

**Mycorrhizal specificity in endemic Western Australian
terrestrial orchids (tribe Diurideae): Implications for
conservation**

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BSc (Hons)**

This thesis is presented for the degree of Doctor of Philosophy of Murdoch University
2004

I declare that this thesis is my own account of my research and contains as its main content work which has not previously been submitted for a degree at any tertiary education institution.

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Abstract

The specificity of fungal isolates from endemic Western Australian orchid species and hybrids in the tribe Diurideae was investigated using symbiotic seed germination and analysis of the fungal DNA by amplified fragment length polymorphism (AFLP). The distribution of the fungal isolates in the field was also assessed using two different seed baiting techniques. The information from these investigations is essential for developing protocols for reintroduction and translocation of orchid species.

Two groups of orchids in the tribe Diurideae were studied. Firstly, a number of *Caladenia* species, their natural hybrids and close relatives from the southwest of Western Australia were selected because orchid species from the genus *Caladenia* are considered to have among the most specific mycorrhizal relationships known in the orchid family – an ideal situation for the investigation of mycorrhizal specificity. Secondly, species of *Drakaea* and close relatives, from the southwest of Western Australia and elsewhere in Australia, which are never common in nature and occur in highly specialised habitats, were selected to investigate the influence of habitat on specificity.

Seed from the common species *Caladenia arenicola* germinated on fungal isolates from adult plants of both *C. arenicola* and its rare and endangered relative *C. huegelii*, while seed from *C. huegelii* only germinated on its own fungal isolates. The AFLP analysis grouped the fungal isolates into three categories: nonefficeous fungi, *C. huegelii* type fungi, and *C. arenicola* type fungi. The group of *C. huegelii* type fungi included some fungal isolates from *C. arenicola*. An analysis of the AFLP fingerprints of *C. arenicola* fungal isolates from different collection locations showed that some, but not all, populations were genetically distinct, and that one population in particular was very variable.

Despite being thought to have very specific mycorrhizal relationships, *Caladenia* species hybridise frequently and prolifically in nature, often forming self-perpetuating hybrid lineages. Five natural hybrids within *Caladenia* and its closest relatives were investigated. Symbiotic cross-germination studies of parental and hybrid seed on fungi from the species and the naturally occurring hybrids were compared with AFLP

analyses of the fungal isolates to answer the question of which fungi the hybrids use. The germination study found that, while hybrid seeds can utilise the fungi from either parental species under laboratory conditions, it is likely that the natural hybrids *in situ* utilise the fungus of only one parental species. Supporting these observations, the AFLP analyses indicated that while the parental species always possessed genetically distinct fungal strains, the hybrids may share the mycorrhizal fungus of one parental species or possess a genetically distinct fungal strain which is more closely related to the fungus of one parental species than the other.

The work on *Caladenia* hybrids revealed that *C. falcata* has a broadly compatible fungus that germinated seeds of *C. falcata*, the hybrid *C. falcata x longicauda*, and species with different degrees of taxonomic affinity to *C. falcata*. In general, germination was greater from species that were more closely related to *C. falcata*: seeds from *Caladenia* species generally germinated well on most *C. falcata* isolates; species from same subtribe (Caladeniinae) germinated well to the stage of trichome development on only some of the fungal isolates and rarely developed further; and seeds from species from different subtribes (Diuridinae, Prasophyllinae, Thelymitrinae) or tribes (Orchideae, Cranichideae) either germinated well to the stage of trichome development but did not develop further, or did not germinate at all. The AFLP analysis of the fungal isolates revealed that the fungi from each location were genetically distinct.

In situ seed baiting was used to study the introduction, growth and persistence of orchid mycorrhizal fungi. A mycorrhizal fungus from *Caladenia arenicola* was introduced to sites within an area from which the orchid and fungus were absent, adjacent to a natural population of *C. arenicola*. In the first growing season, the fungus grew up to 50 cm from its introduction point, usually persisted over the summer drought into the second season and even into the third season, stimulating germination and growth to tuber formation of the seeds in the baits. Watering the inoculated areas significantly increased seed germination.

Mycorrhizal relationships in Drakaeinae were less specific than in Caladeniinae. A study of the species *Spiculaea ciliata* revealed that this species, when germinated symbiotically, develops very rapidly and has photosynthetic protocorms, unlike all

other members of the Drakaeinae. An AFLP analysis of the fungal isolates of this species grouped the isolates according to whether they had been isolated from adult plants or reisolated from protocorms produced *in vitro*. Isolates were genetically distinct when compared before germination and after reisolation. A cross-species symbiotic germination study of seeds of three *Drakaea* species and one *Paracaleana* species against fungal isolates from the same species and several other Drakaeinae species revealed lower specificity in this group than previously thought. A number of fungal isolates from *Drakaea* and *Paracaleana* species germinated two or more seed types, while all seed types germinated on fungal isolates from other species and the seed of *Drakaea thynniphila* germinated to some extent on every fungal isolate tested. An AFLP analysis of the Drakaeinae fungal isolates supported this information, revealing little genetic differentiation between the fungi of different orchid species.

An *ex situ* seed baiting technique was used to examine the role of mycorrhizal fungi in microniche specialisation in the narrow endemic *Drakaea*. Soil samples from within and outside two *Drakaea* populations were tested for germination of the relevant seed types. In both cases, germination was significantly higher on soil samples from within than outside the populations, suggesting that the relevant mycorrhizal fungi may be restricted to the same microniches as the *Drakaea* species. The presence of similar fungi at distant, disjunct locations may be related to the extreme age and geological stability of the Western Australian landscape.

The information from these investigations is essential for developing protocols for reintroduction and translocation of orchid species. It appears that the mycorrhizal relationships in these groups of orchids are not as specific as was previously thought. For reintroduction work, a broad sampling strategy is necessary, as it cannot be assumed that the same orchid species has the same fungus at different locations. A broadly compatible fungus may be of considerable utility in conservation work, such as in situations where a specific fungus appears to have poor saprophytic competence or where soil conditions have been altered. Seed baiting studies provide additional data on fungal distribution *in situ*. In general, molecular data do not provide information about efficacy or fungal distribution, so research programs that combine symbiotic germination studies with seed baiting investigations and genetic analyses of

the fungi will provide the maximum benefit for designing more effective conservation programs.

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Acknowledgements

My supervisors, Professor Jen McComb and Dr Kingsley Dixon, provided excellent guidance, support, encouragement and advice throughout my research.

Dr Andrew Batty and Dr Mark Brundrett provided technical and experimental advice.

Dr Andrew Brown and Dr Steven Hopper advised on the selection of species, particularly *Caladenia* hybrids, as well as photographs and location details (Andrew Brown).

Robyn Taylor provided considerable technical assistance and advice in the genetics lab and ran many gels for me. The genetics work could not have been completed without her.

Dr Siegfried Krauss and Dr Tianhua He provided assistance and advice on the genetics work.

The Master Gardeners of Kings Park provided assistance in the laboratory and field; particular thanks to Ethel Lucas, an exemplary constructor of seed packets, and Philip Shaw, who counted more seedlings than anyone could reasonably be expected to.

Many dedicated volunteers assisted me in the laboratory and field, some over a period of years. They are too many to mention individually, but special thanks go to Rob Holland, Frank Turnbull, Don Smith, Rosalie Wells, Trudy Paap, Christina Mueller, and Nicolas Alleonard.

Many enthusiasts, including the late Ron Heberle, Gary Brockman, Eric Chapman, Clive Malcolm, Greg Bussell, Barbara Thomson, and John and Leonie Brighton, shared their knowledge with me, showed me where to find the species and hybrids I needed, and allowed me to collect on their properties. Other people, including some in Victoria (Helen Richards in particular), collected and provided materials.

Last but not least, thanks to my family and friends for keeping me sane; in particular Sally Craddock and Alex Hollick, and Wally (four years of jokes about *Drakaeas* and *Duckaeas*) and Marion Phoebe.

