Endophytic fungi associated with Australian orchids

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Fungal endophytes of orchids

Australia is rich in orchid flora with over 1000 native species currently recorded. A significant proportion of Australia's terrestrial orchids are critically endangered, endangered or threatened. Threats to many orchid species include habitat destruction, degradation and fragmentation from increased urbanisation, overgrazing, altered fire regimes and unfortunately, excessive collecting by orchid fanciers. Conservation efforts for Australian orchids include both *ex situ* and *in situ* approaches. *Ex situ* efforts involve the growth of orchid species under horticultural conditions and long term storage of plant and associated fungal material in laboratories and herbaria. *In situ* approaches include re-establishing plants in the wild and protection of current populations through management initiatives.

All orchids depend on fungi for their nutritional needs. As the seeds of orchid are minute and contain very few stored reserves, fungal colonisation is essential for further growth and development following germination (Smith and Read 1997). During plant colonisation fungal hyphae penetrate cells and form elaborate coiled structures known as pelotons (see Figure 1). A peloton is the site of nutrient exchange between plant and fungus and it is via these structures that young orchids receive sugars and inorganic substances (eg. P, N) necessary for further growth. Because the fungi grow within plant cells they are called endophytes. Mature photosynthetic orchids remain colonised by fungi and supply their endophytes with sugars but continue to receive inorganic nutrients. A number of orchid species (such as *Dipodium* spp.), the so-called myco-heterotrophic orchids, completely lack photosynthetic capacity and are heavily dependent on a fungal partner to provide both sugars and inorganic nutrients throughout their lifetime.

Fungal endophytes have now been investigated in a large number of orchid species from around the world. The traditional approach to identify the fungal endophytes of orchids has been to isolate pelotons from orchid tissues and to maintain fungal colonies in pure culture. The fungi were then identified on the basis of anatomy and morphology including such features as nucleus number, hyphal cross wall structure and spore dimensions (eg. Perkins *et al.* 1995). Worldwide, the fungi involved with orchids are almost all members of the phylum Basidiomycota group, however many do not produce sexual spores, and are consequently assigned to the form genus *Rhizoctonia* (Rasmussen 2002). Form genera are used in fungi when the sexual spores that are essential in the classification of fungi are not produced. The form genus *Rhizoctonia* produces septate hyphae in culture, but there are few other morphological characters to distinguish different species. The presence of *Rhizoctonia* in orchids is intriguing as fungi in this group are usually renowned as serious pathogens of many agriculturally important plant species. In recent years, analysis of myco-heterotrophic orchid species have shown non-*Rhizoctonia* fungi can also colonise orchids. These are mostly higher basidiomycete genera such as *Thelephora*, *Russula* and *Coprinus*. In addition, various species of *Rhizoctonia* have been matched up to their sexual stages, which occur in genera such as *Thanatephorus* and *Ceratobasidium*. The sexual stage has been induced by altering the culture conditions, often over long periods of growth and with the addition of soil to the cultures, such as by Warcup (1985). Recently, DNA sequence data has also been used to connect *Rhizoctonia* cultures to sexual stages (Bougoure *et al.* 2005)

Although results may vary with the species of orchid examined, it appears that under natural conditions a particular orchid species may associate with only a few or a single fungal species. This specificity for fungal partners has major implications for orchid conservation. Isolation and perpetuation of the fungal endophyte of a rare orchid species is crucial to growing the plant under horticultural conditions, and explains the high failure rates for attempted propagation of fungal-dependent orchids by fanciers. A further implication is that when undertaking long term storage of rare orchid material, the associated fungus should be preserved as well (Batty *et al.* 2001). *In situ* management efforts should ensure the longevity of fungal populations as well as conservation of orchid populations.

Endophytes in Australian Orchids

The fungal endophytes of Australian orchids have been the subject of numerous investigations over the past four decades (eg. Warcup 1971, 1981, Perkins *et al.* 1995, Bougoure *et al.* 2005). In particular, Warcup (1981, 1990) documented the fungal partners of a large number of mostly common terrestrial orchid species (Table 1) and he was the first to isolate and identify the fungal endophyte of the rare subterranean orchid *Rhizanthella gardneri*.

Table 1. Fungal endophytes (bold) found in Australian orchid genera by Warcup*

Ceratobasidium	***Sebacina***	***Tulasnella***
Calanthe	*Acianthus*	*Acianthus*
Prasophyllum	*Caladenia*	*Arthrochilus*
Pterostylis	*Cyrtostylis*	*Caladenia*
Sarcochilus#	*Elythranthera*	*Caleana*
	Eriochilus	*Calochilus*
	Glossodia	*Chiloglottis*
	Leporella	*Corybas*
	Microtis	*Cryptostylis*
		Cymbidium
		Dendrobium
		Dipodium
		Diuris
		Drakaea
		Eriochilus
		Lyperanthus
		Microtis
		Orthoceras
		Thelymitra

*sources Warcup and Talbot (1980), Warcup (1971, 1981, 1988, 1990)
#plus other epiphytic genera listed in Warcup (1981)

Molecular Biology Techniques Identify Orchid Endophytes

A number of recent studies have been conducted on Australian orchid endophytes using molecular biology techniques. These studies have involved isolation of fungal DNA, sequencing of the ITS (internal transcribed spacer) rDNA region and comparison to fungal ITS sequences in GenBank, which is a worldwide database of DNA sequences. Bougoure *et al.* (2005) examined the fungal endophytes of six common SE Queensland terrestrial orchids. Three species of the genus *Pterostylis* ('Greenhood orchids') were shown to be colonised only by *Thanatephorus* species, while two species of *Acianthus* ('Mosquito orchids') were colonised only by a *Tulasnella* species. *Caladenia carnea* ('Pink fingers') was shown to be colonised only by a *Sebacina* species. Bougoure and Dearnaley (2005) and Dearnaley and Le Brocque (2006) have also shown that the myco-heterotrophic orchids, *Dipodium variegatum* and *Dipodium hamiltonianum* (Figure 2) are specifically colonised by *Russulaceae* fungi, a group of fungi that are well known as ectomycorrhizal partners on *Eucalyptus* roots. Recently Dearnaley (in press) has shown that the vine-like myco-heterotrophic orchid, *Erythrorchis cassythoides* is colonized by a number of fungal species including *Russula*, *Sebacina*, *Coltricia* and *Gymnopus*.

Molecular biology techniques have much to contribute to orchid conservation in Australia. The techniques are objective and highly precise and may reveal the identity of fungal endophytes of orchids that defy identification via traditional morphological/anatomical approaches eg. non-sporulating fungi. It is essential that the many *Rhizoctonia*-like endophytes that have previously been identified using the traditional morphological approach are more thoroughly characterised via molecular analysis so that potentially serious pathogens are not released into natural situations during *in situ* conservation efforts. Molecular biology techniques make it possible to view for the first time the populations of previously unculturable fungi that colonise numbers of Australian orchid species, with the promise that identification may provide a guide to appropriate culture methods. At present, the sequences available in GenBank do not fully represent all species of fungi occurring in nature. Therefore the best match to sequences derived from fungi associated with Australian orchids may be to more distantly related fungi. There is a need for more comprehensive inventory of all Australian fungi, so that the full promise of molecular methods in enabling rapid and objective identification of fungi can be realised.

Advances in molecular biology techniques, applied to the study of endophytic fungi-orchid relationships, are poised to contribute significantly to future orchid conservation and cultivation, retaining biodiversity and satisfying the human fascination with this extraordinary plant family.

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Captions for images

Figure 1. Fungal pelotons inside the root of Dipodium hamiltonianum*. Scale bar is 70µm. Photo: John Dearnaley. [Dh3bX20b.jpg]*

Figure 2. The myco-heterotrophic orchids, Dipodium variegatum *(A) and* Dipodium hamiltonianum *(B) – these plants appears to have a specific relationship with ectomycorrhizal fungi that colonise* Eucalyptus *roots. Photos: John Dearnaley. [PICT0048.JPG], [PICT0342.JPG]*