

Recent studies of the biology of the yellow hyacinth orchid – *Dipodium hamiltonianum*

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Abstract

Dipodium hamiltonianum is a rare species of terrestrial orchid in eastern Australia. The fungi that reside in the orchid's roots are critical for nutrition of the plant. By extracting the DNA from whole colonized orchid roots and sequencing the fungal DNA present we have determined the identity of these fungi. These results strongly suggest that the orchid is a parasite on the trees it is usually found close to.

Key words

Orchidaceae, *Dipodium hamiltonianum*, Rare species, fungi, parasite.

Introduction

All orchids depend on soil fungi for seed germination in the natural state. Although most epiphytic orchids have a reduced dependency on fungi as adults, most terrestrial species continuously rely on soil fungi to some extent for their nutrition. Some terrestrial orchids have completely lost the ability to make their own food via photosynthesis and are heavily dependent on soil fungi to provide their nutritional needs via decaying plant matter or a living host. Examples of such genera in Australia include *Rhizanthella*, *Gastrodia*, *Erythrorchis*, *Epipogium* and *Dipodium*.

Dipodium hamiltonianum F.M. Bailey or yellow hyacinth orchid is an impressive yellow flowering member of the *Dipodium* group of Australian terrestrial orchids (Figures 1 & 2). The orchid is endangered in Victoria with only 100 or so known individuals at various sites in the north east of the state (Department of Sustainability and Environment 2004). The range of the species extends to southern Queensland where it is considered uncommon (Riley and Banks 2002). Threats to the species include grazing, weed invasion, collecting and altered fire regimes (Department of Sustainability and Environment 2004). Being a highly myco-heterotrophic (fungus dependent) species, probably indirectly parasitic on tree roots (Bishop 2000; Jones and Jones 2000) the orchid is impossible to cultivate.

This article reports on an investigation into the identity of the fungi that reside in the roots of the yellow hyacinth orchid. As previous studies of fungi in other *Dipodium* species have shown these fungi to be largely unculturable (Bougoure and Dearnaley 2005) molecular biology techniques have been used to answer this question. It is envisaged that identification of the root fungi of the orchid will provide clues about the ecology of the species as well as indicators for improving its conservation status via *ex situ* growth.

Materials and Methods

Roots from 4 plants were sampled from Northern NSW and SE Queensland (Figure 3). DNA was extracted from whole roots and amplified using fungal specific primers. After DNA purification, fungal sequences were inserted into *E. coli* bacteria and amplified via growth in nutrient broth. Following plasmid purification, fungal DNA was sequenced and compared with the GenBank database. For full methods see Dearnaley & Le Brocque (in press)

Results and Discussion

As is typical for *Dipodium* orchids (Riley and Banks 2002) the plants sampled were located at the base of, or close to mature tree species. The potential host species included *Eucalyptus prava*, *E. youmannii*, *E. rossii* and *Callitris endlicheri*, indicating that the orchids are not specific in their tree preferences. Most of the roots sampled were heavily colonised, with brown fungal pelotons (coils) clearly visible in cross sections (Figure 4).

The results of the molecular biology investigations suggest that *D. hamiltonianum* is colonised by a specific group of fungi belonging to the Russulaceae family. DNA matches from GenBank included *Russula* species which have previously been shown to colonise roots of the related *D. variegatum* (Bougoure and Dearnaley 2005) as well as *Gymnomyces* spp. Both of these genera of fungi are commonly ectomycorrhizal on *Eucalyptus* (Bougher 1995) strongly suggesting that *Dipodium* orchid are indeed indirect parasites of tree species ie. they receive nutrients from living tree roots via a fungal connection. The observation that *Gymnomyces* fungi are found in *D. hamiltonianum* roots is interesting as these fungi, otherwise known as native truffles, are highly sort after food of marsupials such as potoroos and bettongs. As these marsupials play a role in fungal spore dispersal (Johnson 1996) it is enticing to conjecture that these animals play a role in the distribution of the orchid as well. Indeed it is noteworthy that few of these marsupials are now found in North east Victoria where the orchid is endangered while in North east NSW and South east Queensland both the orchid and marsupials are more secure.

What does this research mean for the conservation of *D. hamiltonianum*? Now that we have identified the fungi that reside in the roots of the plant we need to investigate the particular methods needed to culture these microorganisms. Once this is achieved it may be possible to grow orchids from seed in the laboratory, presumably together with seedlings of suitable tree species. A large collection of laboratory raised plants will enable release of plants to the wild as well as production of seed to pass to other collections to ensure longevity of the species.

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Figures 1 & 2. *Dipodium hamiltonianum* in Northern NSW. Scale bars are approximately 1cm.



Figure 3. Root from *D. hamiltonianum* from SE Qld. Scale bar is 2cm.

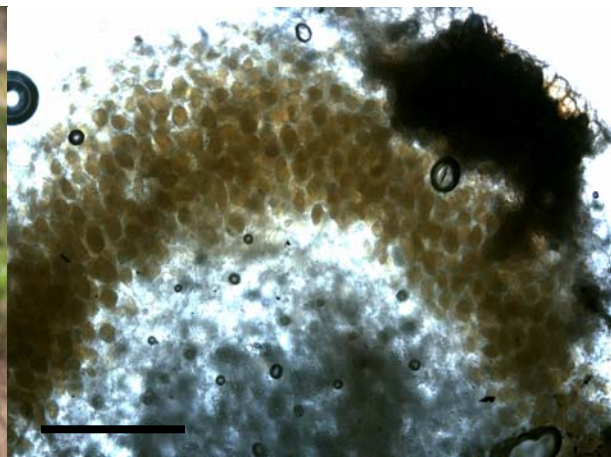


Figure 4. Fungal pelotons inside the root of *D. hamiltonianum*. Scale bar is 1mm.