains an underutilized tool in orchid conservation whereas it could enhance the success rates of orchid emergence and survival when used along with orchid mycorrhizal fungi.

Fig. 1. Inflorescence of *Platanthera cooperi*

Photo: Jyotsna Sharma

Fig. 2. Inflorescence of *Platanthera praeclara*

Photo: Jyotsna Sharma)

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Orchid Conservation in the Age of COVID

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The recently-concluded 23rd World Orchid Conference, originally scheduled for March 2020 in Taichung, Taiwan, serves as a stark reminder of how the COVID pandemic has affected all of us in the orchid conservation community. It is rather remarkable how the conference's organizers were able to reshape WOC23 into a virtual format during the pandemic year, and somehow, they made it work. Well done! The WOCs and IOCCs from pre-pandemic years now seem like a distant memory when meeting people in person and handshakes were the norm.   As effective as virtual delivery may be at showcasing our orchids and the work by specialists to conserve them, my sense of smell still longs for the scent of floral fragrances emitted by these remarkable plants on public display. And nothing can replace talking to other human beings in person and learning from them.

Despite COVID's best efforts to rob us of our lives, our sense of smell, and our dedication to orchid conservation - in the lab and/or in the field - a number of colleagues report progress being made at least on some level. Here at Illinois College, located in rural central North America, several of my undergraduate students have also been able to carry out experiments during the past year aimed at conserving orchids in the Midwest as well as southern Florida, Hawaii and even Palau in the western Pacific (Fig. 1). Below is a summary of some of the highlights of their ongoing work despite restrictions imposed by the COVID pandemic (*e.g.*, social distancing, mask-wearing).

Fig. 1. Three students collecting data in Illinois College's Orchid Recovery Program Laboratory during the COVID pandemic. Pictured (l-r) are Audrey Zettler, Camryn Fryrear, and Hannah Baker. Photo: L. Zettler

Mycorrhizal Fungi and Symbiotic Seed Germination of Midwestern Orchids

Student: Savannah Renken

During the summer of 2020, potential mycorrhizal fungi were isolated from roots of three orchids native to Illinois and surrounding region (Indiana), funded through the American Orchid Society's Dorothy Nieter Trust (Mirenda, 2020). These included the rare Oklahoma grass pink, *Calopogon oklahomensis*, green fringed orchid, *Platanthera lacera*, and orange fringed orchid, *P. ciliaris* (Renken & Zettler, 2021). Fungi from *C. oklahomensis* and *P. lacera* were isolated from pelotons in roots acquired from Goose Lake Prairie (Grundy Co.,

IL), whereas those from *P. ciliaris* were obtained from Kankakee Sands Preserve (IL) and Indiana Dunes National Park, IN. (Fig. 2).

While *P. lacera* yielded fungi assignable to *Ceratobasidium* – the genus most frequently isolated from tallgrass prairies - *C. oklahomensis* harbored *Sebacina* (*Serendipita*) – a genus infrequently isolated from orchids. Fungi from *P. ciliaris* consisted of *Ceratobasisium* and *Tulasnella*. In symbiotic trials involving seeds of *P. ciliaris*, seed germination and development rapidly ensued *in vitro* using fungi from both genera (*Ceratobasidium*, *Tulasnella*) after seeds were exposed to a two month cold moist stratification treatment (Fig. 3). After four months of incubation in darkness at ambient temperature, seedlings are now in the process of initiating leaves and will soon be exposed to a photoperiod for further development. Both genera of fungi facilitated development to the leaf bearing stage suggesting that this orchid may be a mycorrhizal generalist. In a separate experiment, seeds of *P. leucophaea* were also inoculated with these fungi, and seeds are beginning to germinate using the *Ceratobasidium* isolates.

In October (2020), our collaborator, Caleb Dvorak at the Missouri Botanical Garden, inoculated seeds of *Calopogon tuberosus* with *Sebacina* (*Serendipita*) isolated from the Oklahoma grass pink (*C. oklahomensis*) in a pilot study. Among the different mycorrhizal fungi tested, seedling development was most pronounced using the *Sebacina* (*Serendipita*) isolate suggesting that this fungus might be used to propagate *C. oklahomensis*. Considering that the Oklahoma grass pink is rare and restricted in distribution, obtaining seeds in the coming year will be a challenging priority.

Fig. 2 (left). *Platanthera ciliaris* in full bloom, photographed at Indiana Dunes National Park where it grows in isolated *Sphagnum* bogs south of Lake Michigan. Photo: L. Zettler

Fig. 3 (right). A 9 cm diam. petri dish containing protocorms of *P. ciliaris* in symbiotic culture in the process of initiating leaves. The fungus used in this image was identified as a *Ceratobasidium* species acquired from roots of *P. ciliaris* at Indiana Dunes. Photo: S. Renken

Isolation of *Tulasnella* from Cigar Orchid (*Cyrtopodium punctatum*) Seedlings in South Florida

Student: Hannah Baker

Often overshadowed by the ghost orchid (*Dendrophylax lindenii*), the cigar orchid (*Cyrtopodium punctatum*) of south Florida is at least as appealing and equally rare. The decline in this species coincided with the logging of ancient cypress trees dating back to the 1940s. Of the few cigar orchids that remain in the region, all remain vulnerable to poaching. Until recently, very little was known about the mycorrhizal fungi that this orchid utilizes for its seed germination needs *in situ*. During the COVID pandemic, Illinois College received several small *C. punctatum* seedlings from Nick Ewy at the Naples Botanical Garden that he observed growing from beneath the bark of rotting pine logs. Because small orchid seedlings are thought to harbor fungi involved in the early germination process, these seedlings afforded us with a unique opportunity to recover potential mycorrhizal fungi for eventual use in symbiotic germination experiments. The student charged with the

challenge was Hannah Baker who had almost three years of experience isolating orchid fungi for the North American Orchid Conservation Center (NAOCC) and as a summer intern at the Naples Botanical Garden.

Hannah removed roots from the seedlings that she photographed and cut into 1 cm length segments so that she could trace the origin of the fungi she isolated (Fig. 4). Roots of the seedlings were macerated under a sterile hood and immersed in Fungal Isolation Medium (FIM) and incubated at ambient temperature for 1-2 weeks. Most of the root segments harbored pelotons, many of which were composed of intact hyphae that initiated growth in the agar. After subculturing these fungi to Potato Dextrose Agar (PDA), the different types of fungi in pure culture formed colonies that allowed for provisional identification to the genus level. To our surprise, many of these fungi were assignable to the genus *Trichoderma* – a common soil-dwelling genus with explosive growth and not widely assumed to form mycorrhizal associations with orchids. However, she was able to isolate at least one potential mycorrhizal fungus identified as a strain of *Tulasnella* (Fig. 5). Under light microscopy, the growth pattern and hyphal morphology were unusual compared to other members of the genus. In the coming year, we plan to use molecular techniques to identify the fungus further and inoculate seeds *in vitro*.

Fig. 4 (left). One of several small cigar orchid seedlings removed from a rotting pine log in Naples, Florida, for fungal isolations. Each seedling was measured and photographed before roots were macerated and immersed in agar for fungal isolations. Fig. 5 (right). A potential mycorrhizal fungus provisionally identified as a *Tulasnella* isolate acquired from a peloton in the root of a cigar orchid seedling.

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Seed Germination of Two Native Hawaiian Endemic Orchids

Students: Molly Gearin, Jack Lu, Justine Kennedy, Tony Ruiz, Camryn Fryrear, Audrey Zettler

Only three orchids are native to the Hawaiian archipelago: *Anoectochilus sandvicensis*, *Liparis hawaiensis*, and *Peristylus holochila* (Zettler and Oppenheimer, 2012). While all three orchids are vulnerable to extinction, *P. holochila* is currently listed as a U.S. Federally endangered species with less than three dozen individual plants known to occur on the islands of Kauai, Maui, and Molokai. The habitats of all three orchids are restricted in area and distribution, and are vulnerable to invasive pests (*e.g.*, wild pigs, slugs) and poachers that pose an omni-present threat. A decade ago, Illinois College students propagated *P. holochila* from seed *in vitro* on asymbiotic media that led to the reintroduction of seedlings 3.1 years after sowing (Zettler, 2017). Several of the orchids initiated anthesis, but most did not survive. An initial effort to germinate seeds of *L. hawaiensis* was also attempted, but seeds failed to germinate possibly because capsules were not mature at the time of collection. In 2020, in collaboration with Hank Oppenheimer, we began a new series of experiments aimed at germinating these two orchids using existing protocols and seeds acquired more recently (Gearin *et al*. 2021). Our goal was to improve seedling survival of *P. holochila* and propagate *L. hawaiensis* for the first time. For the latter species, we also employed the technique of symbiotic seed germination using two *Tulasnella* fungi acquired from Hawaii and one from Florida (*T. calospora*, UAMH 9824).

For *P. holochila*, seeds acquired from Molokai in 2012 (S209) and the lone plant on Kauai in 2013 (S217), 2018 (S231), and 2019 (S236) were sown on asymbiotic media P723 (*Phyto*Technology Labs) in June 2020. A subsequent sowing occurred in November 2020 when seeds were sown shortly after their collection in Hawaii from Kauai (S247, October) and Maui (S245, November). Similarly, seeds from *Liparis hawaiensis* on Maui were sown shortly after collection and sown on P723. While all seed of *P. holochila* were incubated in complete darkness at ambient temperature, seeds of *L. hawaiensis* were subjected to two treatments: complete darkness vs. exposure to light (photoperiod). All seed

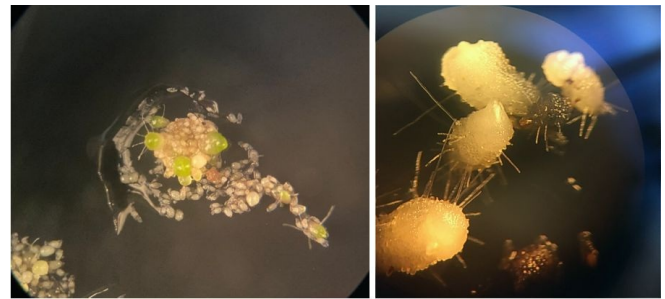sources from the two orchid species germinated after *ca*. 2 months (Figs. 6, 7).

Fig. 6 (left). Light-exposed protocorms of *Liparis hawaiensis* shown in the process of forming leaves on asymbiotic medium P723. Photo: J. Lu; Fig. 7 (right). *Peristylus holochila* protocorms initiating shoots (Stage 3) on P723 ca. 7 months after sowing and incubation in darkness at ambient temperature. Photo: M. Gearin.

Interestingly, seeds of *P. holochila* stored at -7°C since 2013 (S217) resulted in the largest number of protocorms obtained after 7 months of incubation (Fig. 8). Likewise, seeds collected from Kauai and Maui in 2020 also germinated and developed to the protocorm stage. Obtaining seedlings from the Maui population is of special significance because the number of known *P. holochila* individuals is now down to four plants, and our long-term goal is to reintroduce these seedlings in the coming years, augmenting the existing population. For seeds of *L. hawaiensis*, our preliminary findings suggest that more seeds germinate in the presence of light vs. complete darkness (Fig. 9). This would not be surprising considering that *L. hawaiensis* is semi-epiphytic.
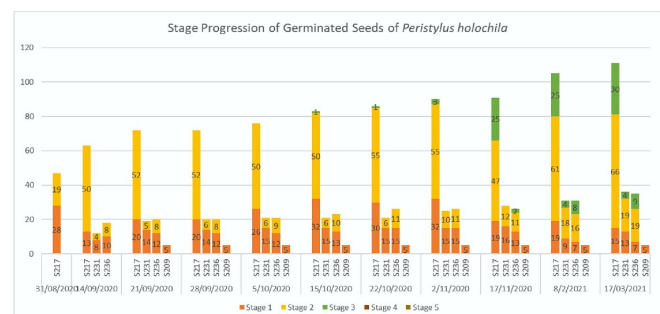

Fig. 8. Seed germination and development of *Peristylus holochila* on asymbiotic medium P723 during the course of 7 months of incubation in darkness at ambient temperature for the four seed sources collected prior to 2020. (M. Gearin)
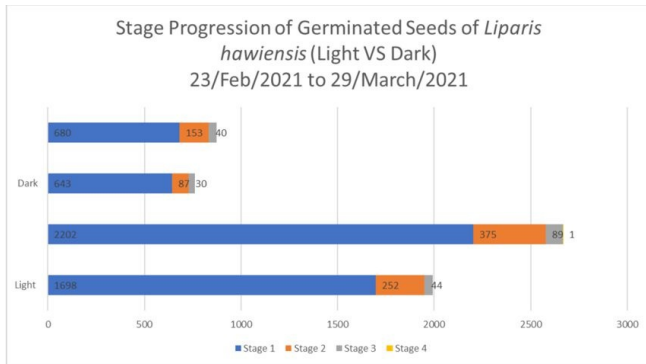
Stage Progression of Germinated Seeds of *Liparis hawiensis* (Light VS Dark) 23/Feb/2021 to 29/March/2021

Fig. 9. Germination and development of *Liparis hawaiensis in vitro* on asymbiotic media for seeds exposed to light photoperiod versus continuous darkness at ambient temperature. Seedings in Stage 4 were characterized by leaf initiation. (J. Lu)

Compared to asymbiotic germination experiments, attempts to germinate seeds of *L. hawaiensis* using fungi took place early in 2021. To date (ca. 4 months after sowing), only one of the three *Tulasnella* isolates tested has facilitated seed germination (*T. calospora* 266) to the advanced protocorm stage. To our knowledge, this is the first record of symbiotic germination in this species.

In Vitro Seed Propagation of Orchids Native to the Republic of Palau (W Pacific)

Students: Charles Veith, Imiezye Fegor, Reilly Hancock, Jacquelin Castellon, Savannah Renken

Despite its small land area (535 km^2), the Republic of Palau in the Western Pacific harbors an unusually large number of native orchid species (Crain 2018). In 2017, the Palau Orchid Conservation Initiative, spearheaded by the North American Orchid Conservation Center (NAOCC), was established to study the mechanisms behind orchid diversity in this forested archipelago, and to develop protocols for conservation. Our collaborators include Drs. Dennis Whigham, Melissa McCormick, and Benjamin Crain from the Smithsonian Environmental Research Center. Other partners include the U.S. Forest Service, Palau Division of Forestry, and Ngardok Nature Reserve. Illinois College's role in this collaboration consisted of the isolation, molecular identification, and storage (cryopreservation) of potential mycorrhizal fungi, and use of select isolates to facilitate seed germination *in vitro*. Another goal is to cultivate the orchids from seed

using conventional (asymbiotic) techniques followed by reintroduction in Palau, with some given to the U.S. Botanic Garden for public (educational) display. Roots and mature capsules of orchids were collected during three trips as a source of fungi and seeds, respectively. To date, 63 fungal endophytes have been isolated in pure culture, and many have been deposited into Canada (UAMH) for safekeeping including several assignable to the genus *Tulasnella*. Using asymbiotic media (P723, *Phyto*Technology Labs), several species have been cultivated from seed *in vitro* to the leaf-bearing stage including *Bulbophyllum membranaceum* (Figs. 10 and 11) *Dendrobium brachyanthum* (endemic), *D. kraemeri*, *D. mirbelianum*, *Pseuderia micronesiaca*, *Robiquetia palawensis* (endemic), and *Spathoglottis micronesiaca* (Veith et al. 2021). Among the species that have been deflasked thus far are *D. kraemeri* and *S. macronesiaca*.

Fig.10 (left). The diminutive *Bulbophyllum membranaceum* photographed in bloom at Ngardok Nature Preserve in October of 2018. Photo: L. Zettler. Fig. 11 (right). Seedlings of *B. membranaceum* growing on asymbiotic medium P748 (*Phyto*Technology Labs) after germination on P723 prior. Photo: C. Veith.

Although the COVID pandemic prevented us from returning to Palau in 2020 as planned, we are optimistic that a return trip may be possible in the next year or two. Until then, we will continue to focus on orchid conservation projects here at Illinois College with the ongoing help of our collaborators.

Training Future Conservationists

It seems that too many specialists in our field and throughout the scientific community are so focused on their research that they lose sight of the 'big picture', and I am often guilty of that myself. We must never forget that, in this Age of Extinction, each scientist should make some attempt to conserve what they study, at least on some level. Studying an organism for the sake of a publication while, at the same time, allowing that species to slip into extinction is (in my opinion) unacceptable. Indifference is not an option anymore. What good is that publication if the species becomes extinct a decade later under their watch?

Since 1996, about 70% of my professional life has been spent teaching at the undergraduate level. When I leave the classroom after teaching 18-22 year-olds about the importance of conservation, I still feel I have not done enough to make the world a better place for the younger generation. For this reason, I have blended undergraduate teaching with orchid conservation. During the 25+ years I have been directing Illinois College's Orchid Recovery Program, over 100 students have carried out research projects here in North America and throughout the world. Some, though not the majority, still study orchids, but at least they have some appreciation for orchids and the natural world. This is the world they will soon inherit – for better or worse.

As I move closer to retirement, nothing would make me happier than for today's generation to disconnect from artificial distractions (e.g., cell phones) – at least some of the time – and conserve the natural beauty that remains, with orchids at the forefront. The dozen students featured in this article provide a small glimpse of what we do by blending teaching and research, and why there is hope for our future even during a pandemic.

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Food for Thought

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Mark your Calendar

IUCN World Conservation Congress 2021

The delayed IUCN World Conservation Congress (WCC) and satellite meetings will take place in Marseille, France, September 2-11, 2021. The format will be partly virtual, with most delegates attending via online platforms. Check website for updates to the programme: https://iucncongress2020.org/.

 The new Quadrennium will officially start at the end of the Congress, and invitations to existing and new members of the Orchid Specialist Group for 2021-2024 will be issued after this.

MONOCOTS VII – 7th International Conference on Comparative Biology of Monocotyledons.

 The 7th International Conference on Comparative Biology of Monocotyledons (Monocots VII), delayed due to the covid-19 situation, will take place in San José, Costa Rica, March 11–15, 2024.

Changes to contact information?

 To maintain effective communication, we need to know of any changes in contact information.

Please inform the OSG Chair, Mike Fay.

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