Muelleria

43 Genomics Special Issue: 60–71

ROYAL BOTANIC
GARDENS VICTORIA

Optimising *ex situ* collections to efficiently conserve genetic variation in *Grevillea jephcottii* (Proteaceae)

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Abstract

Range-restricted species are highly susceptible to threats that impact the whole species in one event. *Grevillea jephcottii* J.H.Willis occurs on two disjunct ranges within Burrowa-Pine Mountain National Park and was severely impacted by bushfire in 2020. To assist with conservation and to ensure genetically representative *ex situ* collections, genetic assessment of *G. jephcottii* was made. Genetic diversity was found to be highly structured between mountain ranges. Populations along Pine Mountain showed high levels of gene flow, while populations on Burrowa Range were structured across geographic distance. Results were used to guide sampling for a genetically representative *ex situ* living collection until plants *in situ* are mature enough to produce seed for inclusion in the Victorian Conservation Seedbank.

Keywords: DArTseq, fire frequency, obligate seeder, population genetics, restricted range

Introduction

Geographic range size is a strong predictor of extinction risk (Gaston & Fuller 2009, Le Breton *et al.* 2019) and includes both extent of occurrence (EEO) and area of occupancy (AOO). When either are limited, species are placed at a high risk of extinction particularly when populations are severely fragmented (IUCN 2012). By their very nature, range-restricted species are more susceptible to catastrophic events that impact all populations. Fire is one such disturbance that can be devastating, however, species such as obligate seeders may require occasional disturbance for regeneration (Knox & Morrison 2005). Short range endemics are often confined to specific habitats (AOO) within their small geographic areas (EEO), further increasing their vulnerability to extinction (Le Breton *et al.* 2019). With ongoing land clearing, these specialised plants may lose the already small niche that they inhabit. Fragmentation of populations from habitat loss may also restrict gene flow and the diversity needed to adapt under changing conditions.

Many plants have evolved to deal with the natural incidence of fire in the landscape, however, human impacts on the characteristics of fire can disrupt natural cycles and plant responses (Gallagher *et al.* 2021). The reliance of obligate seeders on fires for regeneration renders them vulnerable to changes in the occurrence and attributes of wildfire (Muir *et al.* 2014; Pausas & Keeley 2014). Increased frequency and intensity of wildfires in southeastern Australia have been linked to climate change

ISSN: 0077-1813 (print) · ISSN: 2204-2032 (online)

(Abram *et al.* 2021; Canadell *et al.* 2021). When fire frequency increases, the seedbank of obligate seeders may become exhausted and prevent re-establishment if fires recur before seed production is sufficient to replenish it (Muir *et al.* 2014; Gallagher *et al.* 2021). Even if the seedbank is not exhausted, but rather severely diminished, recruitment could be very limited, thus reducing population sizes and impacting the standing genetic diversity available to contribute to subsequent generations. In contrast, if fire frequency is reduced due to human intervention, species risk not having sufficient chance to regenerate and populations may senesce, no longer contributing to the seedbank (Campbell *et al.* 2012; Pausas & Keeley 2014).

The Global Strategy for Plant Conservation has a target of "at least 75% of threatened species in *ex situ* collections" (Convention on Biological Diversity 2012). *Ex situ* seed banks are a feasible means of preserving large amounts of diverse genetic materials for the long term to support conservation efforts (Erickson & Halford 2020; Offord *et al.* 2021). Living *ex situ* collections may also be useful as short term genetic insurance for seed production if *in situ* reproduction is disrupted, and the only *ex situ* option for plants that cannot be effectively seed banked (Guerrant *et al.* 2014; Wyse *et al.* 2018; Martyn Yenson *et al.* 2024). While seed of a threatened species will ideally be collected spatio-temporally to assure appropriate representation of the genetic diversity of a species (Guerrant *et al.* 2014; Hoban & Strand 2015), the reality is that there are not often sufficient resources available to collect and store representatives of all subpopulations. When extreme events such as bushfires impact a range-restricted species, they can highlight the species' precarious position and reinforce the need for appropriate *ex situ* collections. However, after fire, there may be a lag between recruitment and reproductive maturity, thus delaying seed collection for several years (Plumanns-Pouton *et al.* 2024). During this period the plant is also at greater risk of exposure to a subsequent catastrophic event that raises the likelihood of extinction (Plumanns-Pouton *et al.* 2023). In such circumstances, a living *ex situ* collection propagated vegetatively from standing biomass can act as an insurance population preserving some of the genetic diversity of sub-populations and the species until seed can be banked for future conservation.

Ex situ conservation collections aim to preserve the genetic diversity of wild populations with the goal of supporting future *in situ* conservation efforts (Volis 2017). Maintaining genetic diversity is important for the long-term resilience of populations (Sgrò *et al.* 2011). To efficiently and effectively manage for genetic diversity both *in situ* and *ex situ* it is important to first understand the extant levels of genetic diversity and how the genetic diversity is structured across the range (Booy *et al.* 2000). This information can guide sampling for *ex situ* collections to ensure that collections are genetically representative and hold the evolutionary potential for conservation needs (Falk & Holsinger 1991, Hoban & Schlarbaum 2014). Importantly, for species with disjunct distributions, subpopulations may be genetically distinct due to restricted gene flow caused by mate or pollinator limitation (Hoebee *et al.* 2008, Holmes *et al.* 2008) and differences in breeding systems (Caddy & Gross 2006). In addition, dispersal barriers such as limited pollinator range, or environmental or topological features, may prevent seed dispersal (Holmes *et al.* 2009; Nistelberger *et al.* 2015) or establishment of new populations, although unique variation in subpopulations can develop over small geographic distances without obvious disjunctions (Wawrzyczek *et al.* 2023).

The Pine Mountain or Green Grevillea, *Grevillea jephcottii* J.H.Willis, is a rare species endemic to a small area of northeastern Victoria found within Burrowa-Pine Mountain National Park (Department of Climate Change, Energy, the Environment and Water 2023). Increased frequency and intensity of wildfires are considered the major threats to the persistence of *G. jephcottii* (Department of Climate Change, Energy, the Environment and Water 2023). This plant is an example of a species whose limited geographic range makes it vulnerable to catastrophic events such as repeated short-interval or extremely hot wildfires that could encompass the species' entire distribution. This occurred in 2020 when widespread bushfires burnt almost 95% of Burrowa-Pine Mountain National Park and most populations of *G. jephcottii*. As the species is an obligate seeder, with no re-shooting or re-sprouting recorded post-fire, its regeneration is dependent on the presence of a viable soil seedbank and environmental conditions conducive to recruitment after fire. To promote resilience under a changing climate this research assessed genetic diversity and estimated connectivity among the populations of *G. jephcottii* to guide conservation efforts to preserve extant genetic diversity. While our focus was on assessing genetic diversity, an *ex situ* living collection was initiated by applying genetic results to the sampling of three populations.

Methods

Study Species

The Pine Mountain Grevillea is a low, dense to tall, spindly shrub 1–3 m high (Figure 1). The species is restricted to a small area in north-east Victoria in the Burrowa-Pine Mountain National Park where it occurs in rocky locations and on steep hillsides in dry sclerophyll forest, on granite, at elevations of about 550–650 m (Olde & Marriott 1995, Makinson 1996, Australasian Virtual Herbarium 2021). Steep, rugged topography segregates populations on Pine Mountain and Burrowa Range, and land clearing further isolates the two ranges. The restricted distribution of Pine Mountain Grevillea is a consequence of its narrow ecological niche on steep, rocky hillsides (see topographic features, Figure 2). The species is listed as endangered under both the *Flora and Fauna Guarantee Act 1988* (Vic) (Makinson 1996; Department of Environment, Land, Water and Planning 2021) and the *Environmental Protection and Biodiversity Conservation Act 1999* (Cth) (EPBC Act 1999) in part due to data deficiency. The species is locally common within this narrow range solely within the Burrowa-Pine Mountain National Park conservation estate but its distribution is discontinuous due to habitat specificity and topography. *Grevillea jephcottii* is taxonomically distinct and accepted, with dense, entire, oblong to narrowly elliptic leaves 10–35 mm long, 1.5–6(–8) mm wide. The lower leaf surface is glabrous (sparsely hairy along midvein on juvenile leaves) and the upper surface often hairy with a shortly recurved leaf margin. The flowers, which appear in late winter to summer as a terminal conflorescence, are pale lemon or green, with a

Figure 1. (a) Habitat, (b) re-growth after fire, (c) flowers and fruit, and (d) seedling of *Grevillea jephcottii*.

purple style, white hairs and a contrasting green pollen presenter, with pollination most likely effected via birds (Makinson 1996). Subsequent fruits are pilose follicles (Figure 1). The breeding systems of *Grevillea* species are highly variable (Hermanutz *et al.* 1998) although the realised mating systems for many species are predominantly outcrossing. The reproductive biology of *G. jephcottii* has not been investigated to date but the species is considered to be an obligate seeder. The species has been in cultivation for many years, propagating readily from cuttings, and can be grown in a variety of conditions and climates (Hitchcock 2005).

Sample collection

Population sampling of Pine Mountain Grevillea was performed in November 2021 at locations based on National Herbarium of Victoria records, the Victorian Biodiversity Atlas, and other citizen science records retrieved from the Atlas of Living Australia (Atlas of Living Australia 2021). The spatial relationship among populations is represented in Figure 2 and locations are given in Table 1. Where the widespread 2020 Black Summer Fires affected the species, seedlings of *G. jephcottii* were observed in abundance post-fire but no resprouting or re-shooting was observed. Leaf samples were collected from 5 individuals at each of 13 locations selected across the range of the species. Three sampling locations were unburnt: amongst large boulder refugia 1.6 km and 3.1 km along Pine Mountain Track (PM_TR2 & PM_TR4) and a sheltered location past an old mine site (PM_OMS). At these three locations, samples were collected from adult plants, while samples at all other locations were seedlings. Along the Pine Mountain walking track plants are common but not continuously distributed so sampling locations were selected based on geographic complexity, such as the occurrence on opposite sides of major outcrops or minor summits (Figure 2).

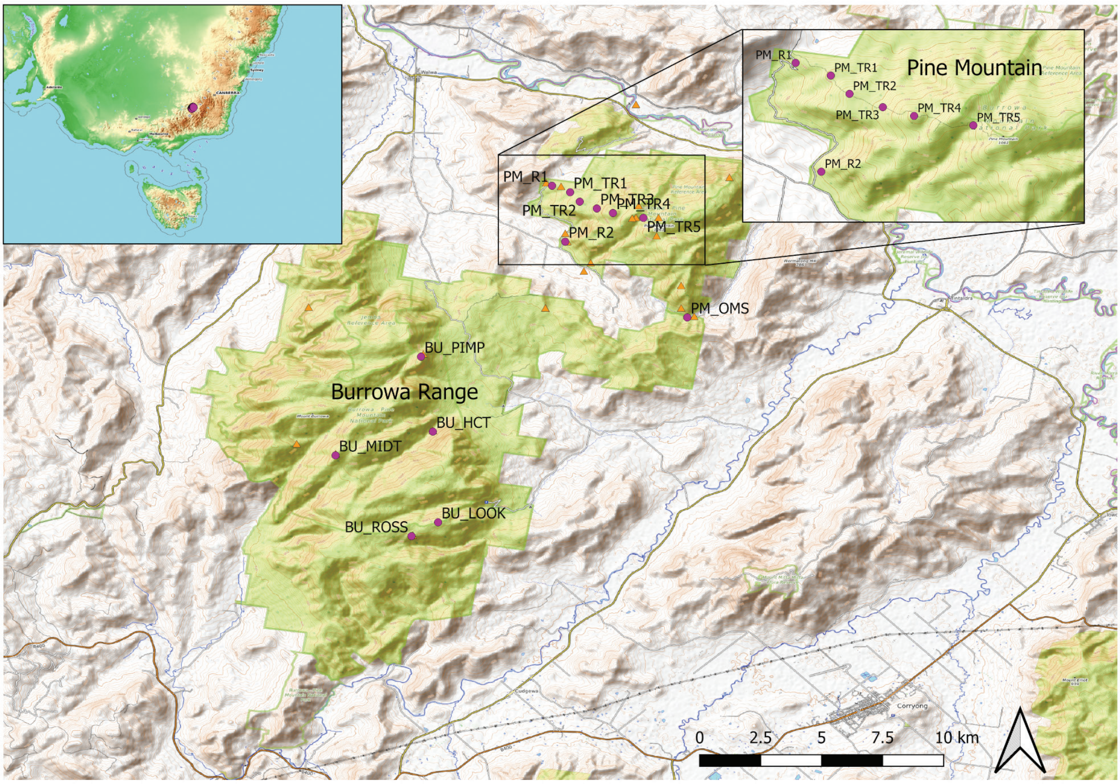

Figure 2. Locations of sampled populations (purple circles) and herbarium records (yellow triangle) of *Grevillea jephcottii*. Inset shows the location of study site in Australia: a) shows the site locations and the vegetation remaining across the landscape, b) shows the topography of the area indicating the steep terrain. Map created using QGIS (QGIS Development Team 2025), herbarium records from Atlas of Living Australia (cultivated and spatial uncertainty greater than 2 km removed).

Table 1. Location details for *Grevillea jephcottii* sample sites.

Site	Latitude	Longitude	Location
BU_MIDT	-36.104	147.706	Burrowa Mid tops
BU_ROSS	-36.134	147.734	Burrowa Ross lookout
BU_PIMP	-36.068	147.738	Burrowa The Pimple
BU_HCT	-36.095	147.742	Burrowa Hinces Creek Track
BU_LOOK	-36.128	147.744	Burrowa Lookout track
PM_R1	-36.005	147.786	Pine Mountain Pine Mountain Track Road near walking track
PM_R2	-36.025	147.791	Pine Mountain Pine Mountain Track Road near intersection with Sandy Creek Rd
PM_TR1	-36.007	147.792	Pine Mountain Pine Mountain Track 1 km along walking track
PM_TR2	-36.010	147.796	Pine Mountain Pine Mountain Track 1.6 km along walking track
PM_TR3	-36.013	147.802	Pine Mountain Pine Mountain Track 2.5 km along walking track
PM_TR4	-36.015	147.808	Pine Mountain Pine Mountain Track 3.1 km along walking track
PM_TR5	-36.016	147.819	Pine Mountain Pine Mountain Track 4.5 km along walking track
PM_OMS	-36.053	147.836	Pine Mountain Past the old Mine Site

DNA extraction, amplification and sequencing

Approximately 10 mg from each of the 65 silica-dried leaf samples were sent to the 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 Plant C-Bead 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. Filtering of poor-quality sequences (Phred score <30) and read assembly were undertaken by DArT's proprietary primary analytical pipeline and the filtered, assembled data were then used in a proprietary secondary pipeline for SNP calling (DarTsoft14).

Data analysis

Results received from DArTseq were filtered using the R packages dartR (Gruber *et al.* 2018; Mijangos *et al.* 2022) and Poppr (Kamvar *et al.* 2014, 2015) in R (R Core Team 2024). The data were filtered to a locus call rate of 0.95, an individual call rate of 0.9, a reproducibility threshold of 0.95, a minor allele frequency greater than 4, removal of monomorphic loci, removal of secondaries, a read depth between 5 and 100, and filtered on Hamming distance to remove potential paralogues.

To identify genetic clusters of individuals and visualise the major axes of variation between clusters, principal component analysis (PCA) was undertaken, implemented in the adegenet package (Jombart 2008; Jombart & Ahmed 2011) in R (R Core Team 2024). Expected and observed heterozygosity, inbreeding coefficients and pairwise population differentiation (F_{ST}) were assessed using the adegenet (Jombart 2008; Jombart & Ahmed 2011), hierfstat (Goudet & Jombart 2020), dartR (Gruber *et al.* 2018; Mijangos *et al.* 2022) 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 and fixed difference analysis were assessed using R package dartR (Gruber *et al.* 2018; Mijangos *et al.* 2022). Population genetic structure was also explored using Structure 2.3.4 (Pritchard *et al.* 2000) using K-values from 1 to 15. Ten independent runs were undertaken for each K value with a burnin of 400,000 and 600,000 MCMC iterations. The R package pophelper (Francis 2017) and ggplot2 (Wickham 2016) were used to visualise results and select the optimal K based on the ΔK metric (Evanno *et al.* 2005).

Results

The results from DArTseq contained 64 samples and 170,648 loci; one individual failed sequencing. After filtering, the filtered SNP data set contained 15,856 loci and 63 individuals across 13 populations; one individual was removed during filtering. *Grevillea jephcottii* was found to have an average observed heterozygosity across all populations of 0.103 and expected heterozygosity of 0.131. Individual population genetic diversity values are shown in Table 2. The observed heterozygosity values ranged from 0.119 to 0.135, with population BU_PIMP having the lowest value and population BU_ROSS having the highest value. Expected heterozygosity levels were consistent across populations. Observed heterozygosity was lower in every population than expected heterozygosity. This is reflected in the inbreeding coefficient, with several populations (PM_R2, BU_PIMP, BU_MIDT) showing high levels of inbreeding. The number of fixed differences between populations ranged from 0 to 16 (Table 3). The highest number of fixed differences (16) was found between populations PM_TR3 and PM_TR4 that are ~600 m apart.

Table 2. Genetic diversity characteristics of the *Grevillea jephcottii* populations (standard error in brackets), * indicates sampled population was unburnt.

Population	# Individuals	Expected heterozygosity	Observed heterozygosity	Inbreeding coefficient
PM_R1*	5	0.123 (0.001)	0.114 (0.002)	0.058
PM_R2*	4	0.128 (0.001)	0.095 (0.001)	0.213
PM_TR1*	5	0.132 (0.001)	0.114 (0.002)	0.108
PM_TR2	5	0.129 (0.001)	0.113 (0.002)	0.099
PM_TR3*	5	0.132 (0.001)	0.112 (0.001)	0.122
PM_TR4	5	0.125 (0.001)	0.107 (0.001)	0.115
PM_TR5*	5	0.129 (0.001)	0.1 (0.001)	0.178
PM_OMS	5	0.129 (0.001)	0.097 (0.001)	0.194
BU_PIMP*	4	0.119 (0.001)	0.079 (0.001)	0.278
BU_HCT*	5	0.124 (0.001)	0.104 (0.001)	0.126
BU_MIDT*	5	0.121 (0.001)	0.078 (0.001)	0.296
BU_LOOK*	5	0.127 (0.001)	0.109 (0.001)	0.117
BU_ROSS*	5	0.135 (0.001)	0.115 (0.001)	0.111

Principal component analysis showed the differentiation among sampled populations (Figure 3). The strongest split is between populations on Pine Mountain and populations on Burrowa Range, accounting for the differentiation seen in axis 1 (4.8%). The second axis identifies the variation amongst the Burrowa Range populations which cluster spatially with BU_HCT being distinct. The third axis explains a similar amount of variation as the second axis, showing the East to West spread of variation amongst the Pine Mountain populations.

Overall species genetic differentiation (F_{ST}) was found to be 0.1075. Measures of between population F_{ST} ranged from a low of 0.056 to a high of 0.177 (Table 3). The highest differentiation was found between

Table 3. Pairwise genetic distance amongst populations of *Grevillea jephcottii* (upper triangle), higher values indicate greater differentiation. Geographic distances (km) between populations (lower triangle).

Population	PM_ R1	PM_ R2	PM_ TR1	PM_ TR2	PM_ TR3	PM_ TR4	PM_ TR5	PM_ OMS	BU_ PIMP	BU_ HCT	BU_ MIDT	BU_ LOOK	BU_ ROSS
PM_R1	-	0.109	0.077	0.099	0.097	0.138	0.118	0.123	0.151	0.177	0.154	0.165	0.138
PM_R2	2.3	-	0.069	0.093	0.07	0.106	0.084	0.093	0.109	0.147	0.117	0.131	0.113
PM_TR1	0.7	2	-	0.056	0.058	0.098	0.079	0.088	0.116	0.139	0.115	0.124	0.103
PM_TR2	1.1	1.7	0.5	-	0.075	0.114	0.099	0.101	0.132	0.155	0.133	0.143	0.121
PM_TR3	1.8	1.7	1.1	0.6	-	0.084	0.071	0.079	0.111	0.136	0.109	0.119	0.098
PM_TR4	2.3	2	1.7	1.2	0.6	-	0.097	0.11	0.139	0.163	0.136	0.147	0.126
PM_TR5	3.3	2.8	2.6	2.2	1.6	1	-	0.085	0.116	0.143	0.115	0.129	0.106
PM_OMS	7	5.1	6.4	5.9	5.4	4.9	4.3	-	0.113	0.141	0.113	0.127	0.103
BU_PIMP	8.2	6.7	8.3	8.2	8.4	8.7	9.3	9	-	0.153	0.105	0.132	0.112
BU_HCT	10.8	8.9	10.8	10.6	10.6	10.7	11.2	9.6	3.1	-	0.141	0.147	0.13
BU_MIDT	13.2	11.6	13.3	13.2	13.3	13.5	14.1	13	4.9	3.4	-	0.125	0.105
BU_LOOK	14.3	12.2	14.2	13.9	13.9	13.9	14.2	11.8	6.8	3.7	4.4	-	0.077
BU_ROSS	15.1	13.1	15	14.8	14.7	14.8	15.1	12.8	7.3	4.3	4.2	1	-

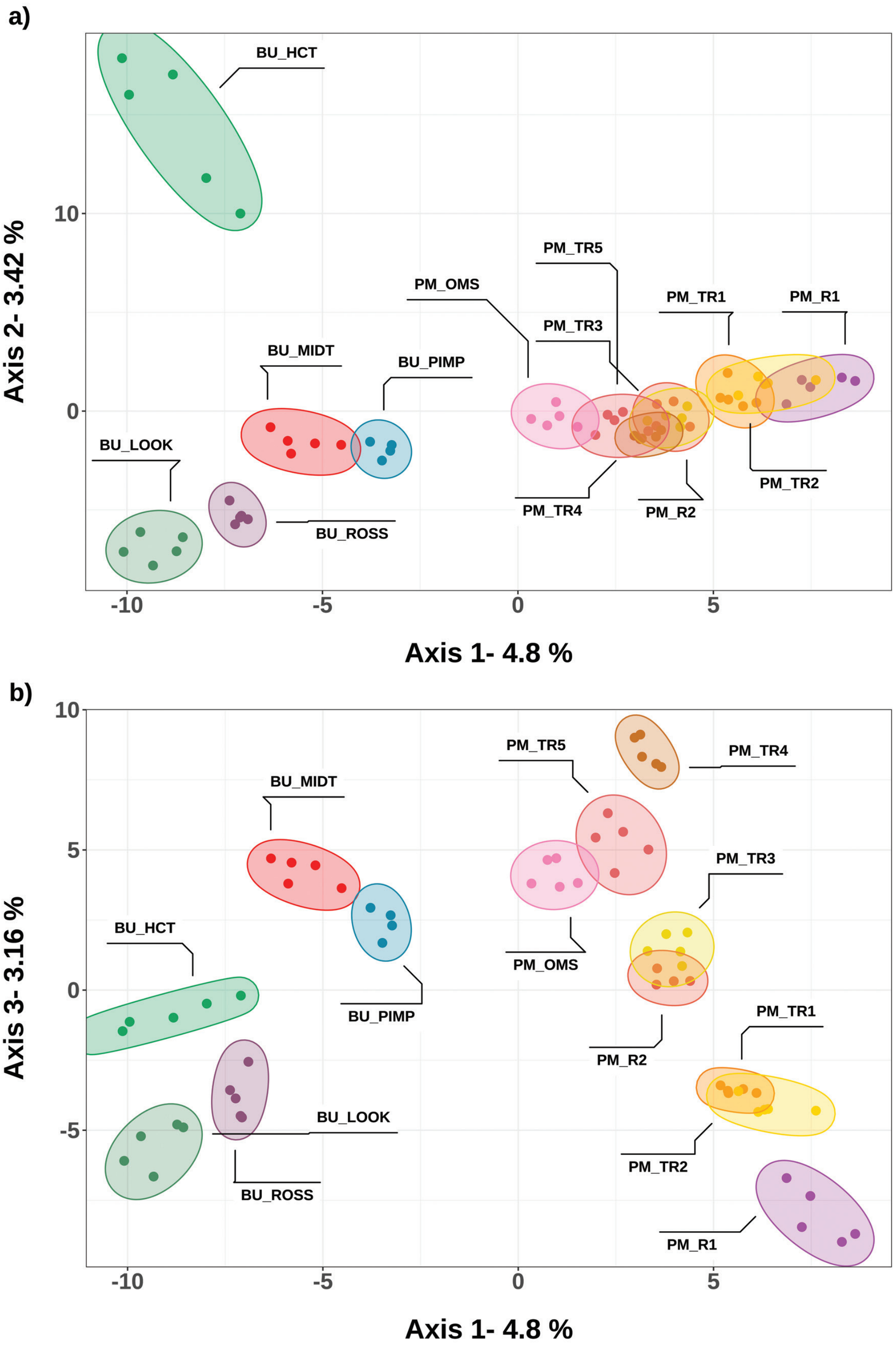

Figure 3. Principal component analysis of genetic differentiation for 13 populations of *Grevillea jephcottii* based on the SNP dataset of 15,856 loci and 63 individuals.

populations PM_R1 and BU_HCT which are located on separate mountain ranges, and the lowest differentiation was found between populations PM_TR1 and PM_TR2, the geographically closest populations. The lowest value between populations in the Burrowa Range was 0.077 between populations BU_LOOK and BU_ROSS, the closest populations on the Burrowa Range. These results support the findings of the genetic diversity and principal component analysis with population BU_HCT in the Burrowa Range showing the greatest differentiation from all other populations. Levels of differentiation amongst populations on Pine Mountain ranged from 0.056 to 0.138, average of 0.092. Levels of differentiation amongst populations on Burrowa Range were generally higher than those amongst populations on Pine Mountain (0.077–0.153), average of 0.123. The isolation by distance result confirms these findings, showing a significant association between geographic and genetic distance (Mantel statistic = 0.557, Significance = 0.0003). Analysis of molecular variance (AMOVA) showed that the majority of genetic variation was found within samples with 70% of the variation explained, while only 11.5% of the variation was explained by the genetic difference between populations and the remainder apportioned between samples within populations (*p* =0.001).

Population genetic structure, assessed in Structure, identified 12 genetic clusters across the 13 populations sampled (Figure 4) (Figure S1). These results show the finer detail of groupings identified in the PCA. Most populations are represented predominately by a single genetic cluster, the exception being populations PM_TR1 and PM_TR2 which share a genetic cluster and show admixture with populations PM_R1 and PM_TR3. The levels of admixture between the Pine Mountain genetic clusters indicate that there is gene flow between close populations, as indicated in the isolation by distance results whereas populations on Burrowa Range generally show less admixture with a small amount indicated only between populations BU_LOOK and BU_ROSS.

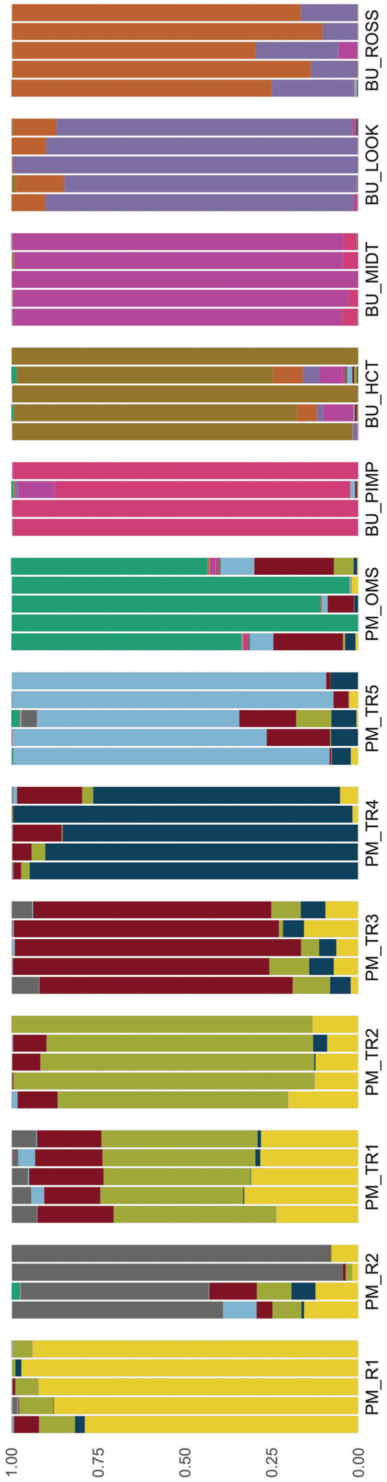

Figure 4. Genetic clustering results from Structure analysis (K = 12) for 13 populations of *Grevillea jephcottii*. Each individual is represented by a horizontal bar that is apportioned into its kinship to each of the 12 identified genetic clusters.

Discussion

The genetic assessment of *G. jephcottii* identified that genetic diversity is highly structured between the two mountain ranges, with each range showing different patterns of genetic structure. This has implications for the collection and storage of germplasm to ensure that *ex situ* holdings encapsulate the available diversity (Guerrant *et al.* 2014, Hoban & Schlarbaum 2014, Offord *et al.* 2021). The scattered distribution along Pine Mountain showed evidence of gene flow amongst neighbouring subpopulations, while populations on Burrowa Range were found to be more structured with geographic distance a good estimate of genetic differentiation. These results provide a valuable contribution to the conservation of *G. jephcottii.* By targeting germplasm collection with knowledge of the underlying genetic diversity and landscape distribution, it becomes possible to use specific collections to augment genetic diversity *in situ* or to experimentally manipulate seed genetics and production *ex situ*.

While regeneration from seed was observed at fire affected sites, the time required for these small plants to reach sexual maturity is unknown. A risk for the species is that the pre-fire seedbank may have been completely depleted at burnt sites or frequent repeat burning in future could exhaust seedbanks and prevent population re-establishment if fires recur before seed production is sufficiently high (Auld & Denham 2006). A reasonable time for replenishment of seedbanks for woody species is 15 years (Auld *et al.* 2022). The large numbers of *G. jephcottii* seedlings seen at all burnt areas indicate a healthy soil seed bank prior to the recent fire but no indication of what remains. Considering all adult plants are killed by fire it is imperative but not guaranteed that these new seedlings have conditions favourable for persistence (Auld *et al.* 2022). Fresh seed from species that require disturbance for germination can have a dormancy that needs to be broken before germination (Pickup *et al.* 2003). Environmental conditions vary from year to year and factors such as temperature influence traits like phenology, bud set and growth, and these changes can subsequently affect the seed production of individual plants (Solvin & Steffenrem 2019). Therefore, it is important to allow for spatio-temporally variable seed to be incorporated into the seedbank to ensure that the genetic diversity of the soil seedbank and any populations derived from it have the best chance of survival and persistence. By combining seed from multiple years, soil seedbanks can accumulate and preserve the genetic diversity of standing populations (Honnay *et al*. 2008, Ottewell *et al*. 2011, Templeton & Levin 1979). This was seen in the results of this study with heterozygosity values similar across burnt and unburnt sites, suggesting a genetically diverse seed bank prior to the fires.

Inbreeding levels in all populations of *G. jephcottii* indicate restricted gene flow and dispersal limitations for the species, findings which are confirmed by the correlation between genetic and geographic distance in the isolation by distance analysis. On Pine Mountain, the species is locally common with populations discrete and scattered across the mountain; as such we did not expect to see restricted gene flow amongst these geographically close populations. Populations that are not geographically isolated are usually expected to maintain genetic connectivity; thus, the results are likely an indication of restricted gene flow that may be a consequence of short distance seed dispersal (Ellstrand & Elam 1993, Ellstrand 2014), limited pollinator movement between subpopulations, and/or possible self-compatibility and selfing.

The genetic results of this study provide a valuable contribution to the conservation of *G. jephcottii* and have been of practical use to design seed collection strategies and guide *in situ* sampling of plants to establish a living insurance collection until it is possible to collect seed. Prior to the 2020 wildfires, the Victorian Conservation Seedbank (VCS) did not hold any seed collections for the Pine Mountain Grevillea. It is important that seed production is sufficient to allow replenishment of the *in situ* seedbank before collecting seed for conservation (Gallagher *et al.* 2022) and it may be some years before seed is produced in *G. jephcottii* populations and in sufficient quantities. Therefore, following initial results from this genetic analysis, cultivated *ex situ* collections were assembled from plants at Pine Mountain Track (PM_PMT), The Pimple (BU_PIMP) and Hinces Creek Track (BU_HCT) with the potential to be used for seed production. In 2024, a small collection of seed was made along Pine Mountain Track for the VCS, the beginning of a seed bank collection for the species, and additional collections will be made as seed production increases. To fully capture the genetic diversity present in *G. jephcottii* we would recommend that seed collections be made from each of the populations on the Burrowa Range and from a number of locations spread across Pine Mountain. We acknowledge that collecting samples from every population may not be feasible, especially when establishing living collections that require substantial space and ongoing maintenance, each with associated costs. Under such constraints, we propose that both the Pine Mountain and Burrowa Range be represented to capture the two main genetic groups, with the aim being to make a few collections from the distribution on Pine Mountain (e.g. PM_OMS, PM_TR3 and PM_R1) and a few from Burrowa Range (i.e. BU_HCT, BU_ROSS or BU_LOOK and BU_PIMP or BU_MIDT). Due to the higher inbreeding at PM_R2, BU_PIMP, and BU_MIDT, *in situ* efforts could promote reconnecting these populations with those close by, likely an easier option at PM_R2. An alternate option would be to augment isolated populations such as BU_PIMP and BU_MIDT with a small number of plants from neighbour populations to increase the diversity at those sites. By introducing only a small number of plants, the risk of any negative outbreeding effects would be minimised while still effecting an increase in genetic diversity.

While these recommendations align with standard conservation practices, particularly by collecting material throughout the distribution and from geographically disjunct populations (Offord *et al.* 2021), the genetic results confirm that populations on Burrowa Range are both geographically and genetically isolated. In such remote and rugged landscapes where the true extent and location of populations may be uncertain, genetic data can serve as a valuable proxy. It can also help infer the presence of undocumented populations that may act as genetic stepping stones to maintain connectivity. The genetic dataset generated by this study can be used as a baseline comparison for future genetic monitoring to estimate the population genetic impacts of fires on *G. jephcottii* and for the management of genetic resources both *in situ* and *ex situ*.

Acknowledgements

This project was supported by funding from the Victorian Government Bushfire Biodiversity Response and Recovery Program. Samples were collected under DELWP Permit Number 10008918 and field work was undertaken with assistance from Parks Victoria. David Cantrill, Elizabeth James and Todd McLay conceived the study and obtained funding.

Supplementary information

Available online via: https://www.rbg.vic.gov.au/science/journal/

Figure S1 K-value plots for selection using the Evanno method from Structure analysis.

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