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Review PhD thesis entitled ‘Factors limiting the distribution of the mycoheterotrophic plants in fragmented landscape’ by Milan Kotlínek

It was with great pleasure and interest that I have read the doctoral thesis of Milan Kotlínek entitled ‘Factors limiting the distribution of the mycoheterotrophic plants in fragmented landscape’. The thesis consists of a brief introduction, five published research papers, one unpublished manuscript and a brief summary of the results. According to the text, the major aims of the thesis were to:

1. investigate differences in mycorrhizal associations between different habitats and ontogenetic stages;
2. assess the effect of habitat fragmentation on the distribution of mycoheterotrophic plants;
3. determine short- and long-distance dispersal in mycoheterotrophic orchid species;
4. investigate whether the distribution of the study species is limited more by habitat than by their dispersion?
5. assess the various factors (mycorrhizal availability, edaphic factors, soil variables) that contribute to habitat suitability in orchids.

While these research questions have been worked out very well in Chapters 5, 6 and 7, they are less obvious in Chapters 2, 3 and 4, which either describe variation in mycorrhizal communities between different *Neottia* species or give a detailed description of the ecology of these species. The link with the other chapters is not always very clear. The thesis ends with a short concluding chapter, which briefly summarizes the results of the previous chapters and provides some (very) general perspectives for future research.

Major remarks

In general the thesis was well written, data collection and analyses were sound and the results are robust and in most cases convincing. However, given the aims and the title of the thesis, I’m not convinced that all papers should be included in this thesis, as some of them do not investigate the various factors that may limit the distribution of mycoheterotrophic plants in fragmented landscapes. Chapter 2, for example, seems redundant in this respect as are chapters 3 and 4. Could you briefly explain why these chapters have been included in this manuscript?

The **Introduction** provides a general overview of the state-of-the-art. However and much to my surprise, this chapter includes almost no references to papers published after 2017 and therefore this introduction seems a bit outdated. Just to list a few examples that are particularly relevant in the context of this thesis:

- Waud, M., Brys, R., Van Landuyt, W., Lievens, B., and Jacquemyn, H. (2017). Mycorrhizal specificity does not limit the distribution of an endangered orchid species. *Mol. Ecol.* 26,



1687–1701. (about the role of mycorrhizal availability in determining the distribution of orchid species and differences in mycorrhizal communities between ontogenetic stages)

- McCormick, M.K., Whigham, D.F. & Canchani-Viruet, A. (2018) Mycorrhizal fungi affect orchid distribution and population dynamics. *New Phytologist* 219: 1207-1215. (on the role of mycorrhizal fungi in determining the abundance and population dynamics of orchid species)
- Shefferson, R. P., Bunch, W., Cowden, C. C., Lee, Y.-I., Kartzinel, T. R., Yukawa, T., et al. (2019). Does evolutionary history determine specificity in broad ecological interactions? *J. Ecol.* 107, 1582–1593. (on mycorrhizal specificity)
- Jacquemyn, H., and Merckx, V. S. F. T. (2019). Mycorrhizal symbioses and the evolution of trophic modes in plants. *J. Ecol.* 107, 1567–1581. (on the evolution of trophic modes in plants and potential associated changes in mycorrhizal communities)
- Xing, X., Gao, Y., Zhao, Z., Waud, M., Duffy, K. J., Selosse, M.-A., et al. (2020). Similarity in mycorrhizal communities associating with two widespread terrestrial orchids decays with distance. *J. Biogeogr.* 47, 421–433. (on variation in mycorrhizal communities in two widespread orchids)

One obvious question therefore is why the most recent literature was not included in this introductory chapter? Could this introduction be updated so that it reflects the most recent state-of-the-art?

The term 'mycoheterotrophic' is misleading here as it may refer to both partially and fully mycoheterotrophic orchids. Could you elaborate on how the trophic mode of a plant is measured and what the difference is between autotrophic, partially mycoheterotrophic and fully mycoheterotrophic plants? Could you speculate about how trophic mode affects mycorrhizal communities and how this potentially affects distribution patterns of plants?

The overview of fungi associating with partially and fully mycoheterotrophic orchids is interesting, but incomplete and hard to read. It would have been better if a table was provided that gives for each species an overview of the fungi that were encountered. This overview also does not include the most recent information, which is a pity. Could this list be updated and reformatted?

In your thesis, you focused particularly on orchid species from tribe Neottieae. Could you briefly explain why you focused on these species? What makes these species relevant for this kind of research? The choice for *E. helleborine* is somewhat surprising as it is a widely distributed species showing even some weedy characteristics. Why did you select this species?

In **Chapters 3 and 4**, you discuss the population biology of two closely related *Neottia* species (*Neottia ovata* and *N. cordata*). These two species tend to have different distribution areas. Could you speculate about the main reasons why they differ in their overall distribution and to what extent mycorrhizal fungi are possibly involved?

In **Chapter 5**, you aimed at identifying the various factors determining the occurrence of forest orchids at the landscape scale. These species often co-occur in forests, yet you found that different habitat characteristics determined the distribution of these species. How can you explain this? How can these species frequently co-occur, and yet different habitat characteristics affect their distribution?

In **Chapter 6**, patterns of seed dispersal and realized gene flow were investigated in *Cephalanthera rubra* and *Epipactis atrorubens*. Results showed that most seeds landed in the immediate vicinity of mother plants, yet gene flow occurred up to 2 km in *C. rubra* and up to 125 km in *Epipactis atrorubens*. While the former result makes sense, the latter is somewhat surprising given that effective dispersal also requires establishment of a seedling and growth into an adult. In Chapter 7, it was demonstrated that the number of seeds developing into a protocorm was fairly low. Assuming that 25 seed packets were buried at each site and 20 sites were used, this resulted in 500 seed packets. About 150 seeds



were included in each seed packet, yielding a total of 75000 seeds that were buried in the soil. Of these, only 580 (0.8%) developed into a protocorm. This suggest that at least 100 seeds need to reach a site to get one protocorm. So, my question here is: what is the possibility that seeds travel distance of >100 km?

While the Chapters 2-6 were well written, this was less the case for **Chapter 7**, which needs additional work, both in terms of data analysis and writing. The text contains several writing mistakes that need correction. It is not clear why analyses were performed with the most abundant OTUs? Is there any evidence that the number of sequences is directly related to OTU abundance in the roots? The discussion also tends to be quite speculative. For example, you state various times that protocorms will not reach adulthood in sites that were deemed unsuitable. What is the evidence for this? How can you conclude this?

Orchids are declining worldwide and many species have become endangered or have already gone locally extinct. What information can be used from your thesis to help conservation managers to adequately protect orchids? What would be your major advice/guidelines for local conservation managers to safeguard our local orchid flora against the detrimental impact of human-induced disturbances and how did you come to this conclusion?

Overall, I enjoyed reading this thesis and I recommend its publication (perhaps with some minor adjustments). I hope the comments and suggestions will be helpful for preparing the defense.

Sincerely,

Prof. Dr. ir. Hans Jacquemyn