

Fungal Endophytes in Australian Myco-heterotrophic Orchids

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Summary

The fungal endophytes of Australian myco-heterotrophic orchids are largely unknown. In this investigation we identified the fungal endophytes of three species of Australian myco-heterotrophic orchid: the terrestrial species *Dipodium variegatum* and *Dipodium hamiltonianum* and the vine-like *Erythrorchis cassythoides*. Similar to studies of myco-heterotrophic orchids in Nth America and Europe the fungal endophytes of *Dipodium* spp. are members of the Russulaceae. *E. cassythoides* contained both ectomycorrhizal and saprotrophic fungi suggesting that it can derive its carbon via fungi from both dead and living plant material.

Introduction

All orchids rely on fungi for seed germination and early growth. A number of orchid species are non-photosynthetic and are termed myco-heterotrophic because of their complete dependence on fungi throughout their life cycle. Molecular identification studies of fungal endophytes of myco-heterotrophic orchids have shown that these plants are colonized by higher basidiomycetes such as members of the Russulaceae and Thelophoraceae (Taylor and Bruns 1997, 1999, Girlanda *et al.* 2006) as well as members of the heterobasidiomycete family Sebacinaceae (Selosse *et al.* 2002, Taylor *et al.* 2003). As these fungal groups contain ectomycorrhizal species it appears that myco-heterotrophic orchids are indirectly parasitic on the tree host partner of ectomycorrhizal partnerships. Evidence supporting this comes from McKendrick *et al.* (2000) who have shown that radiolabelled carbon can flow from tree to myco-heterotrophic orchid via a fungal conduit, additionally, many myco-heterotrophic

orchids share fungal partners with adjacent tree species (Taylor and Bruns 1997, Selosse *et al.* 2002).

Australia is rich in orchid biodiversity. Of the more than 1000 species recorded approximately 20 are fully myco-heterotrophic in nature. The fungal endophytes of Australian myco-heterotrophic orchids have largely remained unidentified to date as they have been difficult to isolate into pure culture. Warcup (1985) succeeded in isolating a *Rhizoctonia*-like species from the underground *Rhizanthella gardneri* which is currently being further characterised with molecular techniques (Bougoure pers. comm.).

In this study, the fungal endophytes of three Australian myco-heterotrophic orchids have been identified using molecular techniques. The orchid species focused on were: *Dipodium variegatum* R.Br., a common terrestrial species, *Dipodium hamiltonianum* F.M. Bailey a rare terrestrial species and *Erythrorchis cassythoides* (Cunn.) Garay a common climbing species.

Materials and Methods

For full methods details see Bougoure and Dearnaley (2005) and Dearnaley and Le Brocque (2006). In brief, genomic DNA was extracted from colonised roots of the three orchid species from sites in Queensland and New South Wales, Australia. Fungal DNA was amplified with ITS1F and ITS4 primers and transformed into *E. coli*. Recombinant plasmids were isolated via blue-white selection and fungal ITS inserts sequenced using T7 primer at the Australian Genome Research Facility. Sequences were compared with fungal ITS sequences in GenBank using BLAST searches.

Results

The fungi associated with *Dipodium variegatum* included *Russula* spp. and the likely soil contaminants, *Verticillium* and *Trichoderma* spp. The fungal community of *Dipodium hamiltonianum* consisted of *Russula* and *Gymnomyces* and the likely soil contaminant, *Penicillium daleae* KM Zalesky. Analysis of the fungal endophytes of *Erythrorchis cassythoides* suggests that the orchid is colonized by both ectomycorrhizal fungi such as *Russula*, *Coltricia* and *Sebacina* as well as the saprotrophic *Gymnopus* (Table 1).

Conclusions

These results suggest that similar to North American and European myco-heterotrophic orchids (Taylor and Bruns 1997, 1999, Girlanda *et al.* 2006), Australian myco-heterotrophic orchids are commonly colonised by members of the Russulaceae. Russulaceae fungi are ectomycorrhizal and the three orchid species typically grow at the base of mature *Eucalyptus* species suggesting the orchids may be indirect tree parasites but this remains to be proven. Conservation strategies for *D. hamiltonianum* will require growth of these Russulaceae fungi under laboratory conditions and, as there are such techniques now available (Sangtien and Schmidt 2002), this is a realistic propo-

Table 1. Fungal root endophytes of Australian myco-heterotrophic orchids

Orchid species	Fungal ITS sequences isolated
<i>Dipodium variegatum</i>	<i>Russula occidentalis</i> <i>Russula solaris</i> <i>Verticillium</i> sp. <i>Trichoderma hamatum</i> <i>Halocyphina villosa</i>
<i>Dipodium hamiltonianum</i>	<i>Gymnomyces fallax</i> <i>Russula lepidicolor</i> <i>Penicillium daleae</i>
<i>Erythrorchis cassythoides</i>	<i>Russula mustelina</i> Uncultured Sebacinaceae <i>Coltricia perennis</i> <i>Gymnopus luxurians</i> <i>Epacris pulchella</i> endophyte (Ascomycete) <i>Metarhizium album</i>

sition. The involvement of *Gymnomyces* spp. as endophytes in *D. hamiltonianum* may also be one reason why this orchid is becoming rare as the fruit bodies of this species are much sort after food of threatened fungivorous marsupials which may act as dispersal agents for fungal spores (Johnson 1996).

The occurrence of both ectomycorrhizal and saprotrophic fungi ie. *Gymnopus* as endophytes in *E. cassythoides* is an intriguing observation. Whereas the majority of *E. cassythoides* plants sampled here grew on living trees, the plant with the *Gymnopus* endophyte was growing on a dead host. Jones (1988) has observed that seed of this orchid can germinate and grow in piles of sawdust. Thus *E. cassythoides* appears to be a member of the small group of myco-heterotrophic orchids that can derive carbon from dead plant material via associated fungi (Leake 2005).

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