Using clonal genetic structures to inform and develop an *ex situ* conservation strategy for *Grevillea renwickiana*

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Grevillea renwickiana F.Muell. (Nerriga Grevillea) is a critically endangered prostrate rhizomatous, root-suckering shrub (Makinson 2002). It is endemic to New South Wales, confined to the Braidwood-Nerriga area where it grows on sandy soils in open dry sclerophyll forest. *In situ* flowering occurs during summer months but appears to be irregular and the flowers putatively sterile.

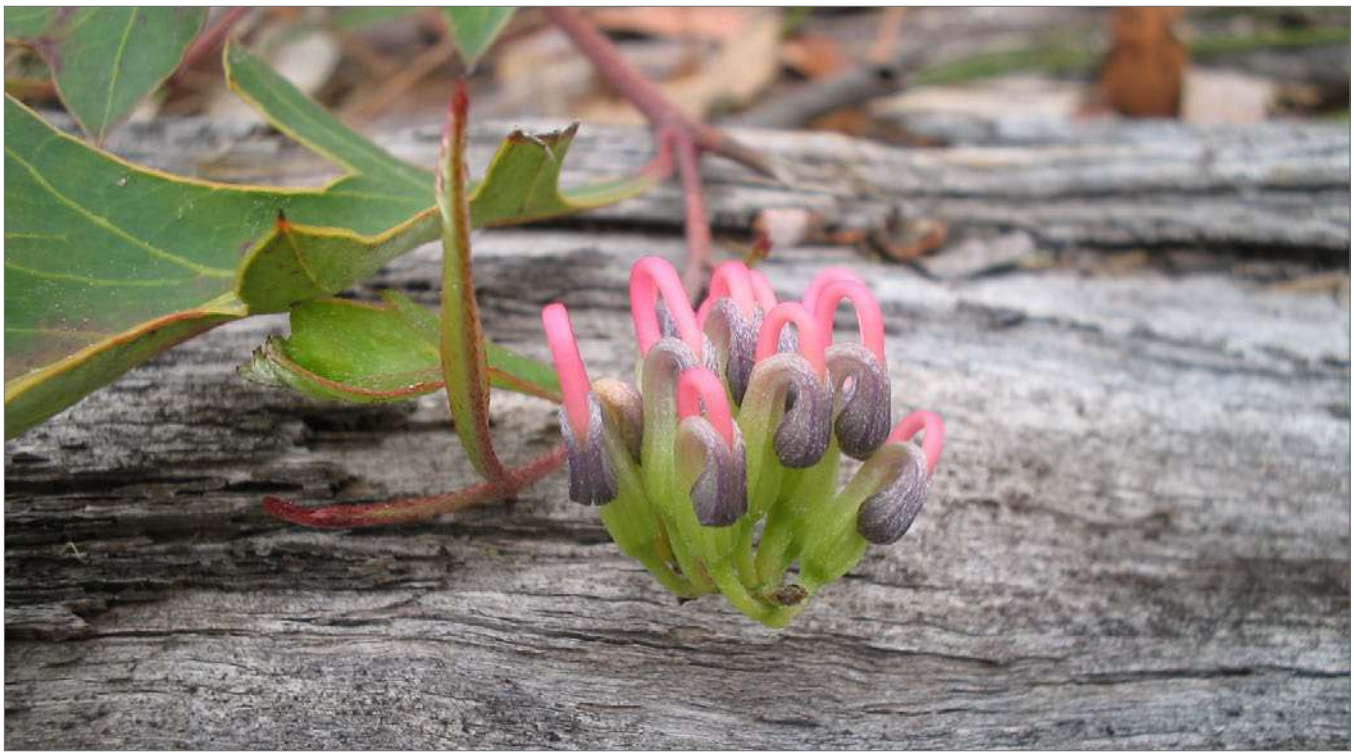
In 2011 members of the Australian National Botanic Gardens' (ANBG) Living Collections staff located a population of the Nerriga Grevillea on private property east of Nerriga. As there were no Australasian Virtual Herbarium records of the species in that area, we alerted the Threatened Species Officers of the then NSW Office for Environment and Heritage (OEH, now the Department of Planning, Industry and Environment (DPIE)), who informed us of relevant research on the species. Elizabeth James from Royal Botanic Gardens Victoria had undertaken genetic analyses of the species and identified eight genetic individuals that were generally restricted to single populations (James & McDougall 2014).

Identifying an opportunity to include the new population in ongoing genetic studies and recognising potential for developing a scientifically backed *ex situ* conservation plan for the species, we discussed a sampling strategy with Elizabeth James and Keith McDougall (DPIE). We modified our standard collection method of taking a herbarium specimen and cuttings from a single plant or area to match the methodology used by James and McDougall (2014), enabling comparisons to be made with previous work.

Samples were collected from three accessible locations over a total distance of 2.5 km, maintaining a minimum distance of 10 m between sampled plants. Two herbarium specimens were taken, one from either end of the collection transect. This resulted in 16 new collections (accessions), which were each given a unique field number, locality description and geocode. For each new collection, samples consisting of four or five leaves were placed into silica gel for genetic analysis and a small number of cuttings were collected and tracked using the same field collection number as the corresponding genetic sample. The cuttings struck well and multiple plants from the 16 collections were later grown and maintained as individual accessions in the ANBG Nursery.

Elizabeth James conducted a genetic analysis of the 16 samples taken from the newly located population and identified two genetic individuals; this is only the second time that more than one genotype has been recorded in a single population of the species. Based on these findings and other scientific advice, the ANBG condensed the 16 accessions into two separate accessions, retaining their genetic identity. This reduces the complexity of short-term management of the lineages and dramatically increases the feasibility of retaining the long-term scientific integrity of the *ex situ* collection.

One of the newly identified genomes of *Grevillea renwickiana* growing in the Australian National Botanic Gardens. Photo J. McAuliffe



***Grevillea renwickiana* sterile flower from Corang B site. Photo E. James**

In designing an *ex situ* management plan for this species, its typical growth habit, characterised by its rhizomatous spread, must be considered. The plant's ability to sucker from rootstock and shed old stems, and to spread with new above-ground stems has the potential to complicate or undermine lineage tracking within any cultivated situation such as a botanic garden. Even without a total understanding of this capability we can design *ex situ* environments that aim to maintain separation of clones, by either physical separation or by using barriers to prevent crossover. We have observed slow growth of plants from an earlier collection cultivated in ANBG for 20 years. In one instance, the actively growing parts of a plant migrated a short distance over several years. We have learnt that once established, plants are likely to be long-lived. This informs collection management decisions such as how many individual plants of an accession are required to maintain clonal lineages over time and provides insights for the development of succession plans.

It is important that planting records facilitate long-term accession identification. The ANBG uses embossed aluminum plant tags to manage identification of planted stock. These are either attached to aluminum stakes or directly to the plants using plastic-coated tie wire. Plants are mapped and details recorded on a database integrated with the herbarium records. The data management system is designed to be able to cope with complications that arise if one or more recording systems fail, typically via the loss of the plant label. By creating and retaining contingency records, the ANBG can later identify unlabelled accessions by using mapping records or other documentation such as section management plans. These systems are only capable of maintaining accurate long-term data if the lineage management plans are sound.

In the case of *Grevillea renwickiana*, the ANBG has deliberately planted a single clonal lineage in a section and separated the plants from other clonal lineages with a physical barrier such as a road. By planting only one clonal lineage per section, the possibility of confusing lineages can be decreased significantly. When appropriate records are maintained, long-term management of clonal lineages is achievable at a relatively low cost and resource effort if the cultivation requirements of a species are straightforward.

However, there are a number of practical factors to consider when managing multiple separate lineages of a species in a botanic garden's living collection. There are always possibilities for human error in data management. Gardens staff need to be well-versed in data management to facilitate long term retention of plant collections of high scientific value. Operating in a public space can add additional challenges to maintaining the scientific integrity of living collections. Botanic gardens also face resource restrictions that can undermine scientific and conservation objectives over time. It is therefore important that botanical institutions with ambitions to contribute to *ex situ* conservation be pragmatic in their overall approach to collection management. One of the key objectives of an *ex situ* collection is to facilitate *in situ* conservation options.

Good conservation outcomes can be achieved through working collaboratively with the scientific and natural resource management communities. This is especially the case with sterile species, such as *Grevillea renwickiana*, where seed banking is not an option and knowledge of genetic structure can have a large impact on collection management decisions and improve the quality of the *ex situ* conservation collections.

A great starting point for securing the future conservation of *Grevillea renwickiana* is ensuring that a botanic garden holds a comprehensive *ex situ* collection. At present, only the two recently collected clonal lineage accessions of *Grevillea renwickiana* are held by the ANBG. Obtaining the eight additional genomes identified in James & McDougall's study for the ANBG collection would provide a comprehensive *ex situ* conservation holding of this threatened species for Australia. In theory, the ANBG has the capacity to hold and maintain all these lineages.

It is important to recognise that the security of both *ex situ* collections and conservation of wild populations of the species will be best achieved if the known diversity of *Grevillea renwickiana* is held in more than one botanical institution. The NSW South-East Bioregion Working Group is a conservation-focused collaboration between the ANBG, the Australian Botanic Garden Mount Annan, Wollongong Botanic Gardens, Booderee Botanic Gardens, Eurobodalla Regional Botanic Gardens and Illawarra Grevillea Park. This group, with support from the NSW DPIE, relevant land authorities, private property owners, communities and other conservation/landcare-oriented groups can develop and enact a feasible *ex situ* security plan for the Nerriga Grevillea. If this can be achieved, developing *ex situ* conservation plans for similar sterile species could be modelled from this example.

Summary

Early and open communication between government agencies and conservation practitioners, in this instance, was a key factor that influenced the ANBG's approach. By considering and contributing to the latest genetic analysis of *Grevillea renwickiana*, the ANBG has been able to apply the most appropriate collection management practices and horticultural knowledge to its *ex situ* holdings of the species. The Gardens now holds 20% of the known diversity of the species. This results in an efficient and effective use of resources. Future investment from the scientific community, government agencies and collaboration between botanic gardens could achieve viable long-term *ex situ* security of the species that may facilitate increased *in situ* management options and draw interest from community groups, promoting conservation awareness in regional communities.

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