

# 博士論文要約 (Summary)

平成 30 年度入学

連合農学研究科 生物生産科学専攻

氏 名 蘭光 健人

タイトル	Identification of mycorrhizal communities and fungal specificities in three epiphytic orchid species
------	--

キーワード (epiphytes) (mycorrhizal preference) (symbiosis)

## General Introduction

Orchids depend primarily on mycorrhizal fungi to obtain nutrients throughout their life cycle. Epiphytic orchids account for 69% of orchid diversity. Mycorrhizal associations may be more important for epiphytic orchids than terrestrial species, as they may aid survival in the canopy under strong environmental stresses such as low water and nutrient availabilities. However, few studies have examined the fungal community associated with epiphytic orchids (mycorrhizal community). Here, the mycorrhizal community of three epiphytic orchid species, *Taeniophyllum glandulosum*, *Thrixspermum japonicum* and *Oberonia japonica*, were assessed using more samples than in any previous study. Because these three species were the most common epiphytic orchids in Japan, a large number of samples can be investigated. Furthermore, this study investigated whether the three orchid species have distinct mycorrhizal preferences by using *in vitro* symbiotic culture and *in situ* individuals co-existing in the same habitats.

## Chapter 1: Identification of mycorrhizal community of *Taeniophyllum glandulosum*

The mycorrhizal community of a leafless epiphytic orchid, *Taeniophyllum glandulosum*, was identified molecularly using 68 mature plants and 17 seedlings. In total, 187 fungal internal transcribed spacers (ITS) sequences were obtained, of which 99% were identified as Ceratobasidiaceae. These sequences were classified into five operational taxonomic units (OTUs) based on 97% sequence similarity. The most frequent sequence was a single OTU, CE1, which accounted for 91% of all Ceratobasidiaceae sequences, although other phylogenetically distinct Ceratobasidiaceae fungi were detected. These

results show that *T. glandulosum* is predominantly associated with a particular group of Ceratobasidiaceae. All mycorrhizal fungi found in *T. glandulosum* seedlings belonged to CE1, which was also found in adult plants on the same host tree. The mycorrhizal fungi from 13 host tree species were compared, and *T. glandulosum* was preferentially associated with CE1 on 11 tree species. Therefore, *T. glandulosum* is predominantly associated with CE1 and this dominant association remains throughout the orchid life cycle on divergent host tree species.

## **Chapter 2: Identification of mycorrhizal community of *Oberonia japonica***

The mycorrhizal community of *Oberonia japonica* was identified using 73 mature plants from 10 tree species at nine sites. In total, 104 fungal ITS sequences were obtained from 87 of 88 root samples. Of these sequences, 89 sequences were assigned to known mycorrhizal fungi of orchids, Tulasnellaceae and Ceratobasidiaceae. The members of Tulasnellaceae were account for 82.7 % of the sequences and assigned to three OTUs (TU3, TU20 and TU21). This shows that *O. japonica* was mainly associated with these three Tulasnellaceae OTUs. Although the dominant Tulasnellaceae OTU was different among the sampling sites, the difference in mycorrhizal communities had no correlation with the sampling sites. Furthermore, no significant difference was found in the components of mycorrhizal communities among the host tree species. Thus, the geographic region and host tree species may less affect the mycorrhizal association of *O. japonica*.

## **Chapter 3: Identification of mycorrhizal community of *Thrixspermum japonicum***

*Thrixspermum japonicum* is the most common epiphytic orchid in the temperate region of Japan. The mycorrhizal community of this species were molecularly identified using 169 root samples obtained from 144 individuals from 28 host tree species at 20 sites across 1300 km. In total, 181 fungal ITS sequences were obtained and assigned to 24 OTUs; 9 belonged to the Ceratobasidiaceae and 15 to the Tulasnellaceae. The phylogenetic analyses of these OTUs belonging to the Ceratobasidiaceae or Tulasnellaceae showed that mycorrhizal fungi associated with this orchid were phylogenetically divergent in these families. However, a single Ceratobasidiaceae OTU, CE1, accounted for 49.7% of all

fungal sequences and was predominant in the samples. These results imply that despite having a broad range of mycorrhizal partners, *T. japonicum* was predominantly associated with a single fungal taxon. This predominant association with CE1 were found in most of the sites among the host-tree species investigated. Furthermore, no significant differences were detected among mycorrhizal communities from different sites or host tree species, showing that these factors may less affect the mycorrhizal association of *T. japonicum*.

#### **Chapter 4: Comparison of mycorrhizal preference among three epiphytic orchid species**

The mycorrhizal communities of the three epiphytic orchid species, *T. glandulosum*, *T. japonicum* and *O. japonica*, were clearly different in Chapter 1-3. This difference may be caused by the plant preferences toward particular fungi (mycorrhizal preferences). In this study, the differences in the mycorrhizal preferences of the three species were assessed using 1) *in vitro* symbiotic germination experiments and 2) identification of mycorrhizal fungi by using co-existing different species under *in situ* condition. For the former assessment, each orchid species was co-cultured with CE1 or TU3 which were main mycorrhizal partners of these orchid species and then compared the growth rates among these fungi. The results showed that the seedlings of the three orchid species were induced only by the dominant fungus among each mycorrhizal community. For the latter assessment, the mycorrhizal fungi of two or three different orchid species co-occurring in the same tree branches (within 10 cm) were compared. The mycorrhizal fungi were clearly different among co-occurring *T. glandulosum* and *O. japonica*, while *T. glandulosum* and *T. japonicum* shared a single fungus CE1. Overall, the examination of *in vitro* cultures and *in situ* co-occurring plants revealed that mycorrhizal preferences are different among the three orchid species.

#### **General discussion**

This study revealed that three epiphytic orchid species, *T. glandulosum*, *T. japonicum* and *O. japonica*, have distinct mycorrhizal communities in natural habitats. Furthermore, these three species show different mycorrhizal preference under *in vitro* and *in situ* conditions. The geographic regions and the

host tree species had no significant effect on the mycorrhizal communities in all three species. Therefore, mycorrhizal preferences may have stronger effects for determination of the mycorrhizal communities than the other environmental factors. The different mycorrhizal preferences could contribute to avoiding the competitions for the mycorrhizal fungi and co-existence in a same habitat. Reducing competitions for nutrient supply from fungi may be more important for epiphytic orchids than terrestrial ones to survive in the canopies under strong environmental stresses such as low water and nutrient availabilities. Furthermore, a resource partitioning in the same site has been regarded as a factor of sympatric speciation, and thus-perhaps the diversification of the mycorrhizal preferences could drive plant speciation in epiphytic orchids. To clarify the relationship between plant phylogeny and mycorrhizal preference will contribute to understanding of mechanisms in diversification of epiphytic orchids.