

# Saving orchids from extinction: the RBGV Orchid Conservation Program *ex situ* collection

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## Introduction

Australia has over 1800 species of orchids, most of which are terrestrial. Orchids are overrepresented on Australia's threatened species list, making up 17% of all nationally listed flora (~197 species). The Royal Botanic Gardens Victoria (RBGV) Orchid Conservation Program aims to prevent extinction by:

- Storing a diverse representation of seed and mycorrhizal fungi.
- Propagating suitable numbers of each of our threatened orchids.
- Undertaking conservation translocations of these species to protected public and private land where the appropriate vegetation, climate conditions and pollinator(s) are present.

The RBGV Orchid Conservation Program undertakes research on all aspects of orchid ecology, including pollination, mycorrhizal associations, propagation, demographics and translocation. For south-eastern Australian orchids, the RBGV Orchid Conservation Program has established a permanent *ex situ* collection of seed, mycorrhizal fungi and living plants propagated from seed.

For each species, plants have been hand-pollinated and the seed collected. With the assistance of volunteers, seed is cleaned, dried at 15% relative humidity (RH) for two weeks, and stored in sealed airtight packages at -20°C. The *ex situ* seed collection now includes diverse seed collections from over 150 orchid species and well over 2,500 accessions of seed. For each species of orchid, we also isolate the mycorrhizal fungi and store pure cultures permanently *ex situ* at -80°C for use in propagation and molecular identification. The RBGV now have over 3,000 isolates of mycorrhizal fungi stored at -80°C for use in future conservation introductions and for taxonomic studies into their identity, ecology and distribution.

Since 2014 the RBGV has grown a large *ex situ* collection of orchids for conservation, introduction and education, which is based at the Cranbourne site. Seeds are germinated symbiotically with their mycorrhizal fungi in the laboratory and grown on in flasks before being potted up in the nursery. This *ex situ* living plant conservation

nursery now consists of over 20,000 plants from 165 species of orchid, including 68 state and nationally threatened species. These species are primarily from the genera *Caladenia*, *Diuris*, *Thelymitra*, *Pterostylis* and *Prasophyllum*. A dedicated group of volunteers assist research staff in the curation and management of the collection. This collection holds many species brought back from the brink of extinction including *Caladenia pumila*, *Thelymitra mackibbinii*, *Caladenia audasii*, *Diuris fragrantissima* and *Prasophyllum correctum*.

This *ex situ* collection is now used extensively for seed orcharding of threatened orchids, pollination studies, taxonomy and conservation introductions. To date this *ex situ* collection of orchids has contributed to well over 50 introductions of threatened orchids across south eastern Australia.

Here we give two examples of *ex situ* collections leading to conservation outcomes. *Caladenia arenaria* is an Endangered species, endemic to the Riverina of southern New South Wales. *Caladenia versicolor* was formerly widespread across Victoria and South Australia and is now extinct in South Australia and is known from two populations with less than 600 individuals in total.

## Propagation

Mycorrhiza were isolated as per the methods of Reiter *et al.* (2020a). Fungal cultures had DNA extracted, sequenced and identified as per Reiter *et al.* (2020a). The mycorrhizal fungi were identified as *Serendipita* OTU A (Reiter *et al.*, 2020a) for both species and did not vary between the remaining sites of this species. Plants were grown symbiotically with their mycorrhizal fungi, from seed to mature flowering individuals using the techniques of Reiter *et al.* (2016).

## Pollinator baiting

Baiting trials were undertaken using the baiting method of Reiter *et al.* (2018). These are summarised here for clarity. Potted plants (20–30 flowers) grown from seed symbiotically from the *ex situ* collection of each of our study species were used for baiting. Using large numbers of flowers increases the stimulus perceived by pollinators for food deceptive or food rewarding species.

Observations of the pollinators were made during the flowering period of wild populations of the orchids. A selection of insects visiting or pollinating the bait flowers were collected for identification.

### Conservation translocation

Translocation data is only presented for *C. versicolor*, as *C. arenaria* is due to be translocated by project partners in 2021. Plants grown symbiotically were translocated into *de novo* (new) sites that were permanently reserved by Trust for Nature. The size of intact native vegetation of the translocation sites in size was chosen as > 100 hectares of suitable vegetation that matched that of the extant sites. Each site had the pollinator present (Reiter *et al.* 2019; 2020a). Each individual orchid was planted with a permanent marker, 10 cm to the north to aid in re-emergence monitoring and identification of the plants in subsequent years. Each plant was caged and watered (up to monthly average rainfall only if rainfall fell below average) for the first season only, until plants entered their first dormancy in the field. Between 2013 and 2017, 798 plants were planted of *C. versicolor*.

### Monitoring

All plants were monitored annually for emergence (July), flowering (September-November) and seed set (November-December). In addition, any recruits were recorded. Each species also had one wild population monitored using this method for comparison against the translocation sites.

## Results

### *Caladenia versicolor*

The pollinator was identified as *Leioproctus maculatus* (Reiter *et al.* 2019) a small colletid bee with the orchid providing a meagre food reward. The pollinator was identified at two of the remaining wild sites and the introduction sites. Over 1,000 plants have been propagated symbiotically with *Serendipita* OTU A for an *ex situ* collection and introduction. Seed orcharding has begun on the *ex situ* collection to ensure that adequate seed is available for future conservation work with this species. For the introduced plants grown symbiotically from seed there was 88% survival, 47% flowering, 30% pollination as of July 2019. The first signs of recruitment were in 2017; and the total number of recruits recorded on the introduction sites thus far is > 60. Emergence on the wild site of the species was 81%.

### *Caladenia arenaria*

*Caladenia arenaria* was found to have at least two pollinator species (Reiter *et al.* 2020b), the thynnine wasps *Tachynomyia* sp. nr *volatilis* and *Aelothynnus westwoodii*. Both of these species removed and deposited pollinia, and were found at suitable translocation sites that matched the vegetation characteristics of existing orchid populations (Reiter *et al.* 2020b). Approximately 2,000 individuals have been cultivated symbiotically with *Serendipita* OTUA from seed from the three largest remaining populations of this species. These plants await introduction into the selected sites in 2021 with project partners. Seed orcharding has begun on the *ex situ* collection to ensure that adequate seed is available for future conservation work with this species.



Figure 1. Examples of *ex situ* living collections at RBGV grown from seed with their mycorrhizal fungi and now flowering in the shade house; *Caladenia cretacea* and *C. cruciformis*. Photo: Richard Dimon, RBGV

## Acknowledgements

RBGV Orchid Conservation Volunteers Charles Young, Bryan Lawrence, Mike Wicks, Eve Almond, Peter Wallace, Neil Freestone, Neil Anderton, Lynda Entwisle, Wendy Bedggood, Gail Pollard, nursery technician Chris Jenek and assistance of the Australasian Native Orchid Society volunteers in planting translocation sites. I would like to thank my co-authors of the pollination papers and the various organisations that support this program including the NSW Government's Saving our Species program, the Wimmera CMA and DELWP. A full list of funding bodies can be found at <https://www.rbg.vic.gov.au/science/projects/orchid-conservation>

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# Conservation Translocation of the endangered Colourful Spider-orchid (*Caladenia colorata*)

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## Abstract

*Caladenia colorata* D.L.Jones is an endemic endangered species from south eastern Australia. 883 plants were symbiotically propagated and translocated into four sites within one property where the pollinator was present, and the vegetation matched wild sites. Demographic monitoring showed an 80% survival of translocated plants with natural pollination and fruit set. In addition, 580 seedlings recruited across these sites, with natural pollination and seed set of the seedling recruits first recorded in 2019. Introduced populations are now considered self-sustaining, with these conservation translocations increasing the total number of *C. colorata* in the wild by 1,286 plants.

## Introduction

The Royal Botanic Gardens Victoria Orchid Conservation Program is working with over 30 nationally threatened orchid species conducting conservation research on their propagation, pollinator identity and distribution, mycorrhizal associations and translocation. *Caladenia colorata* is given here as an example of the species we are working to conserve, that incorporates the pollinators

and mycorrhizal fungi in the conservation translocation program. *Caladenia colorata* is endemic to south eastern Australia where it is now known from a handful of populations in South Australia and Victoria in and surrounding the Little Desert National Park. The species is listed as nationally Endangered (EPBC Act, 1999). The total number of wild plants is thought to be less than 600 plants with pressures from grazing and weed invasion. *Caladenia colorata* typically has one to three flowers that range in colour from pale yellow to pink or yellow with a red lip (Figure 1).

## Methods

### Propagation

Thirty plants from across two wild populations of *C. colorata* were hand pollinated, using pollen from flowers greater than 10 m apart from each other. Seeds were collected four-six weeks after pollination. Seed was cleaned and dried to 15% relative humidity before being stored short term at 4°C, over silica until further use. Mycorrhiza were isolated as per the methods of Reiter *et al.* (2020). Fungal cultures had DNA extracted, sequenced and identified as per Reiter *et al.* (2020).