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Bulahdelah Bypass Orchid Recovery Project Dr Mark A. Clements & Dr Chris Howard









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Overall aims of Project

- Due to the Pacific Highway Upgrade on the footslopes of Alum Mountain, several populations of three threatened Orchid species will be affected
- The NSW RTA has contracted the CSIRO to perform research into the survival and translocation of these species
- CSIRO was awarded the research contract through a competitive process
- The CSIRO is to provide the RTA with strategies to address issues for the survival and translocation of these orchid populations



CSIRO Research Team Introduction

- Centre for Plant Biodiversity Research, Canberra
 - Joint initiative between CSIRO and the Australian National Herbarium
- Dr Mark Clements
 - Research Scientist
 - Approximately 30 years experience in orchid research
 - Expert in:
 - Cultivation & Propagation
 - Taxonomy
 - Systematics
- Dr Chris Howard
 - Postdoctoral Fellow
 - Training in Molecular Biology & Plant Pathology



Brief History of the Project

• 2000 – 2002

- Floristic surveys were commissioned by RTA for route selection and for associated Environmental Impact Statement
 - Cryptostylis hunteriana was discovered on the site
 - Local family relocated *Rhizanthella slateri* plants, a species previously known from the site
 - Detailed surveys for *Rhizanthella slateri* and *Cryptostylis hunteriana* conducted
- 2003 2004
 - Detailed survey for undescribed species (to be *Corybas dowlingii*) with samples sent to CSIRO Canberra for identification and formal naming
- 2003 2008
 - Annual surveys for all three threatened species
 - To gain an understanding of the distribution of the three species on the site
- 2008
 - Orchid Management Plan drafted
 - Addresses issues for the survival and translocation of the affected orchid populations



CSIRO's Involvement in the Project

• 2006 - 2007

- Contacted to observe and record the phenology of the three species
- Also to perform hand pollinations for *Cryptostylis hunteriana* and *Corybas dowlingii* for seed collection and to enhance natural regeneration

• 2008 – 2011

- Contracted to research strategies for propagation and translocation of populations of the three orchid species
- Including additional hand pollinations for seed collection and to enhance natural regeneration



The Orchid Species

Cryptostylis hunteriana (Leafless tongue orchid) EPBC Act – vulnerable



Rhizanthella slateri (Eastern underground orchid) EPBC Act – endangered





Corybas dowlingii

(Red helmet orchid) **NSW TSC Act**

- endangered



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Cryptostylis hunteriana

- Perennial / deciduous terrestrial leafless herbs
- Dry habitat preference Associated with many plant species
- Reproduction sexual, vegetative forming localised clumps
- Wasp Pollination
- Seed dispersal wind









Cryptostylis hunteriana

Distribution



Image source: http://www.environment.gov.au/cgi-bin/sprat/public/publicspecies.pl?taxon_id=19533



Corybas dowlingii

- Small, colony forming, terrestrial herb
- Habit preference restricted to wetter gullies
- Cultivation possible
- Transferable probably
- Fly Pollination
- Seeds wind dispersed
- Formally described in 2004 yet possibly known since 1930s







Corybas dowlingii

Distribution



Image source: http://www.rbg.vic.gov.au/cgi-bin/avhpublic/avh.cgi



Rhizanthella slateri

- Subterranean leafless orchid
- Non-photosynthetic
- Fly Pollination
- Seed maturation 11-12 months
- Seed dispersal animal (bandicoots / birds)
- may be intimately linked to host plant (tree or shrub) by mycorrhizal fungus







Rhizanthella slateri

Distribution





The Orchids - Life History Strategies

- Three orchids with entirely different life-history strategies
- Representing a diverse set of obstacles to overcome in order to achieve project objectives
- Translocating the orchids from the affected area requires knowledge of:
 - mycorrhizal associations
 - flowering times, regimes and pollinators
 - habitat preference
 - reproductive strategies (vegetative, sexual)
 - seed dispersal/longevity



Orchid biology – fungal association

- Reliant on Mycorrhizal Fungi
 - Fungi that interact with and form associations with plant roots
 - Example of a mutualism



Image source: http://www.ipm.iastate.edu/ipm/hortnews/2006/4-5/fungi.html

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Orchid biology – fungal association

- Reliant on Mycorrhizal Fungi Orchids are Heterotrophs
 - Seed germination and Nourishment (myco-heterotrophy)
 - Orchid seed is minute no endosperm (or food source)
 - Mostly wind dispersed
 - Rely on fungal infection and subsequent symbiosis



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Orchid biology - pollination

Pollination by sexual deception





Propagation and Translocation

- Primary aim is to propagate the orchids from seed and replant in an undisturbed area
 - Some physical translocation may be necessary

• Requires knowledge of:

- Habitat Preference
 - Floristic surveys at the location of each orchid species will establish what habitat the orchids prefer
- Mycorrhizal fungi presence
 - Surveys for fungi will reveal likely places to reintroduce the orchids
- Flowering times
 - Work over the last 2 years has established precise flowering times
- Reproductive strategies
 - Each species of orchid utilises different strategies for survival when flowering is limited



What we have achieved

- Now have clearer understanding of the biology of each species
 - Flowering times, seed dispersal mechanisms, reproductive strategies
- Have performed 100s of hand pollinations
 - Resulting in tens of thousands of seed released over the site including adjacent to the road corridor
 - Seed samples have been collected for germination experiments





What we have achieved

- Physical translocation trials have been undertaken
 - Corybas dowlingii and Cryptostylis hunteriana
- Mycorrhizal fungi for each species have been isolated, identified, and are in culture in Canberra
 - DNA sequence comparison reveal distinct species of fungi are involved with all three orchid species







Fungal culture morphology



- Characterisation of each mycorrhizal fungal isolate
 - DNA and growth studies
- DNA analysis of Soil samples from across the site to map the distribution of mycorrhizal fungi on the site to identify reintroduction sites



Image source: http://www.biologyreference.com/Dn-Ep/Electrophoresis.html



• Seed germination trials for *Cryptostylis hunteriana*, *Corybas dowlingii* and *Rhizanthella slateri*



• Monitor trials of translocated *Cryptostylis hunteriana* and *Corybas dowlingii* during their respective growing periods



- With knowledge of fungi and growth requirements, we aim to propagate populations of each orchid species for re-introduction
- Continue orchid propagations for future re-introductions
- Cryogenically store seed and mycorrhizal fungi for long term management
- Specific orchid plants that will be affected will be translocated prior to construction

Source of images: Sommerville, K.D., Siemon, J.P., Wood, C.B. and Offord, C.A. (2008) Simultaneous encapsulation of seed and mycorrhizal fungi for long-term storage and propagation of terrestrial orchids. Australian Journal of Botany, 56: 609 - 615





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- The RTA will continue to facilitate and coordinate quarterly meetings with CSIRO, EcoPro, & relevant NSW and Federal Govt Departments
- Orchid management plan stipulates continual monitoring of the site for a minimum 10 year period
 - Surveys of translocation sites and unaffected populations
 - Weed control
 - Additional hand pollinations
 - Supplementary plantings if required
 - Continual public information sessions
 - Control of fire regime for the site





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