Appendix 1: Monitoring Protocol for Rare Orchid Populations

Mark Brundrett 2017

This protocol is adapted from the open access paper "Using vital statistics and core-habitat maps to manage critically endangered orchids in the Western Australian wheatbelt" by Mark Brundrett that was published in 2016. For orchid translocations, annual monitoring should occur in a fixed area surrounding all the translocated plants to ensure survey effort remains similar each year. This area can be a fenced enclosure, plot or transect. Individual orchids need to be identified by their position and/or tagged, so they can be counted for several years in a row. This is necessary for determining rates of survival, since orchids may remain dormant for one or more years.

To survey larger areas around translocated orchids, a 30-50 m long x 4 m wide transect should be established across the translocation site. The length of a transect is determined by the size of suitable habitat and should be oriented to include as many individuals as possible. Transects should be marked with steel posts at both ends and also with steel pegs as a fixed reference point every 5 m to increase the accuracy of measurements of the position of orchids.

Translocated plants, or those on a transects should be counted during peak flowering (usually spring) to assess plant emergence, flowering and preliminary seed set and again one or two months later to determine final seed set (usually late spring). Grazing should be assessed each time as it often increases during the year. The position of orchid seedlings should also be noted, but they may not be identifiable to species until they flower. A datasheet example is provided below (Table A1-1).

For each orchid plant, the distance along the transect axis and perpendicular distance from it is recorded. These coordinates are used to identify plants that can be assumed to be the same individual if they emerge at the same location on different years (within 2 cm). The identification of individual plants is used to estimate how often each plant emerged or flowered over the period of observation. Data from transect-based surveys can be summarised in a report card format to inform management decisions, as shown in Table A1-2.

Table A1-1. Template for a recording datasheet for orchid monitoring transects (with sample data).

Orchid			**Location**			**Transect no.**		**Date**		**Recorder**
Distance (m) ↓	L ←	R →	Position (cm) ∟	Leave s	Flower s	Buds	Grazed flowers	Grazed leaves	Seed pods	Notes:
2.43		✓	12.5	1	3	1	-			Early flowering

Table A1-2. Example of a report card summarising the status of a rare orchid population. These are real data for a population of *Caladenia graniticola* from a transect in the wheatbelt measured from 2007-2010 (Brundrett 2016). Numbers in bold are sufficiently high or low to suggest recovery actions such as fencing are required.

Factor	**Data**	**Suggested actions**
1. Number of orchids in plot (range over 4 years)	20-53	
2. Proportion of emerging plants that flower	46%	
3. Number of flowers per flowering plant	1.1	
4. Proportion of flowers setting seed	**6.0%**	Supplemental pollination
5. Recruitment from seed	Not seen	
6. Recruitment from clonal division	0	
7. Grazing of leaves, flowers, or seeds	**11%**	Cages or fencing
8. Dormancy frequency (no. events / no. years)	0.32	
9. Dormancy in years, range (average)	1-3 (1.4)	
10. Average annual emergence (proportion of estimated total plants)	37%	
11. Pollinator identity (abundance)	None seen	

Appendix 2: Seed Sowing Trial (Case Study for *Caladenia huegelii* in Banjup in 2016)

Mark Brundrett 2017

A. Seed Availability

Only one of the translocated plants had a flower (2 others were lost due to wind). This flower was hand pollinated and protected by a seed bag as shown in Figure 3 below. This seed capsule was collected at the time of translocation and is available for use. We propose that seed from this plant be sown near the site of tuber translocation in an attempt to increase the size of this small population to a more sustainable level. The remaining seed can be stored in the Threatened Flora Seed Centre for future use.

Figure 3A. A seed bag protecting a hand pollinated flower. **B.** One of the five plants of *Caladenia huegelii* translocated from the Calleya development in Banjup in 2016.

B. Seed Sowing Areas

Vacant positions along the existing transect and two other 50 m long transects will be used to provide sites for sowing of small amounts of orchid seed (see Fig. 4). Transects will be orientated to include as much suitable habitat with a banksia tree overstory as possible. There will also be open areas without tree cover along transects (trees have been impacted by from *Phytophthora* dieback at this site). A small amount of organic matter from near existing orchids will be added to seeds sprinkled at every second seeding position. This treatment will help to determine if fungal inoculum is a factor limiting orchid recruitment in this area. This seeding trial would compare orchid seed germination in shady vs open banksia woodland and in microsites with or without organic matter addition (a potential inoculum source). Seeds will be sown in autumn at precise locations marked with a stake. Only a few seeds will be placed just below the soils surface each site (seeds are <1mm long and there are thousands in one capsule). Germination will be assessed by examining each location in winter and spring at the same time that the survival of translocated plants is assessed. Australian orchid seed does not persist in soil so will only germinate in the first year. *Caladenia* seedlings have a characteristic shape that allows them to be identified.

It has been shown that low rates of pollination and seed set are major factor limiting *Caladenia huegelii* sustainability in Perth (Phillips et al. 2015). However, the frequency of germination of orchid seeds in soil has also been shown to be highly variable in experiments with *Caladenia* in Perth (Batty et al. 2006, Brundrett et al. 2003). This trial will help resolve if supplemental pollination and/or seed dispersal is an effective approach for orchid conservation. Even if germination rates are very low, this would be a valuable tool for orchid conservation since it is relatively easy to pollinate orchids, collect seeds and sow seed, relative to the time and expense required for orchid propagation by laboratory-based methods.

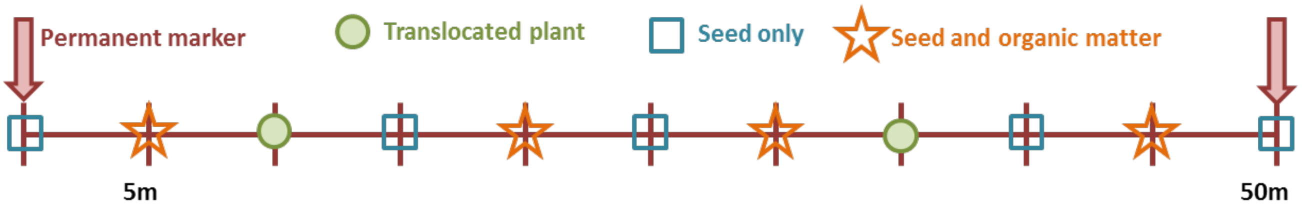

Figure 4. Diagrammatic representation a 50 m transect with two translocated plants and 9 positions for seed sowing. The actual position of plants and seed sites will vary in each transect. A permanent transect is established to ensure translocated plants and seed can be relocated if stakes or tags are lost. The transect position will be marked by steel stakes as well as by accurate geo-referencing with a DGPS. The same transects can be used for future monitoring or orchid populations in the area, as explained in Appendix 2.

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Appendix 3: Generic translocation guidelines for sexually deceptive orchids

Mark Brundrett 2017

These guidelines are based on the pollination requirements of orchids such as *Caladenia* (about 50% of species only), *Drakaea* and *Paracaleana* that are pollinated by a sexual deception syndrome. These orchids attract male thynnid wasps by producing a pheromone that mimics a female wasp (Stoutamire 1983, Menz et al. 2012, Phillips et al. 2016, Weinstein et al. 2016). There may also be some visual mimicry of the female insect by parts of the orchid flower, but this seems to be less important than visual contrast against the background (Gaskett et al. 2016). The concepts described here may also be relevant for visually deceptive orchids which have brightly coloured flowers without nectar (*Diuris*, *Thelymitra,* other caladenias, *Cyanicula*, etc.). These orchids they also get pollinated less frequently when located close together in large groups (Brundrett 2019). Note that the majority of WA orchids have visually or sexually deceptive pollination, but there are some exceptions that produce nectar and thus are more readily pollinated (Brundrett 2014, 2019).

It is recommended that translocated orchids are planted as individuals or small groups that are spaced 20-50 m away from others of the same species (see Fig. 1). The purpose of dispersing orchids over an area is to limit competition for pollinators, while at the same time keeping orchids within the expected wasp home range (territory) of individual pollinators (male wasps) so wasps will travel between orchids. The territorial area that individual male wasps occupy has only been studied for a few species, which were shown to travel up to 500 m when searching for mates, but some species were restricted to smaller distances of 160 or 300 m (Whitehead & Peakall 2012, Menz et al. 2013). It would be expected that cross-pollination will be more likely to occur between orchids that are fairly close together (e.g. <100 m), but this has not been confirmed. Thus, each group or single plant should be fairly close to others to encourage pollen transfer between them for cross-pollination (Fig. 1). However, orchid groups should also be sufficiently far apart to ensure that wasps which become used to the presence of one clump will not also avoid another (10 m is enough). Wasps soon learn that visiting orchids is not productive, so generally will not return to the spot where orchids are located, at least for a few days (Whitehead & Peakall 2012, Brundrett 2019). For example, I have observed that orchid pollinator baiting works best if flowers are moved at least 10 m every 5-10 min. In addition to the insects they parasitise, thynnid wasps require abundant nectar sources for food when they are flying. They are often observed feeding in shrubs or trees with massed flowers in spring or early summer (Brundrett & Brown 2014, Brown & Phillips 2014).

Habitat suitability is also important, but many orchids are surprisingly flexible, provided weeds and disturbance are minimised. They often prefer relatively open areas for efficient photosynthesis and so flowers are visible and accessible to wasps. In other cases, orchids persist best under shrubs or grasstrees, but this is probably a result of grazing pressure. If grazing is expected to occur, it is advisable to place orchids in small cages or fenced enclosures, (e.g. visible signs of kangaroos and rabbits). Both cages for individual orchids and fenced 10 x 10 m or 5 x 5 m enclosures have been shown to substantially reduce grazing of rare orchids, but they did not eliminate it since grazing by invertebrates is also a problem in many locations (Brundrett 2011). Fenced enclosures used should have rabbit proof wire skirting, but the fence only needs to be about 1 m high since kangaroos have rarely been found to graze in a small fenced area. Larger fenced areas can be protected with 2-3 plastic sight lines above the wire fence to avoid the expense of higher fencing. An example of fencing that effectively reduced grazing is shown in Figure 2 below.

Caladenia species have relatively specific relationships with a particular mycorrhizal fungus but many of these fungi seem to be widespread (Brundrett 2007, Phillips et al. 2016). Orchid seed bating is recommended if there is concern about the presence of compatible mycorrhizal fungi (Brundrett et al. 2003).

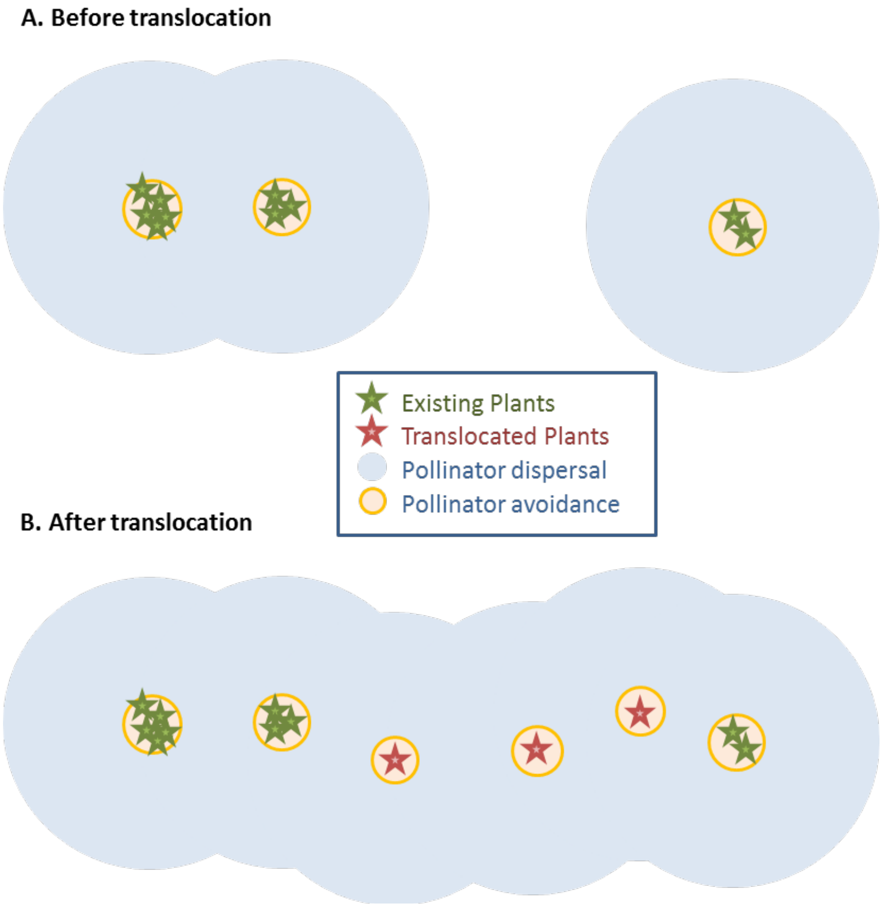

Figure 1. Hypothetical illustration of how translocated orchids could potentially increase the likelihood of cross-pollination for two groups of existing orchids separated by a gap which is larger than the flying range of pollinators. Pollinator dispersal limits and avoidance zones are shown for each group of orchids, as explained in the text.

Figure 2. Example of fencing that excludes rabbits and reduces kangaroo grazing in a restored area that could also be used to protect orchids. This fence has buried skirting to exclude rabbits and three plastic “sight wires” to deter kangaroos. Note the absence of most native plants outside the fence, where regeneration treatments were also applied.