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| **APPLICATION POSTDOCTORAL FELLOWSHIP (junior/senior) PROJECT OUTLINE (MAX. 10 pages)** |

**Which factors affect the distribution of epiphytic orchids in mega-diverse tropical forests?**

# Rationale and positioning with regard to the state-of-the-art

Understanding the various **factors that limit species distribution** is a longstanding question in ecology[[1]](#footnote-1). In land plants, species distribution is simultaneously limited by climate, dispersal, and biotic interactions1. The most relevant climatic factors restricting plant species distribution globally are temperature and humidity. At local scales, microsite conditions such as light and substrate physicochemical quality play an important role1. Dispersal is determined by the dispersal distance of species' diaspores and the presence of barriers impeding their dissemination (ref). Biotic interactions can limit or favour species distributions. Antagonistic interactions such as interspecific competition will exclude some species from a given site, while mutualisms such as mycorrhizal symbioses can contribute to expand plant species distribution areas (ref).

[General paragraph on the effects of interspecific interactions on the distribution]

Whereas the effects of environmental condtions and dispersal on species distributions are relatively well understood, the ecological effects of obligate inter-specific interactions, such as symbioses, are far less understood.[[2]](#footnote-2),[[3]](#footnote-3). Furthermore, a species can interact with more than one obligate partner at the same time, which adds complexity to the determinants of species distribution1. [Partner breadth, availability y concluir.]

In sessile organisms, such as plants, the distribution of adult individuals can largely depend on species' requirements during the earliest developmental life stages (the regeneration niche[[4]](#endnote-1)). The regeneration niche includes preferences for different substrates[[5]](#endnote-2), differential responses of seedlings to light gradients[[6]](#endnote-3), and availability of mutualists[[7]](#endnote-4).

**Epiphytes** live non-parasitically on tree trunks and branches, and therefore they need to find a **suitable host tree** for physical support. Because they live on trees, epiphytes face strong ecological gradients in short distances, such as the **vertical gradient of light** of the forest12, and therefore they have to be adapted to a broad range of environmental conditions6. **Epiphytic orchids,** however, stand out among other epiphytes because of two additional characteristics that might determine their regeneration niche at small scales: i) seed germination fully depends on microscopic **mycorrhizal fungi** that are not inherited from maternal plants[[8]](#endnote-7),[[9]](#endnote-8), and therefore, **free-living fungi** on the substrate might be a key component of microsite quality11; and ii) while early life stages lack chlorophyll and fully depend on mycorrhiza for nutrition, adults of most species are capable of photosynthesis (**partial mycoheterotrophy**), potentially reducing their dependency on mycorrhiza as compared to seedlings[[10]](#endnote-9). Epiphytic orchids thus need to find suitable host trees and mycorrhizal fungi for germination, but also might experience changes in their environmental and nutritional requirements throughout development, meaning that the **regeneration and adult niches might differ substantially**.

Although specialized partnerships with host trees have been reported, most evidence so far indicates that epiphytic orchids can grow on a broad range of tree species[[11]](#footnote-6),[[12]](#footnote-7),[[13]](#footnote-8), suggesting that tree availability may not be a major constraint to the distribution of epiphytic orchids. Less, however, is known about how mycorrhizal fungi determine the distribution of epiphytic orchids. In terrestrial orchids, there is a continuum from specialists[[14]](#footnote-9),[[15]](#footnote-10) to generalists[[16]](#footnote-11),[[17]](#footnote-12). For epiphytic orchids, we do not really know whether they are specialized or generalized[[18]](#footnote-13). The little evidence available suggests that epiphytic orchids may have a higher diversity of mycorrhizal interactions than terrestrial orchids[[19]](#footnote-14) and that they tend to be generalists[[20]](#footnote-15),[[21]](#footnote-16), although highly specialized taxa have also been documented22,[[22]](#footnote-17). Studies over small spatial scales reveal terrestrial orchids to have distinctive mycorrhizal communities and show strong spatial segregation, suggesting that mycorrhizal partners play a role in determining their distribution[[23]](#footnote-18). No such data are available for epiphytic orchids.

An important unknown aspect in this interaction is **how mycorrhizal communities vary** among host trees and over the host tree surface, and how this affects the distribution of epiphytic orchids. It has been proposed that epiphyte turnover among host trees may be mediated by mycorrhizal fungi[[24]](#footnote-19) and that orchids with many mycorrhizal partners have more host tree species[[25]](#footnote-20). However, exceptions to this pattern have been reported29, suggesting that interactions of epiphytic orchids with their two partners can be diverse and complex. Similarly, the vertical turnover in epiphytic orchids is known to relate to changes in bark characteristics as well as epiphytes' requirements for light15. Yet, whether those factors influence the distribution of mycorrhizal partners remains to be assessed29. In general, solid evidence of how host trees and mycorrhiza affect epiphytic orchid distribution is still lacking, because **studies have had a limited spatial scale and rarely included ecological gradients**.

After germination, seedlings experience a high death rate in later stages of development[[26]](#endnote-22),[[27]](#endnote-23). Several factors may cause recruitment failure, from seedling predation to differences between the *regeneration* and *adult niches* of the species[[28]](#endnote-24). Plant physiological needs often change over ontogeny, and the successful transition from seedling (protocorm) to adult might depend on acquiring new mycorrhizal partners that help fullfill those new needs19. Such ontogenetic partner turnover can result from complementarity or sampling effects in time19. **Complementarity** consists of a replacement of partners from the seedling to the adult stage, under the assumption that new partners potentially play complementary roles. On the other hand, **sampling effects** consist of adults retaining a subset of their partners from the seedling stage. Ontogenetic partner turnover through total complementarity is risky because a lack of suitable new partners can compromise survival to adulthood. Evidence from terrestrial orchids suggests that partner gains are common11, while losses are less well documented[[29]](#endnote-25). In epiphytic orchids, the role of mycorrhiza turnover on the successful transition to adulthood remains to be assessed9,15.

# Scientific research objectives

The major aim of this research is to understand **how multiple partners interacting with abiotic conditions influence the distribution of epiphytic orchids in hyper-diverse tropical forests**. . Specifically, the project aims at understanding how mycorrhizal fungi availability affects germination and recruitment of epiphytic orchids along natural light gradients. To this end, I will address three key aspects of the interaction: i) the availability of free-living fungi on the substrate as a key component of microsite quality; ii) changes in the interaction over the vertical gradient of light of the forest; and iii) ontogenetic turnover of mycorrhizal partners as a putative barrier to post-germination establishment.

In particular, I will test the hypotheses that:

H1: the influence of partner availability on epiphytic orchid distribution depends on partner breadth and abiotic conditions.

H2: the availability of free-living fungi changes over the vertical gradient of light. The composition of free-living fungi communities will change over the trunk of the host tree.

H3: seedlings will have a greater diversity of mycorrhizal fungi than adults. This would indicate that germination is opportunistic, using the fungi at hand in each sector of the host tree trunk.

H4: ontogenetic changes in mycorrhizal partners are due to sampling effects rather than total complementarity.

This study will provide **three innovative aspects to the field**:

**(1) Address an unresolved question in plant ecology**: how more than one partner affects plant species' distribution. This is not trivial because a considerable proportion of tropical plant diversity relies on more than one partner for successful establishment. In epiphytic orchids in particular, most studies have focused on bipartite interactions, *i.e.*, epiphyte-host tree or epiphyte-fungi interactions, while a tripartite network approach (epiphyte-mycorrhiza-host tree) better reflects the actual situation.

**(2)** The **first experimental test** of how tripartite interactions affect orchid distribution within a vertical light gradient. Combining **careful field observations and experiments with cutting-edge analyses and molecular techniques**, I will be able to decipher:

• how mycorrhizal fungi are distributed over the host tree trunk.

• how the vertical gradient of light within a host-tree affects epiphytic orchid germination.

• whether orchid mycorrhizal partners are replaced or retained over an individual's lifetime, and the underlying mechanisms.

**(3)** The **first comprehensive picture of the patterns and potential drivers of tropical epiphytic orchid distribution**. This research **will push the state-of-the-art forward**, moving from local studies of focal orchid species towards an integrative approach over larger scales, and providing novel insights into:

• how climatic factors influence the interaction network and community structure of epiphytic orchids.

• how orchid partner breadth and partner availability influence the large-scale distribution of epiphytic orchids.

I will build and analyse tripartite interaction networks to infer changes in the orchid-mycorrhiza-host tree interactions over a geographical gradient of temperature and moisture. I expect to find a continuum of orchid-partner interactions, from strict generalists to strict specialists. Forests with more stressful abiotic conditions (e.g., warm and dry) will harbour orchid species that depend more strongly on their partners, while moist forests will host a wider variety of strategies. This project will address for the first time the effect of two partners on the distribution of epiphytic orchids in megadiverse communities across geographical and local ecological gradients.

Solid evidence of how light gradients affect fungi availability and mycorrhizal symbioses in epiphytic orchids is still lacking . Furthermore, the few studies available deal mainly with adult plants, but neglect germination or transitions between ontogenetic stages. Such knowledge is key to design effective, evidence-based orchid conservation actions.

Our knowledge on how seedling and adult niche requirements ultimately affect epiphytic orchid distribution is only fragmentary. This is because the studies addressing this problem have focused on adults, which are more conspicuous than earlier life stages, in local studies in a few species. In addition, the technology for massive DNA sequencing of microscopic fungi has been developed only in the last 15-10 years, which now allows to quantify the molecular diversity of fungal partners with an unprecedented resolution. A major current need in the field is an integrated approach that addresses (i) how do host trees and mycorrhizal fungi limit epiphytic orchid distribution across geographical ecological gradients, (ii) how the regeneration niche as defined by mycorrhizal fungi and the abiotic environment, and the transition to the adult stage determine individual establishment in epiphytic orchid populations6 .

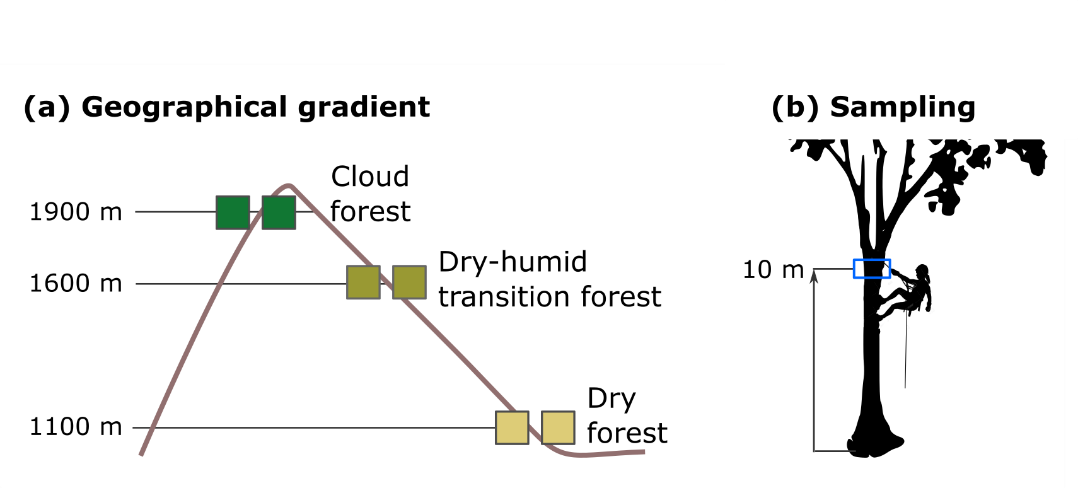
# Research methodology and work plan

The work will be structured in three work packages (WP). In WP 1, I will study how the tripartite interaction between orchid, host tree, and mycorrhizal fungi varies across a geographical gradient of temperature and humidity (H1), that includes three forest types (Fig. X). In WP 2, I will investigate the distribution of free-living mycorrhizal fungi over a local gradient of light (H2) represented by three trunk heights. Field sampling for this WP will be conducted in dry-humid transition forests. In WP 3, I will assess whether mycorrhizal fungi communities change between the seedling and the adult stages of epiphytic orchids (H3) and infer potential underlying ecological mechanisms (H4). To this end, I will perform seed germination experiments in dry-humid transition forest plots. [State the cohesion of the work packages in a phrase.]

**WP1 Assessing the influence of partner availability on epiphytic orchid distribution.**

It is reasonable to assume that orchid germination frequency will be higher in species that associate selectively with common instead of rare fungal partners, so that **partner availability in the substrate** is probably an important determinant of orchid occurrence. Yet, orchid species differ in their degree of specialization in the interaction (**partner breadth**) , meaning that they can associate selectively with a few fungal partners (*specialist species*), or indistinctively with many of them (*generalist species*). Theory predicts that natural selection favours highly specialized interactions as a way to avoid cheaters . This view has been recently challenged on the basis that generalist interactions can be advantageous under environmental heterogeneity, where species should not be very choosy in order to associate with the partners at hand19,20. In this way, generalist species can broaden their distribution by shifting partners (**partner turnover**) when the preferred partner is not locally available. Species exhibiting such ability have more chances of germinating in a variety of microsites than highly-specialized species.

The study will be conducted in natural, mostly undisturbed tropical forest ecosystems. I will sample communities of epiphytic orchids in three forest types along a humidity-altitude gradient in the western mountain range of the Colombian Andes (Fig. 3a). The forest types encompass dry forests in the Cauca River Valley, dry-humid transition forests (DHTF) in the valley slope, and cloud forests in the Pacific slope. In each forest type I will choose two plots 5 km apart and 10 host trees per plot. I will sample plants growing at a height of 10 m on tree trunks using the single-rope climbing method, collecting three 2-cm root fragments in up to five individuals per orchid species of all the species found. Sampling will not destroy the plants.

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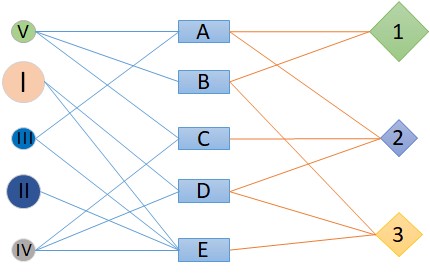
**Fig.** **3** Sampling design. **(a)** Geographical gradient including three forest types in the Andes Mountain Range, with two plots per altitude. **(b)** Sampling method.

Based on diversity inventories in the study locations, I expect to find approximately 120 orchid species in total (García-Revelo, S. unpubl.; Ospina, NH, unpubl.). Some orchid species are expected to be unique to a particular forest type, while others are expected to occur in at least two of them. I will register the **host tree species** where the different epiphytic orchid species occur (host-tree breadth), and the **mycorrhizal partners** in orchid roots (mycorrhizal fungi breadth). With this information, I will be able to test H1, which states that the influence of partner availability on epiphytic orchid distribution depends on partner breadth and abiotic conditions.

**Measuring abiotic conditions -** To characterize **regional climate** I will use climatic data from meteorological stations near the plots. To describe the **light environment of host trees** I will measure photosynthetically active radiation (PAR, mol·m-2·day-1) by taking three hemispheric photographs in each tree at 10 m, where orchid roots will be sampled. To improve PAR estimation accuracy, I will measure the diffuse to direct PAR ratio with BF5 diffuse PAR sensors (Delta-T Devices, UK) in three randomly chosen host trees.

**Assessing partner breadth -** Since mycorrhizal fungi are microscopic and may form complex interactions with both host trees and orchids, I will combine **meta-barcoding of mycorrhizal DNA** with **network analysis tools**[[30]](#footnote-21) to assess mycorrhizal partner breadth[[31]](#footnote-22),[[32]](#footnote-23). An individual orchid can host 1-30 different fungal partners[[33]](#footnote-24). To accurately describe **mycorrhizal diversity** associated with each individual plant I will extract DNA from 0.5 g mycorrhizal root fragments using UltraClean Plant DNA Isolation Kit (Mo Bio Laboratories Inc., CA, USA). I will use two complementary primer pairs (ITS3/ITS4OF and ITS86F/ITS4) for detailed characterization of diverse orchid mycorrhizal communities[[34]](#footnote-25). I will use Illumina sequencing to obtain mycorrhizal operational taxonomic units (OTUs), which are the commonly used units of microbial diversity. I will **identify orchids and host trees** with the help of a local taxonomist (Nhora Ospina, Universidad del Valle, Cali) and confirm ambiguous tree and orchid records using genetic barcoding.

**Describing inter-specific interactions -** Network analysis capture patterns of interactions between species[[35]](#footnote-26). I will build **tripartite networks** comprised of three types of nodes: i) epiphytic orchids, ii) mycorrhizal fungi, and iii) host trees (Fig. 4). Tripartite network analysis provides information on the *number of links per species* (which species are more connected) and *layer interdependence* (a measure of how much information about one layer predicts information in another layer)[[36]](#footnote-27), and allows to *detect communities within the network* ('blocks', a metric similar to modularity of bipartite networks)42,[[37]](#footnote-28),[[38]](#footnote-29). Tripartite network analysis is only recently being used to address multiple interactions and, despite the field evolves rapidly43, networks involving more than two partners have to be decomposed into bipartite networks to obtain other relevant metrics[[39]](#footnote-30), such as *modularity*[[40]](#footnote-31), *nestedness*[[41]](#footnote-32), and *specialization*[[42]](#footnote-33). A high modularity indicates there are subsets of strongly connected species interlinked through a few interactions46. A high nestedness indicates that there is a core of the most generalist species interacting among them47. Partner breadth of a species can be quantified with the degree of interaction specialization at the species (d') and network (H') level48.



**Fig. 4** Schematic representation of a tripartite interaction network between orchids (circles), mycorrhiza (squares) and host trees (rhombi). Characters depict different species; lines depict inter-specific interactions.

I expect nestedness and specialization to vary between forests. In cloud forests, where abiotic stress is lowest, epiphyte abundance is high and competition for resources is strong9, orchid-mycorrhiza-host tree interactions will be weaker and orchids will establish generalist associations with the most abundant partner species39. In dry forests, with opposite characteristics, physiological adaptation of the orchid will be a strong determinant of its distribution and also interaction networks will probably be more specialized9,17.

**WP2 Assessing how mycorrhizal fungi are distributed over the host tree trunk.**

The interaction between orchids and mycorrhizal fungi is asymmetric. Orchid seeds have to find suitable mycorrhizal fungi for germinating, while fungi have a microscopic, free-living form which is independent from the orchid. Therefore, the distribution of free-living mycorrhizal fungi in the substrate can determine orchid distribution. In particular, the composition of mycorrhizal fungi communities is likely a key aspect of microsite quality for orchid seed germination. If the composition of fungal communities is heterogeneous over the substrate, then a microsite can be limiting for orchid species specialized in fungi taxa that are locally absent. By contrast, generalist species are expected to occupy a broader range of microhabitats by associating with a variety of mycorrhizal fungi.

Orchids interact with a subset of the mycorrhizal fungi available in the local pool, i.e. those present in the substrate as free-living fungi. Currently, there is some knowledge of the fungi interacting with orchids but barely any knowledge of the composition of free-living mycorrhizal fungal communities. This project will provide information about the mycorrhizal fungi pool and the proportion that actually engages in interactions with orchids, in a local study exploring a vertical gradient in environmental conditions.

I will work with the 20 trees in the dry-humid transition forest plots from WP 1. I will establish three sampling heights at 10, 6 and 2 m in the trunk, representing a vertical light gradient (Fig. X). In each height, I will measure light using hemispheric photography as described in WP 1. I will collect three bark samples per height to obtain bark fungi. Fungal DNA extraction and sequencing analyses will be performed as described in WP 1. This will provide a picture of the pool of mycorrhizal fungi locally available at each height. The proportion of mycorrhizal fungi actually interacting with orchids will be identified by combining three pieces of information: 1) OTUs lists derived from the regional scanning in WP 1, 2) the mycorrhizal fungi associated to the adult orchids present nearby, and 3) the mycorrhizal fungi identified from seedlings derived from *in-situ* germination assays.

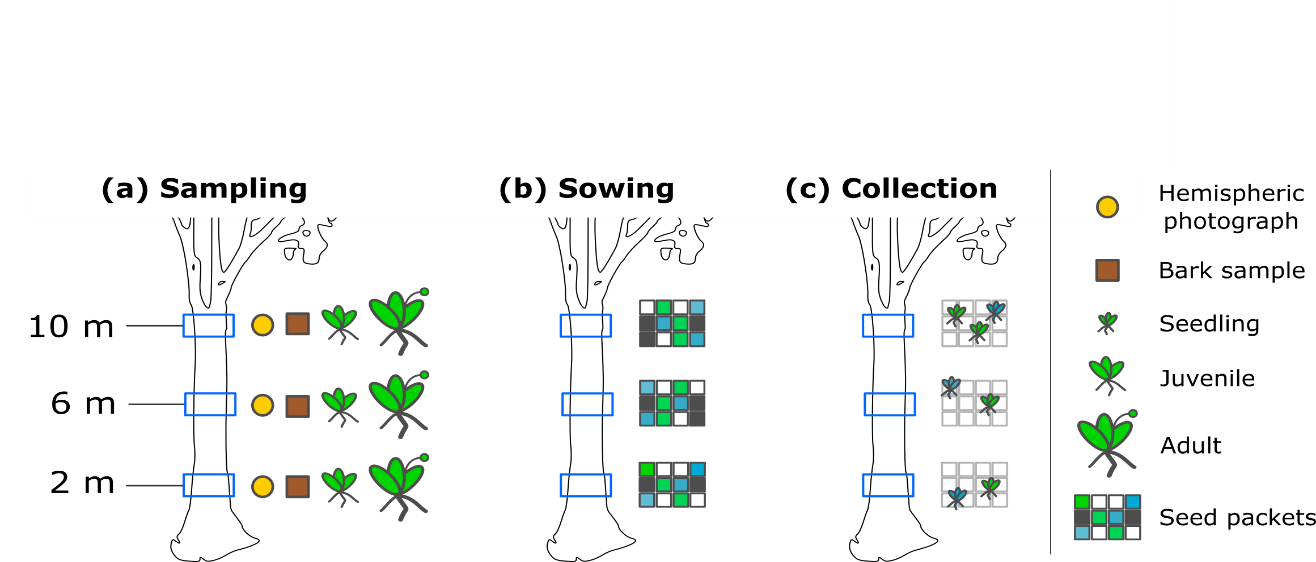
***In-situ germination assays***

*Study species*

The selection of the study species will be based on results from a previous study conducted in 2017 in the two plots in Andean dry-humid transition forests (Ventre-Lespiaucq, A. unpubl.).I investigated how two factors, light and host tree identity, influenced orchid community structure within the host tree[[43]](#footnote-34). Sampling 500 individuals from 37 orchid species in 18 host trees at five trunk heights, I found that light decreased by 34% from 10 to 1 m, with species turnover among heights exceeding 60%, and among host trees exceeding 80%, due mainly to replacement of rare species. These results reveal that there is a small number of common species with weak host tree specificity occurring across all the range of light conditions, and many rare species with probably more restricted requirements. These communities are ideal for testing the hypotheses of WP 2 and 3, since they harbour known species at both extremes of the generalist-specialist continuum towards host trees and environments. I will select four species: two occurring in the three trunk heights (broadly distributed), and the other two occurring in one extreme of the trunk (narrowly distributed) (Fig. X).

*Experimental design*

In the 20 trees, I will sample the three heights for the 4 species selected and collect 3 root fragments in 3-5 adult individuals, in order to identify mycorrhizal fungi in the adult stage. During this sampling, I will collect ripe capsules of these species and extract the seeds to prepare seed packets[[44]](#endnote-26). I will use plastic wraps31 to attach 3 seed packets per orchid species at the 3 heights in 10 trees per plot (720 seed packets in total). This experiment will be monitored every three months by a member of Dr. Flanagan's lab. Eight to ten months after sowing I will harvest the seedlings (Fig. 5c) to collect protocorm fragments for mycorrhizal DNA extraction and sequencing, which will be performed as described in WP 1.



**Fig.** **5** Study design. **(a)** Sampling at 3 trunk heights, **(b)** *in-situ* germination assays (each colour denotes a different species), **(c)** collection of seedlings.

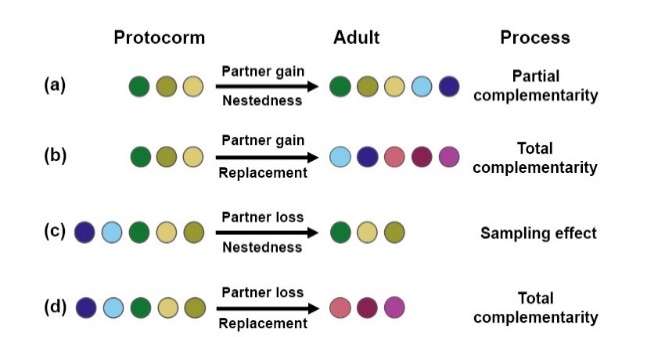
***Data analysis***

With this information, I will be able to test H2, which states that the availability of free-living fungi changes over the vertical gradient of light. To this end, I will first describe mycorrhiza community composition, richness and turnover across tree heights. I will perform non-metric multidimensional scaling (NMDS) analysis over a mycorrhizal OTU presence-absence matrix and assess differences in species composition with PERMANOVA, estimate richness with rarefaction curves[[45]](#footnote-35), and turnover using Jaccard index[[46]](#footnote-36). Second, I will test whether mycorrhizal fungi communities vary over the tree trunk building linear models controlled by the host tree species.

**WP3 Assessing turnover in mycorrhizal fungi over epiphytic orchid ontogeny.**

The study species and experimental design will be the same as in WP2. To assess whether mycorrhiza communities differ between ontogenetic stages, I will compare the diversity and composition of mycorrhizal fungi communities extracted from seedling and adult roots. In particular, to test the hypothesis that seedlings will have a greater diversity of mycorrhizal fungi than adults (H3), I will calculate mycorrhizal OTU diversity in seedlings and adults for each species, and test whether seedlings associate with a higher diversity of fungi. A confirmation of this hypothesis would indicate that seedlings are more generalist than adults, suggesting that the establishment of mycorrhizal associations is opportunistic during early life stages.

I derived four testable hypothetical scenarios of ontogenetic partner turnover and their putative driving processes (Fig. 1). Individuals can gain (Fig. 1a,b) or lose (Fig. 1c,d) partners throughout ontogeny. Ontogenetic partner gains would indicate a change in adult requirements relative to the seedling's, while partner losses would indicate that germination is opportunistic, and that at least some partners become dispensable at later ontogenetic stages. Partner gains can be due to **partial** (Fig. 1a) or **total complementarity** (Fig. 1b), and partner loss due to subsampling (**sampling effect**; Fig. 1c) or **total complementarity** (Fig. 1d). Partner turnover due to complementarity would allow the plant to fulfill its adult requirements, which differ from the seedling requirements; whereas partner turnover due to sampling effects would indicate that the regeneration and adult niches (as related to the mycorrhizal interaction) are similar.



**Fig. 1** Hypothetical scenarios of ontogenetic partner turnover and their putative driving processes. Colours denote different partners. Text above arrows indicates changes in partner number; text below arrows indicates the prevailing component of composition turnover (nestedness or replacement)

To test the hypothesis that ontogenetic partner turnover occurs through sampling effects rather than complementarity (H4), I will calculate OTU turnover from seedlings to adults for each species, and partition total OTU turnover into its nestedness and replacement components[[47]](#endnote-27). I expect nestedness to represent a greater proportion of OTU turnover (Fig. 1x). This result would support the general hypothesis that switching partners over ontogeny is a risky strategy.

This research raises ethical issues regarding the collection of plant material in a non-EU country. However, sampling will not destroy the plants, and I will comply with Access and Benefit Sharing regulations as well as with the local and EU law.

**RISKS AND CONTINGENCY PLANS**

I do not foresee major risks in the completion of this project given the supervisor's expertise in orchid-mycorrhiza interactions, statistical tools and molecular techniques; my experience in the study model and fieldwork in the chosen sites; and my solid contacts in Colombia, that will provide practical and administrative support during fieldwork stays. Nevertheless, I have identified potential contingencies for some WPs (Table 1). I will re-assess each risk and alternative strategies by monitoring tasks progress along with my supervisor and other team members.

**Table 1** Potential risks to the completion of work packages (WP), likelihood and contingency plan.

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| **WP** | **Risk** | **Likelihood** | **Contingency plan** |
| 1, 2, 3 | Fieldwork is not finished in time | very low | Two extra weeks planned to buffer sampling delays |
| 1,2 | Delayed export licenses | low | Use Dr. Flanagan's licenses backed by PUJ. Extract mycorrhiza DNA in her molecular biology lab and export PCR products. |
| 3 | Not enough seeds in the wild | medium | Obtain seeds from local orchidaria |
| 3 | Low seed germination rate | medium | Large sample sizes to obtain workable numbers |

**WORK PLAN**

The feasibility and effectiveness of this project is supported by the experience of the host institution, the supervisor, and the candidate. The proposal consists of four work packages (**WP**), nine milestones (**M**) and four deliverables (**D**) (*Table 2*). Ideal start date is October 1st, 2019.

**Table 2** Gantt chart showing work packages (WP), milestones (M) and deliverables (D) of the project. Blue: research packages; yellow: training; green: seminars. The chart is not exhaustive.



In ***months 2 to 4*** we will sample the six plots for roots and collect fruits in DHTF plots **(M1)**. The second half of ***month 4*** will be used to request export licenses and up-load orchid species inventories to SiB database in order to comply with the Colombian law. Licenses for research purposes are often granted within 1-3 months (N Ospina, Pers. comm.), so by applying at the end of month 3 I leave sufficient time for the process to complete (over three months). During ***months 5 to 7*** I will prepare and sow the seed packets **(WP3, WP4)** with the help of a M.Sc. student. Completion of these tasks will mark the end of fieldwork **(M2)**. In the first half of ***month 8*** export licenses should have been granted, allowing me to ship the samples to Belgium **(M3)**. If licenses delay further I have two alternative contingency plans (Table 1). The first half of ***month 8*** is also intended for closing administrative tasks and as a contingency period for potential risks during fieldwork (*e.g*., delays for bad weather) (Table 1). In the second half of ***month 8*** I will return to the host institution in Belgium, where I will extract mycorrhizal DNA in Prof. Jacquemyn's lab **(M4)**. If delays do not occur I will advance my return to Belgium. I will offer a seminar to discuss fieldwork outcomes with the team members and discuss further steps in data analysis **(S1)**.

In ***month 9*** I will send samples for high-throughput gene sequencing in the University of Antwerp (Belgium). Meanwhile I will analyse environmental, orchid community and host-tree data **(WP1)**. I will also enrol in selected modules of the MSc. degree in Bioinformatics at the host **(T1),** where I will learn how to handle and analyse multiple gene sequence data. I will receive hands-on training on sequence alignment and network analyses **(T2)** during ***months 11 and 12***. Upon analyses completion **(M5)**, I will offer a seminar to discuss results and possible approaches to the papers **(S2)**. I will spend ***months 14 to 16*** writing the manuscript corresponding to **WP1** (**D1**). I will perform the analyses for **WP2** between ***months 17-19*** **(M6)**, offer a seminar to discuss results and possible approaches to the papers **(S3),** and write the manuscript between ***months 20-22*** **(D2)**.

Field and lab work for **WP3** and **WP4** will be in ***months 23 and 24.***I will return to Colombia with a M.Sc. student to collect the germinated seedling roots and ship the samples to Belgium **(M7)**. Back in Belgium (***month 25***), I will extract mycorrhiza DNA and send it for sequencing. While I wait for sequence data, I will meet with team members **(S4)** to discuss the germination assays data and the approach of the manuscript of **WP3** and work in the analyses. I will submit the resulting manuscript in ***month 30*** **(D3)**. From ***months 31 to 34*** I will analyse data of **WP4**. I will submit the last manuscript on ***month 36*** **(D4)** and offer a seminar to present an overview of the results of the whole project **(S5)**.

# References

*Give an overview of the bibliographical references that are relevant for your research proposal.*

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