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

*The titles below provide a list of aspects that should be discussed in the project outline. This is followed by a brief description of the expected content in italics. Please retain these titles in the final project description, but remove the description. You may add extra titles and subtitles as necessary. Please stick to the maximum number of 10 pages, without changing text layout (font Calibri 11, line distance 1, page margins etc.). Please also remove this explanatory paragraph before submitting this project description.*

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

**WP 3 -** **Which factors limit the recruitment of epiphytic orchids in tropical forests?**

Understanding the factors influencing community assembly is a longstanding problem in ecology[[1]](#endnote-1),[[2]](#endnote-2). In sessile organisms, such as plants, species coexistence can be promoted by differences in their regeneration niche[[3]](#endnote-3). The regeneration niche includes preferences for different substrates[[4]](#endnote-4), differential responses of seedlings to light gradients[[5]](#endnote-5), and availability of mutualists[[6]](#endnote-6). The classic experiment by Connell[[7]](#endnote-7) illustrated how a combination of abiotic (dessication) and biotic factors (competiton) influenced barnacle recruitment in rocky intertidal coasts at small scales. In WP3, I use a similar approach to disentangle how microsite conditions affect the germination and recruitment of epiphytic orchids in tropical forests, which is a major knowledge gap in orchid conservation and restoration[[8]](#endnote-8).

**Epiphytic orchids** have three characteristics that might determine their regeneration niche at small scales: i) seed germination fully depends on **mycorrhizal fungi** that are not inherited from maternal plants[[9]](#endnote-9),[[10]](#endnote-10), and therefore, **free-living fungi** on the substrate might be a key component of microsite quality11; ii) epiphytes face strong ecological gradients in short distances, such as the **vertical gradient of light** of the forest12, ; iii) in most orchid species, adult plants rely on mycorrhizal fungi but are capable of photosynthesis (**partial mycoheterotrophy**), potentially reducing their dependency on mycorrhiza as compared to seedlings[[11]](#endnote-11).

**How does fungi availability affect germination across ecological gradients?** Most studies of orchid-mycorrhiza interactions assess **mycorrhizal communities**, which give information on which partners are selected from the local pool, instead of **free-living fungi** in the soil or the bark of the host[[12]](#endnote-12),[[13]](#endnote-13). Studies over small spatial scales revealed terrestrial orchids have distinctive mycorrhizal communities and show strong spatial segregation, suggesting that mycorrhizal partners play a role in determining their distribution16,[[14]](#endnote-14),[[15]](#endnote-15). No such data are available for epiphytic orchids.

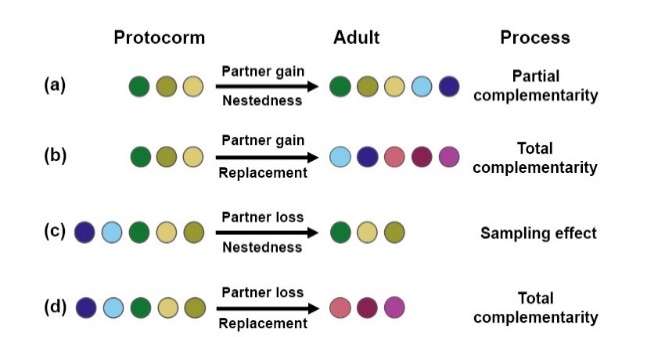
It is reasonable to assume that orchid germination frequency will be higher in species that associate selectively with common instead of rare fungal partners, so that **partner availability** is probably an important determinant of orchid germination. Yet, orchid species also differ in their degree of specialization in the interaction (**partner breadth**)[[16]](#endnote-16), 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[[17]](#endnote-17). 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. We ignore whether epiphytic orchids establish generalist or specialist interactions[[18]](#endnote-18). Recent studies suggest that they tend to associate with many mycorrhizal partners[[19]](#endnote-19),[[20]](#endnote-20), although highly specialized interactions also were documented21,[[21]](#endnote-21). A handful of comparative studies between seedlings and adults did not show a single pattern, highlighting the complexity of the regeneration niche of epiphytic orchids11,[[22]](#endnote-22). The presence of fungal symbionts in the substrate15 and partner breadth9 are major knowledge gaps towards understanding what limits the germination and recruitment of epiphytic orchids.

Solid evidence of how light gradients affect fungi availability and mycorrhizal symbioses in epiphytic orchids is still lacking[[23]](#endnote-23). 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.

**Which factors determine a successful transition from seedlings to adult****s?** After germination, seedlings experience a high death rate in later stages of development[[24]](#endnote-24),[[25]](#endnote-25). Several factors may cause recruitment failure, from seedling predation to differences between the *regeneration* and *adult niches* of the species[[26]](#endnote-26). 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.

Using these concepts, 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.

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 (Fig. 1a, b) are common11, while losses are less well documented[[27]](#endnote-27). [Develop this evidence a bit more] The role of mycorrhiza in germination and recruitment of epiphytic orchids remains to be assessed9,15.



**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)

# Scientific research objectives

*Describe explicitly the scientific objective(s) and the research hypothesis. Explain whether and how the research is specifically challenging and inventive, describing in particular the innovative aspects of the envisaged results. Discuss in detail the results (or partial results) that you aim to achieve, such as specific knowledge and academic breakthroughs.*

The scientific objective is to understand 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. Specifically, I will test three hypotheses:

**H1: The availability of free-living fungi changes over the vertical gradient of light.** The composition of free-living fungi communities will change over the host tree trunk.

**H2: 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.

**H3: Ontogenetic changes in mycorrhizal partners are due to sampling effects rather than total complementarity.** As total ontogenetic complementarity is risky, the nestedness component of mycorrhiza turnover (Fig. 1 a, c) will prevail over the replacement component (Fig. 1 b, d), regardless of whether adults gain or lose partners.

# Research methodology and work plan

*Elaborate the different envisaged steps (experiments/activities) in your research, and motivate your strategic choices with the aim of reaching the objectives. Describe the set-up and cohesion of the work packages including intermediate goals (milestones).*

*Show where the proposed methodology (research approach) is according to the state of the art and where it is novel. Discuss risks that might endanger reaching project objectives and the contingency plans to be put in place should this risk occur.*

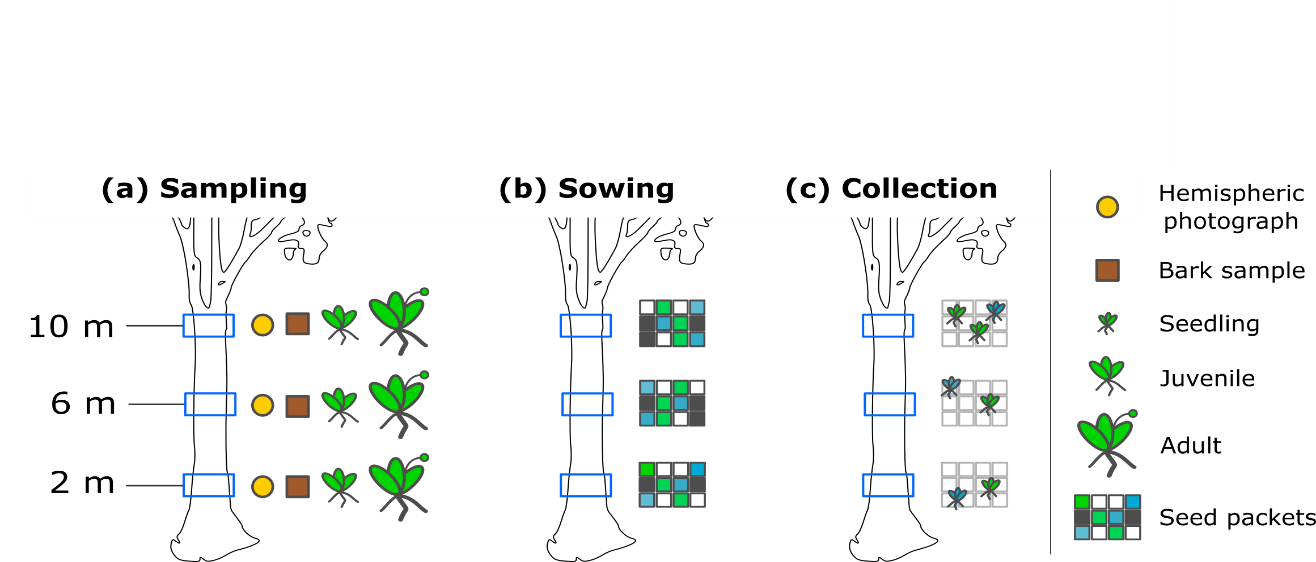
*Use a table or graphic representation of the planned course of activities (timing work packages, milestones, critical path) over the 3-years grant period.*

To assess how light, partner breadth, and mycorrhizal fungi availability affect orchid germination and recruitment, I will use a design analogous to Connell's experiment of barnacle distribution over the intertidal zone10. I will compare the spatial distribution of adult plants of 4 species, 2 broadly- and 2 narrowly-distributed over the tree trunk, with the distribution of conspecific juveniles, and seedlings from *in-situ* germination assays.

The study will be conducted in natural, dry-humid transition tropical forests in the western mountain range of the Colombian Andes. I will work in two plots 5 km apart and select 20 host trees in each. I will sample juvenile and adult plants growing on tree trunks at heights of 2, 6, and 10 m (Fig. 2). At each height, I will collect (Fig. 2a): i) 3 root fragments in 3-5 individuals per species and age to identify mycorrhizal fungi; ii) 3 bark samples to assess fungal availability on the host tree; and iii) 3 hemispheric photographs to describe the vertical gradient of light of host trees. 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.

For germination assays, I will collect seeds from ripe capsules of the 4 species and prepare seed packets[[28]](#endnote-28). I will attach 3 seed packets per orchid species in the same trees at 3 heights (1440 packets in total) (Fig. 2b) using plastic wraps31. In this way, I will evaluate whether seedlings and adults show the same vertical pattern of mycorrhizal partners. Eight months after sowing I will harvest the seedlings (Fig. 2c) to collect protocorm fragments for mycorrhizal DNA extraction. I will assess whether complementarity or sampling effects better explain ontogenetic partner turnover, by assuming that all partners are equally beneficial and by partitioning partner turnover into its nestedness and replacement components[[29]](#endnote-29) (Fig. 1).

An individual orchid can host 1-30 different fungal partners[[30]](#endnote-30). To accurately describe **fungal diversity** associated to individual plants and host’s bark, I will extract DNA from root fragments and bark using two complementary primer pairs (ITS3/ITS4OF and ITS86F/ITS4). I will use Illumina sequencing to obtain fungi operational taxonomic units (OTUs), which are the commonly used units of microbial diversity.



**Fig.** **2** Study design. **(a)** Sampling at 3 trunk heights, **(b)** *in-situ* germination assays, **(c)** collection of seedlings.

# References

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

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