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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.

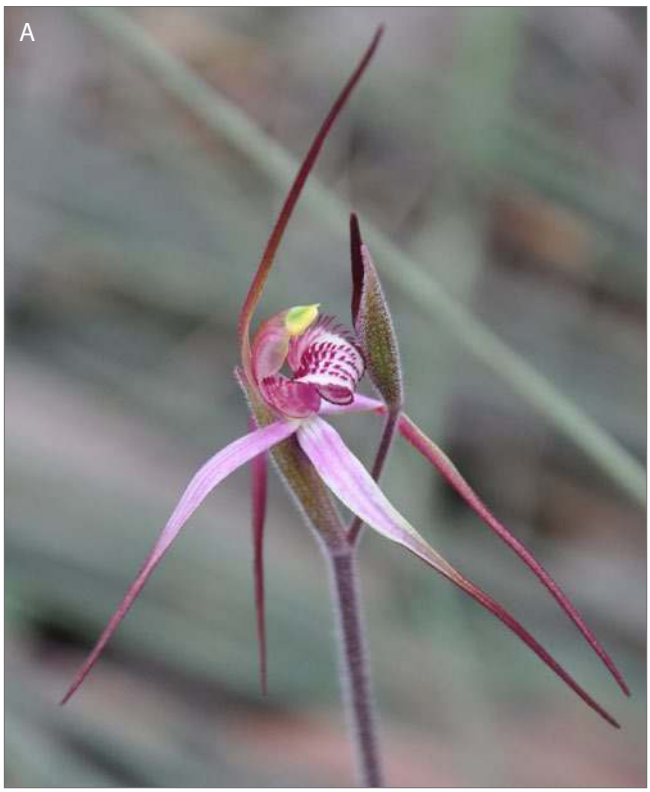
Introduction

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).



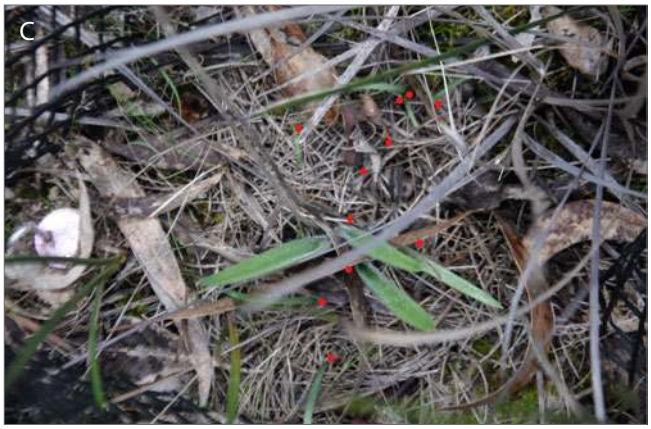


Figure 1. A) *Caladenia colorata* flowering (pink form), B) *C. colorata* grown in nursery and C) *C. colorata* translocation seedling recruits as marked by red dot. Photos: N. Reiter

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). Potted plants grown from seed (as described above) of *C. colorata* were used for baiting for the presence of the pollinator. Observations of the pollinators were made during the flowering period of wild populations of the orchid. A selection of thynnine wasps from each site were identified using a series of unpublished keys to the *Australian thynnine* wasp fauna (G Brown, unpubl. data).

Site selection

Introduction sites were selected that were greater than 100 hectares, had a vegetation and soil match to extant sites of *C. colorata*, were permanently protected land tenure and had the pollinator present. In addition, each site was fenced with rabbit proof fencing and any weed or herbivore control was undertaken on each site as required.

Plants were translocated into sites where *C. colorata* had not previously been known to occur that were covenanted through Trust for Nature.

Each individual orchid was planted with a permanent marker, number and tag 10 cm to the north to aid in re-emergence monitoring and identification of the plants in subsequent years. The location of each plant was triangulated in the field again to aid future detection. 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, 883 plants were introduced of *C. colorata*.

Monitoring

All translocated plants of *C. colorata* were monitored annually for emergence (July), flowering (September-November) and seed set (November-December). In addition, any recruits were recorded. A wild population was also monitored using this method for comparison against the translocation sites.

Results

The mycorrhizal fungi were identified as *Serendipita* Operational Taxonomic Unit A (Reiter *et al.* 2020) and did not vary between the remaining wild sites of this species. Over 1,000 plants were propagated symbiotically for introduction and as a permanent *ex situ* collection. Seed orcharding has begun on the *ex situ* collection to ensure that adequate seed is available for future conservation work with this species.

The pollinator was identified as the thynnine wasp species *Phymatothynous pygidialis* which removed and deposited pollinia (Reiter *et al.* 2018). The pollinator was present at both the remaining wild sites in Victoria and the introduction sites (Reiter *et al.* 2018).

Of the 883 plants that were introduced between 2013 and 2017, 80% survived with an additional 580 recruits. The control wild population had on average 83% emergence +/- 18% SE.

Discussion

Successful large-scale conservation translocations are underpinned by matching suitable habitat, ensuring that translocation sites are weed and predator free and permanently protected. This alongside an understanding of both the mycorrhizal and pollinator associations (Phillips *et al.* 2020), and large founder populations (Silcock *et al.* 2019) has ensured the success of translocations of *C. colorata*.

This research highlights the importance of *ex situ* collections to facilitate research into pollinators and their distribution. This *ex situ* collection allowed large quantities of seed to be banked for future conservation, and permanent *ex situ* collections of living plants have been developed. Introductions of endangered orchids on a large scale incorporating knowledge of both the pollinators and mycorrhizal fungi is both possible and can provide significant conservation outcomes for threatened species. These conservation translocations have more than doubled the number of wild plants of this species, with substantial natural recruitment seen. Indeed, the introduced populations are now self-sustaining with a population growth rate >1, within 7 years of starting conservation translocations. This is faster than our prediction of up to 15 years (Reiter *et al.* 2016).

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