The Importance of Diversity in Natural Breeding©

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CONTEXT FOR THE THREATENED SPECIES COLLECTION AT THE ROYAL BOTANIC GARDENS MELBOURNE

The Victorian Flora and Fauna Guarantee Act (1988) has as its principle objective: "to guarantee that all taxa of Victoria's flora and fauna…can survive, flourish, and retain their potential for evolutionary development in the wild."

Target 8 of the Global Strategy for Plant Conservation to which Australia is party states that "60% of threatened species (are to be) in accessible ex-situ collections."

The Royal Botanic Gardens Melbourne, as a member of Botanic Gardens Conservation International, adopts the guidelines of this organisation in its ex situ plant conservation activities. We are increasingly playing a critical role in threatened species conservation in Victoria.

Unlike many approaches to plant breeding where uniformity is the desideratum, best practice plant breeding for conservation allows for inclusion of maximum genetic variability (e.g., Falk and Holsinger, 1991; Guerrant et al., 2004; Vallee et al., 2004). The Royal Botanic Gardens Melbourne (RBGM) is involved in a number of species recovery actions for threatened Victorian plant species and in these we aim to maximise genetic representation while restricting source material to individual populations. There are two main thrusts of our native species conservation activities.

The Victorian Conservation Seedbank. The Victorian Conservation Seedbank (VCS) is housed at the RBGM and is a partnership with the Millennium Seed Bank (MSB) Project based at The Royal Botanic Gardens, Kew in England. The first phase of Kew's MSB Project had a target of seedbanking 10% of the species of the world's dryland flora by 2010. Through the collaboration and funding of 17 countries around the world, we are confident that this target will be achieved. A new post-2010 target has been established to seedbank 25% of the world's dryland flora by 2020.

Fleshy fruited, mostly tropical species are generally considered unsuitable for the storage techniques we adopt — drying seeds to around 5% moisture content then hermetically sealing and storing them at -20 °C. Under these conditions, most dryland (= "orthodox") species retain viability for many years (perhaps centuries) beyond their natural longevity.

The value of a seedbank versus a collection of living plants is that an enormous amount of genetic diversity may be stored in just a handful (or even a thimbleful) of seeds. Furthermore, the seeds are kept under easily maintainable conditions, not prone to accidental losses caused by hot dry spells, failure of automatic watering systems, failure to repot, etc., etc.

The first phase of our 4-year funded project aims to collect seed from 450 species of Victorian plants, focussing on those that are threatened in the state. The seedlots are duplicated, one staying here in Victoria, and one adding to Kew's substantial seed bank, currently holding nearly 20,000 of their targeted 24,000 species.

A standard protocol, established through over 40 years of research into seed collecting and banking, suggests that by collecting seed from around 50 randomly selected plants in any population, 95% or better of the genetic diversity within that population may be captured (e.g., Guerrant et al., 2004). This is the method adopted by the VCS. Kew's protocol requires that a collection of at least 4000 seeds per population is needed to ensure that the seedlot is of sufficient size to split it into two (one for Kew, one remaining here) to embark on reintroduction programs or establishment of new populations in "safe" sites if that is what species recovery plans recommend. We are well underway toward our initial target and currently hold nearly 300 Victorian species in our seedbank.

Plants produced at Royal Botanic Gardens Melbourne for Conservation Purposes. The living conservation/research collection at RBGM consists of plants that are held as ex situ plants for research, conservation, or as stock plants from which further plants are to be propagated. Historically, the conservation collection of threatened species has consisted of one or a few plants of each species and these are subject to the vagaries of potted plant maintenance alluded to previously.

The selection of species has been a result of targeted collection, a consequence of a research project at the National Herbarium of Victoria (one of the branches of the RBGM), or unplanned, serendipitous acquisition. Generally speaking, space and staff constraints preclude a collection of dozens of plants that are genetically representative of a particular threatened species or population. However, more recently, RBGM has been called upon to produce plants for translocation or reintroduction activities associated with recovery actions such as those prepared under the state Flora and Fauna Guarantee Act (1988) or the federal Environment Protection and Biodiversity Conservation Act (1999). These actions generally (and correctly) require translocated or reintroduced plants to be genetically diverse so that plants "can survive, flourish and retain their potential for evolutionary development in the wild" (FFG Act, 1988). To achieve this, collections are taken from at least 10% of all mature plants in a population and across the geographical and ecological extent of the population. Generally the exact genetics of the collection is unknown — the breadth and nature of the sites occupied by the plants are used as a surrogate for their known genetic variability.

In some cases, where there is suspicion or knowledge that plants are clonal, that is the plants are all of the same genotype, genetic fingerprinting techniques are employed to ensure that plants collected for translocation or reintroduction capture a genetically representative sample of the species' entire gene pool.

CASE STUDIES

Grampians Pincushion Lily (*Borya mirabilis*). *Borya mirabilis* was thought to be extinct until rediscovered in 1982. It is known from a single site in the Grampians National Park in western Victoria. Only four colonies of the plant are known over an area of less than 40 × 20 m. The total cumulative area occupied by the four colonies would be in the order of 1 m^2. It is one of Australia's most endangered plant species. The species appears to be incapable of setting seed, presumably through a self-incompatability mechanism (Coates et al., 2002). It had been concluded, from earlier genetic studies, that the plants were clonal and genetically identical or of only two genotypes (Cropper, 1993). A series of hundreds of hand cross-pollination

experiments carried out during Noushka Reiter's Ph.D. studies last year produced a single seed. This seed turned out to be nonviable, but its production suggests that given enough attempts, there is potential for seed production within this species.

More advanced techniques of detecting genetic variability via Random Amplification of Polymorphic DNA (RAPDs) analysis have allowed more of the genome to be explored and we are now confident that there is genetic variation present in the population (Reiter, 2008, unpublished data). We assume that the best chance of seed production and self-sustainability of the plants in the wild will occur with plants that are somewhat genetically distant.

Because of the vulnerability of the single site to extinction (it was reduced by about half after the 2005 wildfire in the Grampians), a second population, grown from plants held in the RBGM collection, has been established about 15 km from the original population. So far, these plants have survived and grown, but as they were propagated before the new genetic testing, this new population does not contain the range of genetic variation present in the original population. Knowledge of the genetic makeup of the natural population will guide us in selecting material from which to propagate and translocate into the newly established population.

Stiff Groundsel (*Senecio behrianus*). Like *Borya mirabilis*, this species was believed to be extinct until rediscovered near Shepparton in northern Victoria in 1991. Much of the surrounding land has been cleared for farming and the plants are confined to an area in and near an irrigation ditch. Further searches have discovered another three populations in the nearby area, and a further population was discovered at Miners Rest near Ballarat in 2004. Again, this species was believed to be largely clonal, although distances of some hundreds of metres (or hundreds of kilometres in the case of the Miners Rest population) between stands suggested a low likelihood of plants in different colonies being from the same parent stock. Seed production is rarely observed, and the plant appears to be self-incompatible. However, occasional recruitment events amongst the northern Victorian populations indicate that seed is sometimes produced and we believe this to occur on the relatively rare occasions when pollen is transported between colonies (most likely by an insect vector) in times of synchronous flowering of different colonies (E. James, pers. commun.).

Genetic studies employing Intersimple sequence repeat polymorphisms by RBGM's conservation geneticist, Liz James, has shown the individual populations in the Shepparton area to be genetically distinct, whereas the Ballarat population, although very extensive, shows no genetic variation at all (James 2007, unpub. data). Sites have been selected for the establishment of new populations in protected sites and the genetic studies will guide the selection of plants to maximise the likelihood of seed production and so, evolutionary potential.

Shining Nematolepis (*Nematolepis wilsonii*). Unlike the two preceding examples, shining nematolepis produces copious seed. It is a handsome, erect shrub confined to a single population of around 300 mature plants and numerous seedlings over an area of a few hectares in the Yarra Ranges National Park where it occurs in an ecotone between mountain ash (*Eucalyptus regnans*) forest and cool-temperate rainforest dominated by myrtle beech (*Nothofagus cunninghamii*). The site occurs in a deep valley where it is naturally fire-protected, but global warming predictions suggest that even these sites are likely to be burnt by wildfires someday. A more immediate threat to the species has been identified from a surprising source.

Sambar (*Cervus unicolor*), a large deer native to India and prized as a game species in south-eastern Australia, have undergone a significant population increase in the past decade or more and their activities in native forests are now listed as a Potentially Threatening Process under the Flora and Fauna Guarantee (FFG) Act. They impact particularly on shining nematolepis by the males using the trunks of the nematolepis plants to develvet their antlers, causing the plants to become ring-barked and dying soon after. It is not known just why they select the nematolepis for this purpose, but it is one of a few species that are targeted for this activity. As part of an action plan being prepared for shining nematolepis, it has been recommended that new populations be established in suitable habitat in a new, nearby catchment. The RBGM has been contracted to source material and propagate several hundred plants for these new populations. Ideally we would develop the new plants from seed, and we hold a representative collection of several thousand seeds in the Victorian Conservation Seedbank, but, like many of our native Rutaceae, germination of this species has proven to be a tough nut to crack. Germination trials are continuing with new techniques being tested, but in the interim, vegetative production of plants has been undertaken for the populations to be established. Material from 30 tagged individuals has been collected from across the range of the population and the stock material carefully labelled. Cuttings (930) have been prepared and we are waiting for these to establish (and for the drought to break) before incorporating them into three new sites that have been identified as suitable in the Yarra Ranges National Park. The new populations will be fenced to exclude Sambar. Currently a management plan for the control of Sambar is also being prepared, and it is hoped that through these two conservation actions, the future of shining nematolepis will be ensured.

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