LANKESTERIANA 7(1-2): 219-222. 2007.

DOES INTEGRATED CONSERVATION OF TERRESTRIAL ORCHIDS WORK?

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KEY WORDS: terrestrial orchids, integrated conservation, microsatellites, mycorrhiza, pollination

Introduction to integrated conservation

Effective plant conservation involves careful consideration and difficult choices when investing limited resources to conservation programs and policies. The conservation practice must integrate the understanding of existing and future environmental threats, taxonomic distinctiveness, numbers of individuals in populations, reproductive biology, *ex situ* propagation and the maintenance of evolutionary processes influencing population distribution patterns. For this to be possible, conservation should involve detailed experimentation directed at continued survival of the species in both an on site (*in situ)* ecological context and off site (*ex situ*) laboratory based context (Ramsay & Dixon, 2003). Thus the development of effective conservation strategies, must strike a balance between the need for urgent action to avoid further loss and the search for essential information and understanding of the species or ecosystem to be conserved. The integrated conservation strategy emphasizes the study of interactions among land conservation, biological management, *ex situ* research and propagation and (re)introduction and habitat restoration (Hopper, 1997). Integrated conservation approaches vary according to different species with their habitats and distribution characteristics; however the basic concept remains the same (Fig 1).

Orchid Conservation

Many orchids are characterised by a symbiotic relationship with a mycorrhizal fungus and variety of pollination syndromes (Le Tacon & Selosse 1994, Rasmussen 1995). The interaction of these attributes with often specialised habitat requirements has played a significant role in the evolutionary diversification and the present distribution of these flowering plants (Cozzolino & Wedmer 2005, Otero & Flanagan 2006).

To recover a rare or threatened orchid species in a timely and effective manner, an integrated conservation approach must be applied to the understanding of plant-fungus interactions, pollination syndromes, population genetic structure and evolutionary processes, *in situ* habitat requirements and *ex situ* conservation concepts. Many workers have effective-

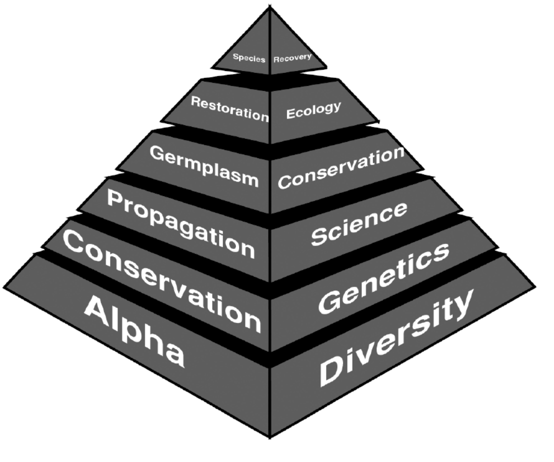

FIGURE 1. A pyramid model of the integrated conservation concept (Dixon & Batty 2003). Species recovery (top step) is an integrated process requiring all the five lower steps shown in this figure.

ly researched these principles as singular entities in a conservation context, however, over the last decade with the successful development of a raft of new conservation technologies can the integrated approach be adopted at a whole species level. The aim of this study is to test the principle of a science-based integrated conservation approach (Falk 1990) towards the recovery of nationally endangered orchid *Caladenia huegelii* H. G. Reichb. Key elements of the study include understanding the basis of rarity in the species, defining and abating threatening processes and developing translocation and management strategies to enhance species resilience and persistence in extant locations.

Caladenia huegelii occurs from Perth to Busselton in Western Australia along the Bassendean Sand geological system characterized by deep, highly infertile siliceous sands in species rich banksias woodland (Hoffman & Brown 1992). Extensive surveying of the all known populations and potential habitat for new populations over three successive growing seasons revealed that the species is significantly reduced to just 18 locations, seven of these are new populations and four of the 18 contain just one plant. Urban development contributed to the loss of populations and large-scale loss of suitable habitat for recruitment opportunities. Grazing of leaves and buds by caterpillars, tuber diggings by bandicoots and the grazing of developing seed capsules by kangaroos were observed as other threatening processes and key management issues.

Population Genetics

Conservation strategies for the maintenance of genetic diversity rely greatly on detailed study of the genetic structure, demographic history and evolutionary potential of plant populations (Fay & Krauss 2003). To develop an understanding of the genetic structure and variability of *C. huegelii* populations, microsatellite markers were used to screen 460 samples representing all populations over its geographical range for polymorphism across seven loci. Genetic divergence among populations was correlated with geographical distance investigating genetic isolation on both population and regional levels. In results comparable to another sexually deceptive *Caladenia* species, *C. huegelii* exhibited similar low levels of genetic differentiation (Fst = 0.102), with 90% of genetic variation partitioned within populations (Peakall & Beattie 1996).

Fungal Associations

Fungal associates of *Caladenia* species show a distinctively fragmented distribution in the landscape, a feature that may be contributing to the rarity of these orchids (Batty *et al*. 2001a, Brundrett *et al*. 2003). Working with *C. huegelii* and common sympatric congeners, we investigated the diversity and specificity of associated endophytes isolated from adult plants over the geographical range of the orchid through large scale *in situ* and *in vitro* matrix germination experiments. These studies demonstrated a highly specific plant-endophyte relationship in *C. huegelii* indicating that orchid distribution may be limited to the type and availability of a specific symbiont at a site, a possible driver of rarity in this species. Endophytes isolated from *C. huegelii* adult plants over the range of the taxon and germinated *in situ* protcorms were found to effectively germinate seed from across the habitat range of the orchid. No significant home site advantage was observed for orchid seed germinating on an endophyte collected from the same plant. Common congeneric species utilized a range of available endophytes in both *in situ* and *in vitro* germination trials including the *C. huegelii* fungus suggesting potential implications for competitive niche occupancy.

The results of high specificity of *C. huegelii* endophytes were reinforced by sequencing the highly variable ITS region of 50 endophytes isolated from both adult plants of *C. huegelii* and *in situ* protocorms, using the fungal specific primers ITS1 and ITS4, finding identical sequences of all endophytes sequenced. Further sequencing of endophytes isolated from common congeners revealed sequence variation when analyzed with those from *C. huegelii* providing additional support to germination results. Analysis of the ITS sequences isolated from the range of *Caladenia* species showed high similarities with a sequence from a *Sebacina vermifera* originally isolated from *Caladenia dilatata* (Bougoure *et al*. 2005) and matching sexual stages identified by Warcup (1971) for *Caladenia*.

Pollination Syndrome

This species adopts a sexually deceptive pollination syndrome employing male thynnid wasps for the transfer of pollen from one plant to another (Stoutamire 1983). We investigated the natural abundance of the specialized wasp pollinators at the key study site at Ken Hurst Park site using the established 'baiting' techniques used in sexual deceptive systems. A detailed study of the natural pollination success for *C. huegelii* showed for the species a low rate of seed set with <4% of flowering plants producing seed in both the 2005 and 2006 seasons. The study compared the success to other sexually deceptive pollinated orchids and showed that *C. huegelii* is on the lower end of the pollination success scale compared with related genera such as *Drakaea* where pollination success rates can be in excess of 80% (R. Phillips, pers. comm. 2006) and was very low compared to most other sexually deceptive orchids with a mean pollination rate of 33.1% (Tremblay *et al*. 2005, J. See, pers. comm). All baiting trials indicated an absence of the pollinator at the site based on the sampling method used. Comparison with pollination success in other *Caladenia* species showed the low levels of pollination recorded in *C. huegelii* fall within the pollination range for *Caladenia* indicating that the low seed set in *C. huegelii* is not unexpected. Clearly to sustain suitable seed output from wild and reintroduced populations of the orchid, there may be either the need to increase pollinator abundance or have intervention management involving artificial pollination.

Reintroduction and transplanting

Protocorms generated from germination experiments were used in subsequent pot and field trials to optimize tuberisation in developing seedlings thus increasing the transfer success of plants *in situ*. The reintroduction of orchids to field sites was markedly enhanced by outplanting two-year old seedlings over one-year old seedlings with irrigation and protective cages enhancing plant growth and overall survival. Out-plants were monitored fortnightly over the 2006 growing season and 100% survival was observed for two-year old seedlings compared to 5% survival in the seedlings out-planted in their first season of growth. Increased survival and seedling growth was observed in seedlings outplanted in close proximity to adult plants in comparison with artificially inoculated substrate and the no inoculum control further reinforcing endophytic potential for reintroduction success.

Ex situ conservation

Although *in situ* conservation of threatened taxa takes precedence in management priorities, the requirement for *ex situ* conservation and storage of germplasm is increasingly recognized as an imperative tool in the preservation and maintenance of biodiversity (Batty *et al.* 2001b, Fay & Kruass, 2003). Using the results of the molecular study to determine significant populations and the methods of Batty *et al* (2001b) to ensure long term survival of stored germplasm, a representative sample of viable *C. huegelii* seed was placed in liquid nitrogen (-196°C). Although no significant sequence variation of *C. huegelii* associated endophytes was observed, a range of isolates that best promoted seed germination across all populations have also been successfully preserved in liquid nitrogen.

Conclusion

This study has demonstrated that within a relatively short time frame it is possible to develop a comprehensive and focused data set leading to effective integrated conservation. Using seedling establishment in the wild as a key benchmark of success the study results provided confidence that recovery operations can be effectively undertaken if there is an understanding of:

- Mycorrhizal specificity
- Biogeographic variation in mycorrhiza
- Genetic diversity of plants and fungi
- Seed banking and endophyte banking
- Pollination ecology.

The challenge now is to adopt and test these key principles in terms of multi-species recovery operations for orchids possibly starting with congeneric taxa and then working on phylogenetically related taxa. The future of orchid conservation depends on effective and timely delivery of orchid conservation where there are active links between on-site conservation actions and research programs.

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Stephen Hopper is director of the Royal Botanic Gardens, Kew. He has worked on Australian orchid systematics and conservation since 1973. Current interests include generic classification of Australian orchids, and the evolution of southwest Australian orchids.