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Genetic rescue a viable option for the range-restricted endemic shrub *Grevillea celata* Molyneux (Proteaceae) with history of ineffective translocations

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Introduction

Recent decades have seen a push towards understanding the conservation status and threats to plant species, in large part due to the Global Strategy for Plant Conservation which had a target of assessing the conservation status of all known plant species by 2020 (Convention on Biological Diversity, 2012). This is an ongoing process with an estimated 21–26% of known plant species assessed by 2016 (Bachman *et al*. 2018), and 37–44% of assessed plant species considered threatened (Nic Lughadha *et al*. 2020). Australia has more than 18,000 endemic species, the second- highest ranked country, and just under 40% of those species had threat assessments by 2022 (Gallagher *et al*. 2023). A meta-analysis of 1,135 threatened Australian taxa found nearly 40% (418) are still declining and, of these, a quarter were ranked as being at risk of extinction under current management regimes (Silcock & Fensham 2018).

Species geographic range size includes both extent of occurrence and area of occupancy, either of which, when limited, place species at a high risk of extinction particularly when populations are severely fragmented (IUCN, 2012). Conservation of range-restricted species is challenging as they are more susceptible to threats impacting the whole

Abstract

Restricted geographic range is a strong indicator of a species' extinction risk and is often coupled with small, isolated populations that are susceptible to genetic decline. Genetic analysis of the critically endangered *Grevillea celata* Molyneux, restricted to an area of 60 km^2 in eastern Victoria, reveals that genetic connectivity diminishes with distance between its sites. One small population was found to harbour a unique genetic signature that merits preservation. One translocation site was found to be monoclonal and this location in particular would be a good candidate for genetic augmentation. Root-suckering was observed and must be considered when collecting germplasm for conservation.

***Keywords*:** Conservation, endangered, ex situ, in situ, population genomics, DArTseq

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species in one event. Larger range and large population size can safeguard a species against localised threats reducing the chance of range-wide impacts. With the increasing risk of extreme weather, particularly fires in Australia, the chances of a range-wide threat are heightened (Collins et al. 2022; Ellis et al. 2022). Within the geographic range for a species, subpopulations that are small and relatively isolated may be at greater risk of extinction (Broadhurst et al. 2017). In small and isolated subpopulations, a loss of genetic diversity may lead to an increased incidence of inbreeding and production of plants that lack vigour or are susceptible to pathogens (Keller & Waller 2002). Fragmentation reduces gene flow among subpopulations and increases the chance of local adaptation which can reduce the fitness of populations if climatic conditions change at a faster rate than species can adapt or migrate (Aitken & Whitlock, 2013). Adaptation to harsh conditions often encountered at the edge of range distributions can develop, and this local adaptation may harbour unique diversity that could support survival through future extreme predicted conditions. Too much gene flow into such marginal populations could result in outbreeding depression if it leads to disruption of local adaptations (Forrest et al. 2011). Alternatively, small, isolated populations may be genetically depauperate, lacking fitness and evolutionary potential (Ellstrand & Elam 1993).

Population genetic processes critically depend on plant species mating system and life history traits. When reproduction is asexual only, genetic diversity within populations becomes static, removing the ongoing process of adaptation via recombination that is conducive to persistence in a changing environment. Asexual reproduction, such as resprouting or root-suckering can be an adaptive mechanism for maintaining landscape persistence under temporary or ongoing conditions sub-optimal for seed production or recruitment (Vallejo-Marín *et al*. 2010). Resprouting or root-suckering in response to fire will result in pre-fire genetic diversity being maintained, alleviating the reliance on a seedbank for survival. Shorter fire intervals in the absence of resprouting could lead to local extinction if the seedbank does not have time to replenish between fires. If fire frequency increases, resprouting plants will be able to maintain levels of genetic diversity post-fire but this advantage will only last until plants senesce, while there is also the risk that repeated fires or high intensity fires may kill plants (Fairman *et al*. 2019).

Assessment of a species' extinction risk and identification of key threats inform conservation actions to mitigate threats. While some threats such as wild fires are challenging to predict and prepare for, smaller more predictable threats, such as road works, may be alleviated using a combination of methods such as *ex situ* propagule storage and translocations. Translocations may be composed of multiple different propagule sources such as seeds, whole plants, or cuttings. Most historic translocations in Australia have been performed with the intention of reducing extinction risk, however the majority have lacked guidance from genetic studies (Silcock *et al*. 2019). Commonly, many propagules are used (Silcock *et al*. 2019) but there is the risk that propagules may have been sourced from a limited number of genetically diverse plants such as cuttings, or seed taken from a single, few or closely related individuals (Broadhurst *et al*. 2021). Similarly, some *ex situ* germplasm collections have not necessarily incorporated the extent of genetic variation present in natural populations and diversity can be lost from *ex situ* collections over time (Diaz-Martin *et al*. 2023). Genetic assessment and monitoring of historic translocations can provide insight into the methods used to establish populations, assess the diversity in translocations against that of natural subpopulations and, if required, provide guidance for augmentations aimed to boost the genetic diversity within subpopulations (Van Rossum & Hardy 2022).

One of many threatened species in Victoria with a restricted range is *Grevillea celata* Molyneux (Nowa Nowa/Colquhoun grevillea) which is limited to an area of only 60 km^2 in eastern Victoria and listed as Critically Endangered under the Victorian Government *Flora and Fauna Guarantee Act 1988* (FFG) (DEWLP, 2021). A translocation was undertaken prior to 2009 in response to roadworks (DSE, 2009) but only 3–5% of translocated plants remain. This research examines the genetic diversity across the range of *G. celata* and estimates the level of connectivity amongst remaining natural subpopulations. This information can be used to prioritise management actions that preserve

genetic diversity *in situ* and guide *ex situ* collections to maximise genetic diversity conserved. A complete picture of the genetic diversity in *G. celata* can also guide genetic rescue of small, isolated subpopulations by ensuring that the source for augmentation is the most appropriate to maintain any unique diversity or potential local adaptation that may be present within subpopulations.

Methods

Species

Grevillea celata (Nowa Nowa/Colquhoun grevillea) is endemic to Victoria where it is restricted to well-drained sandy soils in the Colquhoun State Forest between Nowa Nowa and Bruthen in central east Gippsland. *Grevillea celata* is an erect and open, low and dense, root-suckering shrub 0.4–1.8 m high (Makinson, 1996). Red and yellow flowers up to 12 mm long appear from July to February (Figure 1). The species is most likely pollinated by birds and insects with ants observed collecting and burying seed, potentially an important component for germination (Molyneux, 1995). It is very similar to two other more widespread grevilleas, *G. chrysophaea* and *G. alpina*. *Grevillea chrysophaea* (FFG Vulnerable) has yellow flowers and does not root-sucker. It grows in eucalypt woodland or heath on silty sand to sandy loam with a disjunct distribution in the Brisbane Ranges (Anakie-Steiglitz area) and Gippsland, in the area roughly enclosed by Traralgon, Woodside and Sperm Whale Head-Licola. *Grevillea alpina* is widespread in Victoria and on the southern Tablelands of New South Wales (Australia's Virtual Herbarium, 2022). Preliminary phylogenetic studies place *G. celata* nested within Gippsland collections of *G. chrysophaea*, however, the relationship between the two has not been fully resolved (Holmes 2021).

Figure 1 *Grevillea celata*.

Surveys of *G. celata* in 2006 estimated 1,500 plants occurring in nine wild populations across a range of 11 km (60 km^2) (Carter & Walsh, 2006), with no populations occurring in conservation reserves. Due to extreme range restriction and small population size, *G. celata* has been listed as critically endangered under the *Victorian Flora and Fauna Guarantee Act* (Vic) (DELWP, 2021). This species was assigned a genetic risk rating of high in the 2020 report assessing genetic risk to Victorian flora and fauna (Kriesner *et al*. 2020).

Sampling for population genetic analyses

Leaf samples were collected from *G. celata* plants targeting the nine discrete locations described in the National Reco55very Plan (Carter & Walsh 2006) and an additional five locations based on National Herbarium of Victoria records or Atlas of Living Australia observation records (Australia's Virtual Herbarium, 2022) (Figure 2). The Action Statement for *G. celata* described the destruction of a population while upgrading the Bruthen-Nowa Nowa Road (DSE 2009). To offset this loss, a translocation of 1000 plants was undertaken at a nearby location with the area fenced off for protection. The assumed location was scouted during field surveys, with approximately 30–50 plants observed in August 2021 within a damaged fence. Translocated plants were purposefully not sampled for this genetic study to reduce the chance of bias of including clonal (cutting-propagated plants) samples in the data. The widespread bushfires in Australia in 2019/2020 affected the following five collection locations in Colquhoun State Forest north of Bruthen-Nowa Nowa Rd: Watershed Rd, sites 1 and 2 (WATR1/WATR2), Unnamed track, North and South side

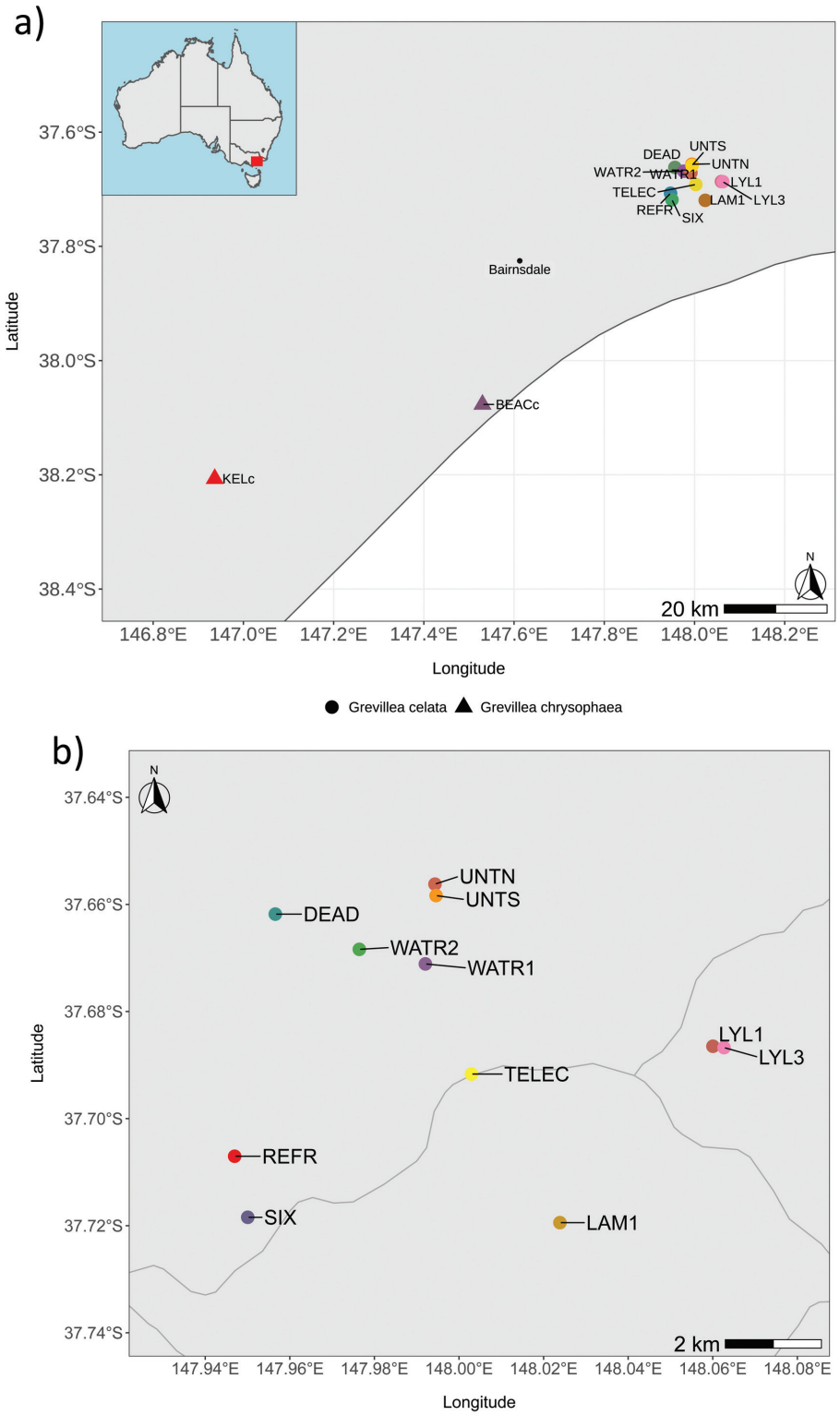

Figure 2 Map of study area and locations of sampled populations, a) *Grevillea chrysophaea* populations in relation to *Grevillea celata* study area, b) sampled *Grevillea celata* populations across its distribution.

of Stony Creek Crossing (UNTN/UNTS) and Dead Horse Creek Rd (DEAD). In these locations, plants appeared to be resprouting and suckering from root stock, with regrowth to 1 m tall and flowering observed at all locations. In many unburned locations, plants were also observed to be suckering, with large plants surrounded by small plants assumed to be ramets within a 2 m area. More recent surveys confirmed that plants were setting fruit, indicating that the species is not reliant on asexual reproduction for persistence; seed collections have been banked at the Victorian Conservation Seedbank (MEL 2510915, 2372894).

Clonal reproduction via root-suckering was confirmed by genetic analysis of 4 ramets sampled less than 2 metres apart. These samples were later removed from further analyses. In all other locations, sampling was undertaken of plants believed to be separate individuals spaced at least 10 m apart across the geographic extent of a site. At two small and isolated sites, the Corner of Lambournes and Nowa Nowa-Bruthen Road (LAMBRU, 20 stems), and the edge of Nowa Nowa-Bruthen Road (8MIL, 6 stems), it was necessary to sample at intervals of less than 10 m. At Six Mile Track (SIX), plants occurred at a lower density and were spread across a greater distance than at other locations. It was necessary to spread individual collections across 1.35 km to sample five individuals that appeared to be separate plants.

During field collections, it was noted that in some large populations, plants formed discrete patches separated by 100–200 m. Due to this intermittent distribution, two patches were sampled at two locations to quantify the differentiation between patches within locations. At both Lyles Break/Smarts Break intersection (LYL1/LYL3) and Unnamed track, N and S side of Stony Creek Crossing (UNTN/UNTS), we collected from two discrete patches located approximately 250 m apart at LYL1/LYL3, and approximately 100 m at UNTN/UNTS. In addition, two of the geographically closest populations of *G. chrysophaea*, Holey Plains State Park (KELc) and Gippsland Lakes Coastal Park (BEACc) were sampled to test whether populations of *G. celata* were distinct entities from *G. chrysophaea* (Figure 2a).

DNA extraction, amplification, and sequencing

Silica-dried leaf samples (94 samples including technical repeats) were sent to a commercial genotyping service, Diversity Arrays Technology ('DArT', Canberra, Australia), for DNA extraction and DArTSeq analysis, a reduced representation sequencing method (Kilian *et al*. 2012). DNA was extracted at DArT using the Nucleo Mag kit (Machery Nagel, Germany), on a Tecan 100 platform following the manufacturers protocols. Library preparation involved DNA digestion and ligation using methylation-sensitive restriction enzymes PstI and MseI and uniquely barcoded adaptors. Following PCR and quantification, the samples were standardised and pooled for sequencing in a single lane of an Illumina NovaSeq X+ sequencer. Sequences were processed using proprietary DArT analytical pipelines to filter poor quality sequences. Resultant sequences were used in DArTsoft14, DArT PL's proprietary SNP calling algorithms.

Data analysis

The unfiltered SNP dataset received from DArT consisted of 101665 SNPs and 94 samples. To identify clonal samples, the dataset was assessed for (1) genetic distance, using the R package 'stats' to calculate pairwise Euclidean distances between samples, (2) kinship, using KING method of moments using the R package 'SNPRelate', and (3) proportion of shared alleles, using the R package dartR. For the genetic distance and proportion of shared alleles methods, the included repeats (known clonal samples) were used to set a threshold to select clonal samples. The threshold was calculated as the average value between repeat pairs plus/minus three standard deviations of the repeat pair values. Any sample pairs below these values in the genetic distance measure or above in the proportion of shared alleles were considered clonal samples for that method. KING method of moments identified monoclonal individuals. If pairs of samples were identified as clonal in at least two of these methods, they were considered clonal. Monoclonal populations (LAMBRU and 8MIL), and one individual from each pair of samples identified as ramets, and the samples used to test for assumed clonal growth (LAM2) were removed from further analysis, see results.

Two datasets were designated and analysed: the first dataset contained all remaining *G. celata* populations and the two *G. chrysophaea* populations; the second dataset contained only *G. celata* populations. The resultant datasets were filtered using the R package

dartR (Gruber *et al*. 2018) in R (R Core Team, 2019). Data were filtered to a locus call rate of 80% and individual call rate of 75%, a reproducibility score of 1, a Hardy-Weinberg equilibrium with a 5% level of significance, a minor allele frequency greater than 4%, secondaries were removed as were monomorphic loci and then filtered on Hamming distance to remove potential paralogues.

For the first dataset, a principal component analysis (PCA) was undertaken on all *G. celata* and *G. chrysophaea* samples (clones removed) to determine whether *G. celata* populations are genetically distinct entities from *G. chrysophaea*. The following analyses were undertaken on the second dataset. To identify genetic clusters of individuals and visualise the major axes of variation between clusters, PCA was undertaken, implemented in the adegenet package (Jombart 2008; Jombart & Ahmed 2011) in R (R Core Team, 2019). Expected and observed heterozygosity, private alleles, inbreeding coefficients and pairwise population differentiation (F_{ST}) were assessed using the adegenet (Jombart & Ahmed 2011), hierfstat (Goudet & Jombart, 2020) and Poppr (Kamvar *et al*. 2014, 2015) packages in R. Analysis of molecular variance (AMOVA) was tested using the Poppr (Kamvar *et al*. 2014, 2015) package in R. Isolation by distance (IBD) was assessed using R package dartR (Gruber *et al*. 2018). Population genetic structure was explored using Structure 2.3.4 (Pritchard *et al*. 2000). Ten independent runs were undertaken for each K value from 1 to 13 with a burn-in of 200,000 and 300,000 MCMC iterations. The R package pophelper (Francis 2017) was used to visualise results and select the most probable K based on the ΔK metric (Evanno *et al*. 2005). The R package ggplot2 (Wickham 2016) was used to visualise results. The R package diverRsity (Sundqvist *et al.* 2016) was used to estimate migration rates, with plotting using igraph (Csardi & Nepusz 2006) and qgraph (Epskamp *et al*. 2012).

Results

Clonal/suckering growth

The test for clonality at site LAM2 demonstrated that all four stems presumed to be sucker growth were found to be ramets from the same genetic individual, confirming that suckering is a strategy for this species. The four samples had an average pairwise genetic distance of 80.6 and an average proportion of shared alleles value of 0.97, while the average values between the unrelated individual and these samples were 217.6 and 0.85 respectively.

Clonal analysis across all samples identified two sites as monoclonal, LAMBRU and 8MIL, with average pairwise genetic distance values within the site of 60.6 and 89.4 and average proportion of shared alleles value of 0.98 and 0.97 for LAMBRU and 8MIL respectively. In contrast, the average pairwise genetic distance amongst all unrelated samples was 236 and average proportion of shared alleles values was 0.83. Two other pairs of samples in two separate subpopulations (SIX, LAM1) were also identified as clonal, with pairwise genetic distance values of 71.3 and 99.6 and proportion of shared alleles value of 0.98 and 0.96.

Species delimitation

The results from DArTSeq contained 63 samples and 97013 loci. After filtering, as outlined above, the SNP data set contained 10129 loci and 59 individuals across 13 locations. The PCA of the *G. celata*/*G. chrysophaea* dataset confirms that the *G. celata* samples are genetically distinct from the two sampled locations of *G. chrysophaea* (Figure 3). The first axis of the principal component analysis differentiates the two species, explaining 17.16% of the variation in the dataset. The second axis explains 4.68% of the differentiation and separates the discrete locations of *G. chrysophaea* sampled.

Genetic diversity of *Grevillea celata*

The results from DArTSeq contained 53 samples and 73743 loci. After filtering, as outlined above, the SNP data set contained 9290 loci and 50 individuals across 11 locations. Spread of final locations in the analysis are shown in Figure 2b. Analysis of *G. celata* found overall nuclear genetic diversity to be 0.236. Gene diversity values at individual locations are shown in Table 1. Expected heterozygosity levels were consistent across all locations, except for LAM1 which was lower. Observed heterozygosity was more variable (ranging from 0.106 to 0.162 +/- 0.002 SE) and lower in every site than expected heterozygosity (ranging from 0.155 to 0.192 +/- 0.002 SE). This is reflected in the inbreeding coefficient

Table 1 Genetic diversity characteristics of the sites including number of sampled individuals in final dataset, number of private alleles, expected heterozygosity, observed heterozygosity and inbreeding coefficient for each of the populations, standard error in brackets.

	Number of individuals	No. private alleles	Expected heterozygosity	Observed heterozygosity	Inbreeding coefficient
REFR	5	15	0.180 (0.002)	0.125 (0.002)	0.305
SIX	4	44	0.176 (0.002)	0.162 (0.002)	0.079
DEAD	5	19	0.187 (0.002)	0.133 (0.002)	0.290
WATR2	5	24	0.189 (0.002)	0.161 (0.002)	0.147
WATR1	5	24	0.184 (0.002)	0.146 (0.002)	0.208
UNTN	5	10	0.192 (0.002)	0.157 (0.002)	0.178
UNTS	4	6	0.182 (0.002)	0.154 (0.002)	0.154
TELEC	5	11	0.187 (0.002)	0.143 (0.002)	0.232
LAM1	4	7	0.155 (0.002)	0.106 (0.002)	0.313
LYL1	4	9	0.175 (0.002)	0.153 (0.002)	0.128
LYL3	4	12	0.175 (0.002)	0.150 (0.002)	0.142

(ranging from 0.079 to 0.313), with LAM1, DEAD and REFR showing the highest levels of inbreeding. Site SIX showed the lowest inbreeding value while also having the highest number of private alleles.

Genetic structure and differentiation

The first two principal components explained 8.9% of variance in the genotypic data (Figure 4). The first axis of the PCA (4.79%) identifies the variation amongst individuals at all locations, which cluster according to the sampling location, spread across the first axis resembling the spatial spread of the locations from East to West. The second axis indicates there is a large amount of variation within population SIX which accounts for the differentiation seen in axis 2 (4.11%). The variation within SIX is greater than that which separates UNTN/UNTS from locations to its south (WATR1/2). The paired locations (LYL1/LYL3 and UNTN/UNTS) cluster together as expected based on their geographic proximity, as do samples from WATR1, WATR2 and DEAD.

Population genetic structure, assessed in Structure,

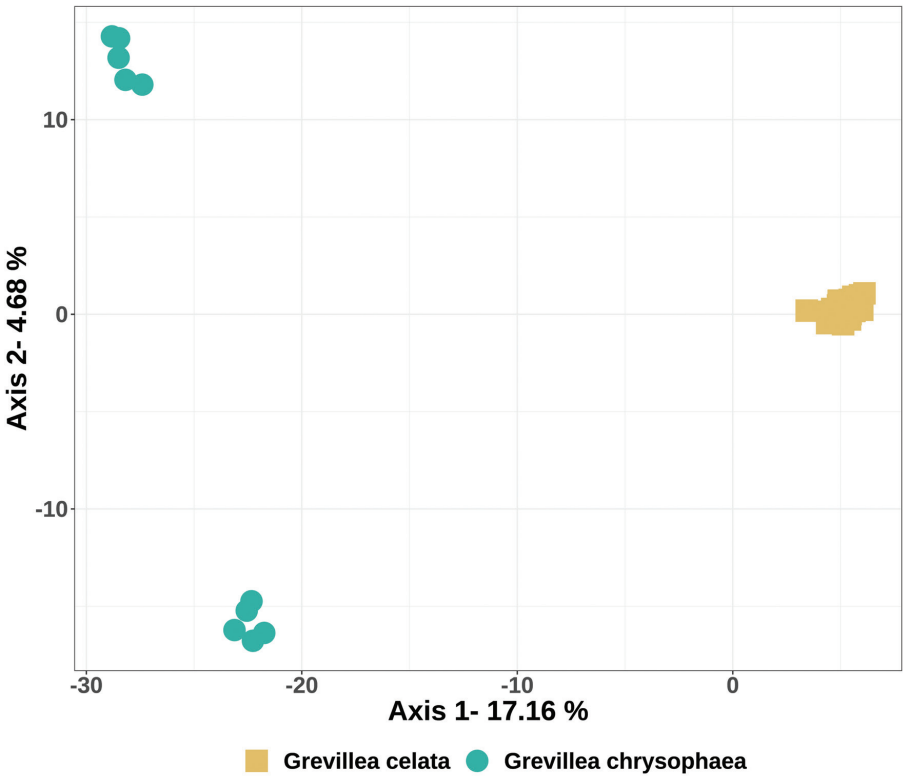

Figure 3 Plot of first and second component of principal component analysis of genetic differentiation for species delimitation between *Grevillea chrysophaea* and *Grevillea celata*.

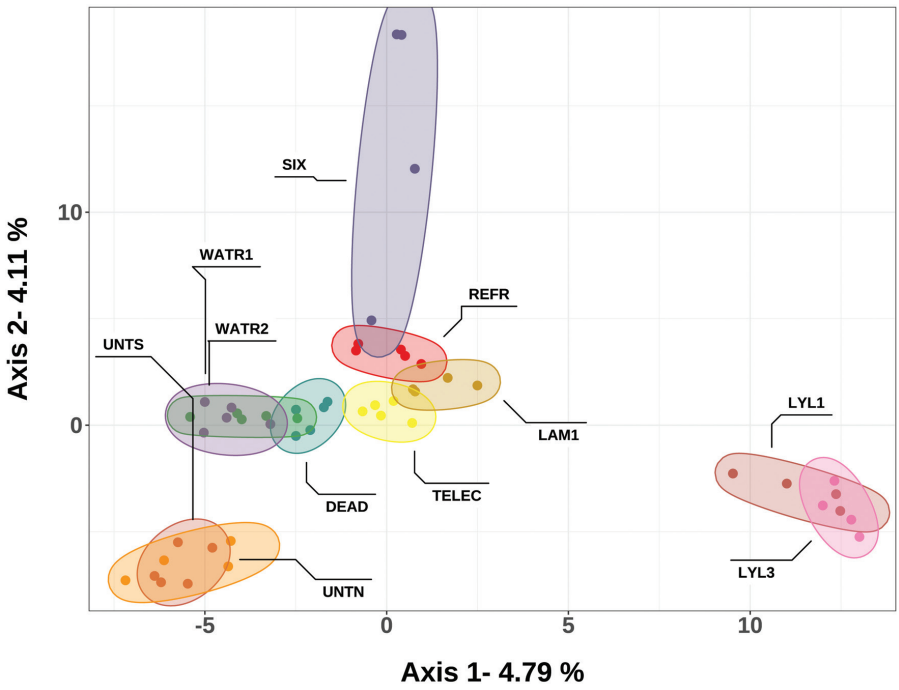

Figure 4 Plot of first and second axis of results from principal component analysis of genetic differentiation for *Grevillea celata*.

identified four genetic clusters with similar genetic patterns to the PCA results (Figure 5). The paired locations UNTN/UNTS and LYL1/LYL3 each form a cluster and most individuals from SIX form a distinct genetic cluster. The remaining locations are primarily composed of the same genetic cluster, with admixture in many individuals across these locations. Individuals at DEAD and WATR1/2 show admixture primarily with the UNTN/UNTS genetic group reflecting their close geographic proximity. Individuals at TELEC and LAM1 show admixture largely with the LYL genetic group. REFR shows admixture with the LYL group to a lesser extent, indicating a reduced gene flow at a greater distance, supporting the isolation by distance results.

Overall species genetic differentiation (F_{ST}) was found to be 0.0865. Measures of pairwise F_{ST} ranged from a low of 0.004 to a high of 0.16 (Figure 6). The highest differentiation values were between SIX and LYL, locations 10 km apart at opposite longitudinal ends of the species distribution. SIX also had higher F_{ST} values with locations half that distance apart (6-7.9 km, UNTN/UNTS and WATR1/WATR2. The lowest differentiation was between the paired locations UNTN and UNTS (0.004) which were the closest in geographic distance. The paired populations LYL1 and LYL3 also had a low differentiation (0.046), but this was greater than that between TELEC and REFR (0.035) which are located a greater distance apart. This is supported by the isolation

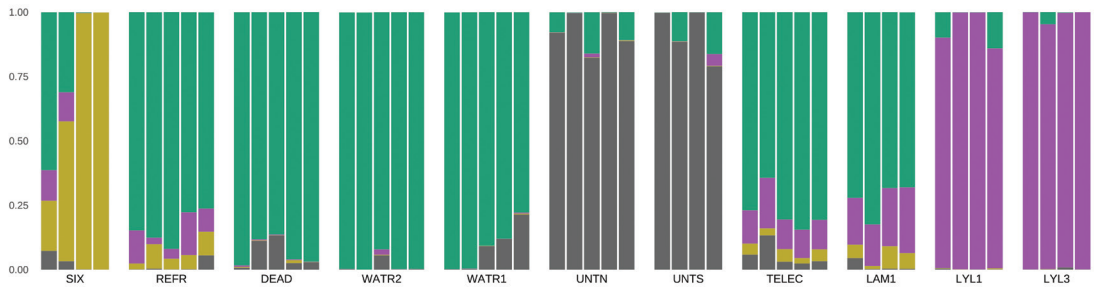

Figure 5 Genetic clustering results from Structure analysis for *Grevillea celata* sites. Each individual is represented by a vertical bar which is apportioned into its membership to each of the identified four genetic clusters.

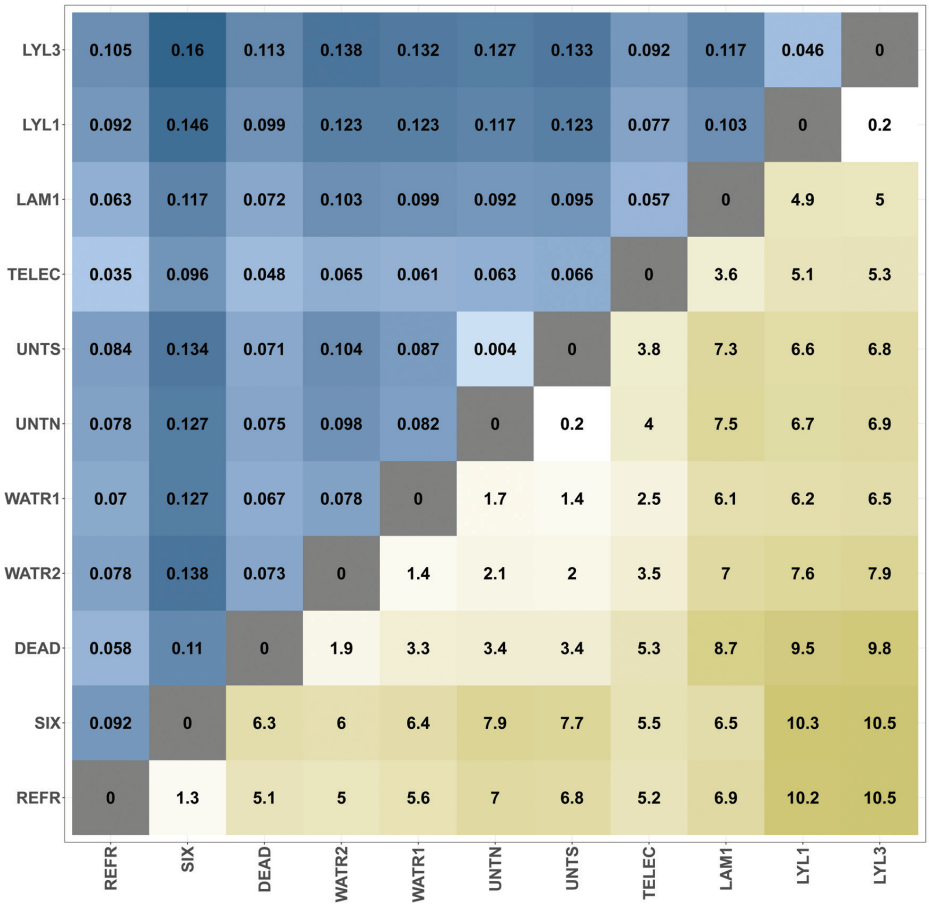

Figure 6 Pairwise genetic distance (F_{ST}) among populations of *Grevillea celata* coloured in blue in the upper triangle and geographic distance (km) among populations coloured in khaki in the lower triangle. Higher values (greater differentiation/ distance) are shaded darker.

by distance (IBD) results which found a highly significant relationship between genetic and geographic distance (Mantel statistic = 0.587, $p = 0.001$), suggesting that gene flow diminishes with distance. Analysis of molecular variance (AMOVA) showed that the majority of genetic variation was found within samples with 61.76% ($p = 0.001$) of the variation explained whereas only 9% ($p = 0.001$) was explained between populations.

Discussion

Grevillea celata has a restricted range and is represented by a small number of subpopulations. Whilst the species delimitation undertaken in this research was not a formal phylogenetic analysis, the result indicates that the *G. celata* subpopulations are genetically distinct from the nearest *G. chrysophaea* subpopulations and thus represent a conservation unit deserving of management. Asexual reproduction, through root-suckering and resprouting, was confirmed in the species, so population census counts may not reflect the effective population size. This root-suckering trait is useful after fires enabling standing numbers to rebound quickly, avoiding the loss of genetic diversity and a reliance on a seedbank. However, sexual reproduction is important as it allows for genetic recombination needed for evolution and adaptation to changing environments (Bürger 1999).

Genetic analysis has revealed the relationships both within sites and across the overall population and the importance of understanding reproductive strategies prior to the development of *ex situ* living collections or seedbanks for restoration activities. Genetic assessment of *G. celata* has highlighted locations that are genetically depauperate and could benefit from genetic rescue practices which should improve plant fitness and resilience (Frankham 2015) while also improving the evolutionary potential by reducing loss of genetic diversity from small populations through drift.

In a review of Australian threatened flora, 40% of 1,135 threatened taxa were assessed as actively declining and of the highest conservation concern (Silcock & Fensham 2018). A further 40% were narrow-range endemics that meet the IUCN assessment criteria for EN (Endangered) or CR (Critically Endangered) but are not considered to be actively declining (Silcock & Fensham 2018). *Grevillea celata* is a naturally range-restricted species and while numbers in remnant populations may not be actively declining, such narrow-range species are at a high risk of being affected by a single catastrophic event such as a fire that could affect the entire range. The main fire response strategy of *G. celata* is to resprout or root-sucker, which was observed in locations north of Bruthen-Nowa Nowa Rd that were severely impacted by fire. Whilst this indicates successful post-fire recovery, if a large fire occurred across the entire species' range or repeated fires occurred at the same locations, such events could result in an overall decline of the species.

Globally, fire activity is projected to increase, and fires are predicted to become larger and more severe in south-eastern Australia (Collins *et al*. 2022; Ellis *et al*. 2022). A species' reproductive success is critical to landscape persistence and is influenced by fire regimes, seedbanks, and disturbance. Managers require a better understanding of those interactions to guide conservation of endangered species particularly in the face of global environmental changes (Silcock & Fensham 2018). The recovery plan for the species (Carter & Walsh 2006) suggests that a 10-year fire cycle may be appropriate for *G. celata* as this would allow time for plants to mature and seed to accumulate in the soil and would reduce competition with fire-promoted species such as Bracken (*Pteridium esculentum*). More frequent fires may promote suckering of plants rather than recruitment via seed as demonstrated in *G. rhizomatosa* (Gross & Caddy 2006). The relative contribution of sexual and asexual reproduction in *G. celata* is not known, however viable seed is produced. Although *G. celata* is capable of resprouting after fire, it is expected that long term population persistence requires some sexual reproduction to occur between fires.

All sampled sites of *G. celata* show some level of inbreeding, indicating that gene flow is restricted across the range, confirmed by the positive correlation between genetic and geographic distance. Smaller occurrences or clusters of plants in the west of the range (SIX, 8MIL) may be less attractive to birds as pollinators, suggesting pollination may be primarily mediated by insects that travel shorter distances. The collection and dispersal of seed by ants limits long distance dispersal, however pollination by insects and birds should lead to longer distance pollen-mediated gene flow (Molyneux 1995). While more isolated, LYL1 and LYL3 in the east of the range is large with denser occurrences of stems and therefore should be capable of attracting pollinators and temper inbreeding within the site. The isolation of this site may be preventing the longer-range movement between populations. While it appears that migration levels amongst *G. celata* are great enough to prevent the formation of high levels of genetic differentiation, distinct genetic clusters are present, likely driven by localised breeding and adaptation in response to local conditions. It is important to preserve the natural genetic clustering within the species, whilst undertaking management actions such as augmentation to ensure local adaptation is not lost through genetic swamping.

Our results showed that some *G. celata* locations with a small number of stems (LAMBRU, 8MIL) can represent either a single or very few genetic individuals. While plants were flowering at these locations, they may be persisting primarily through asexual reproduction. Unrecognised clonality through root-suckering means that census counts could over-estimate the number of genetic individuals present at any given location of *G. celata*. Plants at SIX harbour unique genetic diversity and greater variation within this location despite occurring at a low density across a large distance. Here, the plants do not seem to have spread asexually and only a few stems/plants are present, potentially a reflection of a lack of disturbance or fire in this part of the species range. A subpopulation with so few individuals is prone to inbreeding effects and the associated long term risks affecting persistence (Ellstrand & Elam 1993). The group of individuals from SIX on the edge of the species range is not geographically isolated as another sampled population occurs just over a kilometre away and yet it has three individuals with a unique genetic signature. It is important to assist the small population of *G. celata* (SIX) to become sustainable by increasing the number of individuals through genetic rescue, whilst ensuring that any augmentation does not swamp the unique

diversity represented by just three individuals (Bragg *et al*. 2021; Frankham 2015; Whiteley *et al*. 2015). In such a scenario, plants could be sourced from REFR, which has historical admixture with the unique genetic cluster detected at SIX.

Genetic monitoring is an important tool for assessing translocation success, as sexual or asexual recruitment methods can be determined (Van Rossum & Hardy 2022). Historic translocations of *G. celata* were undertaken with the best intention of establishing plants at a new location to replace those lost due to roadworks. Through discussions with botanists at Royal Botanic Gardens Victoria (RBGV) about interpreting the *G. celata* results, it was revealed that the genetically identical site LAMBRU, is the surviving remains of a translocation to compensate for plants destroyed by road upgrades (DSE 2009). The genetic uniformity of the samples collected from this location indicates that the translocated plants were propagated from cuttings of one individual and do not reflect a genetically healthy population. Rapid genetic decline of translocated populations is likely if the founder individuals are from a limited genetic background (Krauss *et al*. 2002). It is therefore recommended for any species that any translocations incorporate a genetically diverse germplasm to alleviate the chance of inbreeding (Commander *et al*. 2018). The LAMBRU site is an ideal candidate for augmentation to 'genetically rescue' this established subpopulation (Whiteley *et al*. 2015). The single genotype at LAMBRU clusters with the central genetic group (data not shown) making DEAD, WATR, REFR ideal source locations. This finding demonstrates the direct application of genetic analysis in assisting appropriate conservation actions, as the clonal nature of LAMBRU would not have been known without genetic assessment as it otherwise looks to be a healthy population when assessed by population census alone.

Implications for conservation

The establishment of an *ex situ* collection of at least 30 mature plants in cultivation is detailed in the recovery actions for *Grevillea celata* (Carter & Walsh 2006). While cutting propagation techniques are a successful way to produce large numbers of plants in a short period of time, those plants are genetic clones of their source plant. Therefore, efforts must be made to collect from multiple plants to maximise sampling unique individuals to capture sufficient diversity from a site and to reduce the chance of over-representation of one clone in the *ex situ* collection (Commander *et al*. 2018; Guerrant *et al*. 2014). *Ex situ* collections of *G. celata* should encompass germplasm collected from multiple plants within the four geographic clusters described above (LYL, LAM, SIX and DEAD/WATR/REFR) to maximise the *ex situ* representation of genetic diversity. Seed collections should be made from as many plants as possible within each geographic cluster with seed lots from locations stored separately, ideally as maternal lines, to maintain control over genotype selection (Commander *et al*. 2018). The populations identified with low, or no genetic diversity will be best managed by augmentations with genetically diverse plants. Due to the genetic structuring, it would be advisable that any augmentation is done with plants from the same genetic cluster, unless this is not a viable option (SIX). Utilising the genetic findings from this study in future management activities should maximise the genetic diversity in populations resulting in healthier populations best prepared for future challenges.

Further phylogenetic research is underway on the *Grevillea Arenaria* and *Floribunda* subgroups and future work may resolve *G. celata* at a different taxonomic rank (Holmes 2021). However, the work undertaken here indicates that *G. celata* as currently recognised constitutes a discrete conservation unit and thus *in-* and *ex situ* management actions are warranted to preserve the unique diversity. Conservation of this range-restricted species needs to incorporate the above recommendations derived from the results of this genetic analysis. *In situ* management, such as the augmentations detailed above, will strengthen genetic diversity across the range, ensuring the greatest potential for future survival. These results have already guided the establishment of *ex situ* conservation collections at RBGV aimed at preserving maximum genetic diversity of the species. A living collection representative of the four major genetic clusters is currently being cultivated at RBGV, with material taken from multiple plants spread well across locations to avoid the collection of root-suckering individuals. Currently seed is held in the Victorian Conservation Seedbank from two of the four genetic clusters, with future additional seed collections

guided by the above results to support the long term *ex situ* conservation of *G. celata*. These *ex situ* collections provide insurance for this Critically Endangered, range-restricted species in the case of disastrous loss of individuals through range wide catastrophic events.

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