

Native orchid mycorrhizal investigations

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Orchid mycorrhizal connections are as essential for orchids as breath is for us. These mycorrhizal connections are seen in both chlorophyllous and achlorophyllous orchids. The fungi and orchid form a connection that may or may not be specific to the orchid. Some orchids in Aotearoa New Zealand are observed to follow the distribution patterns of an ecosystem dominated by a specific tree. Are the orchid mycorrhizae the factor in an ecosystem that specifically makes these orchids germinate and thrive only there?

I tested this on chlorophyllous and achlorophyllous orchids in Aotearoa New Zealand because one has a lesser dependence on mycorrhizal fungus than the other throughout its lifetime. *Pterostylis agathicola* follows the distribution of *Agathis australis*, while *Danhatchia australis* (Fig. 1) closely follows the distribution of *Beilschmiedia tarairi* and *Rhopalostylis sapida*. I examined the co-occurrence of the orchids and co-locating trees, and the fungal communities of the orchids. In the field, I examined the spatial locations of these orchids in relation to that of their co-occurring tree species, and other features of their environments. I also cultured fungi from pelotons present in the orchid roots on agar media. Once I had achieved pure fungal cultures, these were characterized using Sanger sequencing. I selected some of these fungal cultures and I used them in an attempt to germinate the orchid seeds. In addition to Sanger sequencing, I performed metagenomic analysis of the internal fungal microbiome within roots of both the orchid species. This analysis returned a wider range of the fungal communities present in the roots of each species than did the culturing.

I only observed *Pterostylis agathicola* growing specifically around *Agathis australis*-dominated ecosystems. The closest tree to several *Danhatchia australis* plants was not always *Beilschmiedia tarairi* or *Rhopalostylis sapida*, although these were still in the vicinity. This suggests that further evidence is necessary to confirm which tree

species *D. australis* is associating with. The most dominant species from the cultures and the most abundant sequences (reads) in my metagenomic analyses for *P. agathicola* were from the Ceratobasidiaceae family. The Sanger sequences of cultured fungi from *D. australis* were of families different from those seen for other similar orchids. Seed germination cultures using Ceratobasidiaceae for both the orchids produced protocorm-like structures. The fungal communities of *Pterostylis agathicola* were as expected but those of *Danhatchia australis* suggest that there is more to be learnt to uncover their connection to the surrounding ecosystems.



Fig. 1. *Danhatchia australis*, with a closed bud (above) and an open flower – a rare sight in this inconspicuous orchid. Tawharanui, 5 Jan 2019. Photo: Jack Warden. <https://inaturalist.nz/observations/19433815>