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43 Genomics Special Issue: 47–59

ROYAL BOTANIC
GARDENS VICTORIA

Seeing the tree through the forest, *Eucalyptus mackintii* (Myrtaceae), how to conserve the hard to ID

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Introduction

Genetic diversity underpins the health and fitness of populations and the ability they have to adapt to future challenges (Booy *et al.* 2000). Genetic diversity is a commonly used criterion-based evaluation of a species' conservation status and is included in the Global Biodiversity Framework (Stephens 2023). While not incorporated into the IUCN Red List risk assessments, other assessment criteria such as population size, population fragmentation and range size are included and are often related to genetic diversity (Schmidt *et al.* 2023). Extent of occurrence and area of occupancy are included in evaluating a species' geographic range size which, when limited, places a species at a high risk of extinction particularly when populations are severely fragmented (IUCN 2012). Range sizes that are large can secure a species against localised threats and reduce the chance of range-wide impacts. Conserving species that have restricted ranges is not easy as they are more vulnerable to threats impacting the whole species. Australia has an increasing risk of extreme weather and fires, raising the chances of a range-wide catastrophic event for species with small ranges (Collins *et al.* 2022; Ellis *et al.* 2022). Loss, or a reduction in size, of populations of range-restricted species can reduce genetic diversity if gene flow between populations is restricted (Ellstrand & Elam 1993; Oostermeijer *et al.* 2003).

Population genetic studies can provide practical information on landscape genetic patterns to assist in the design of collection strategies

Abstract

Eucalyptus mackintii Kottek (Gippsland stringybark) is endemic to East Gippsland, Victoria and is recovering after widespread fires in 2019/2020 severely impacted the species at several locations. This study estimated levels of genetic diversity and connectivity among nine locations across the species' range. With high levels of gene flow across the distribution, on-ground management should focus on maintaining this connectivity and preventing population fragmentation. The greatest challenge to managing this species is accurate identification. It is recommended that sampling is expanded, spatially and temporally, for *ex situ* seed collections so they represent the genetic diversity found in this study and that collections are made by those trained in *Eucalyptus* identification to ensure propagules are collected from the correct species.

***Keywords*:** conservation, DArTseq, genetics, restricted distribution, resprouting

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

for *ex situ* germplasm and its use for threatened species conservation. Naturally high gene flow maintains genetic diversity, reduces the risk of inbreeding (Frankham 2015) and reduces the genetic differentiation between populations. If populations are not naturally fragmented and gene flow is high, differences in local adaptation may be minimal (Lenormand 2002; Savolainen *et al.* 2007) but there could still be potential for adaptation resulting from additive genetic variance (Noble *et al.* 2019). An understanding of patterns of genetic diversity as a proxy for local adaptation can highlight populations most susceptible to climate-induced stressors (Meek *et al.* 2023). Historical patterns of genetic connectivity are not necessarily maintained under contemporary land-use pressures. Agriculture, timber harvesting, and changing climates have the potential to disrupt gene flow. This may necessitate targeted conservation efforts to re-establish connectivity and maintain genetic diversity. Where site conditions have changed, conservation decisions regarding the use of germplasm are likely to be a compromise between restoring the presumed historical landscape genetic patterns and providing highly diverse genetic material for restoration under the premise that greater diversity will ensure that some genotypes will be suited to the modified habitat or climatic conditions (Meek *et al.* 2023). However, standing genetic variation in range-restricted species, or some populations therein, may be insufficient to facilitate local adaptation under new conditions (Barrett & Schluter 2008).

Genetic diversity is essential for maintaining adaptive potential which enables populations to persist under changing environmental conditions (Barrett & Schluter 2008). Globally, fire activity is projected to increase, and fires are predicted to become larger and more severe in southeastern Australia (Collins 2020; Ellis *et al.* 2022). Under conditions sub-optimal for seed production or recruitment, asexual reproduction, such as resprouting or root-suckering is considered an adaptive mechanism for persistence (Vallejo-Marín *et al.* 2010). In response to fire, some plants will resprout or root-sucker, thus maintaining pre-fire genetic diversity and alleviating the reliance solely on a seed bank for survival. Asexual reproduction can maintain genetic diversity or slow down its loss, but the production of novel genotypes is reliant on sexual reproduction (Silvertown 2008). Dominant across many vegetation communities in Australia, *Eucalyptus* species are well known across fire prone landscapes. In general, they are split into two fire response groups, post-fire resprouters and seeders (Burrows 2013). While resprouting can maintain extant genomic diversity, repeated fires may reduce the likelihood of resprouting and eventually plants will senesce (Fairman *et al.* 2019) resulting in erosion of standing genetic diversity and adaptive potential.

Given the variable nature of *Eucalyptus* species, it is important to understand the genetic, reproductive and gene flow dynamics on a case-by-case basis to guide appropriate management and seed collection. Gene flow via pollen and seed in *Eucalyptus* generally occurs over relatively short distances. Dispersal of pollen is typically less than 200 m from its source but dispersal over 1 km has been recorded (Ford *et al.* 1979; House 1997; Hingston *et al.* 2004; Griffin *et al.* 2009; Booth 2017). The majority of seed from *Eucalyptus* species falls within a distance equivalent to the height of the tree (Booth 2017), so long distance gene flow is likely to be mediated by pollination (Potts & Wiltshire 1997). Gene flow and genetic structuring in *Eucalyptus* is also variable, with genetic structuring and restricted gene flow found at short distance in *E. camaldulensis* Dehnh. (Butcher *et al.* 2009) but, more commonly, little genetic structuring and high gene flow, as identified in *E. marginata* Donn ex Sm., *E. pauciflora* Sieber ex Spreng. and *E. wandoo* Blakely (Wheeler *et al.* 2003; Byrne *et al.* 2008; Gauli *et al.* 2014).

Eucalyptus mackintii Kottek is a rare tree restricted to a small area (Area of Occupancy (AOO) is estimated to be 120 km^2) in East Gippsland, Victoria, and due to its small range it is listed as Vulnerable under the Victorian Government Flora and Fauna Guarantee Act 1988 (DEWLP 2021a; DEWLP 2021b). The widespread 2019/2020 Black Summer Fires impacted approximately 75% of its range, in many locations consuming the entire canopy (DEECA 2025). The species is likely to be insect-pollinated and is a post-fire resprouter. The species now appears to be on a trajectory to recovery as existing trees are resprouting across its range. Eucalypt seedlings have also been observed during post-fire surveys but these could not be identified to species level.

Among recommendations for the future management of *Eucalyptus mackintii*, seed collection from multiple sites for the Victorian Conservation Seedbank (VCS) was prioritised (DEWLP 2022). The restricted and sporadic distribution of *E. mackintii* and its occurrence among other morphologically similar eucalypt species make it difficult to discern the population dynamics and connectivity across the species' distribution in the absence of genetic data. The species has not been included in any genetic studies to date. An understanding of the genetic landscape of *E. mackintii* can guide conservation management activities such as the collection of genetically representative seed for the VCS and the sourcing of germplasm for use in restoration. This study assessed the broadscale genetic diversity of *E. mackintii* and identified localised genetic patterns.

Methods

Study species

Eucalyptus mackintii, commonly known as the Gippsland or blue-crowned stringybark, is a rare tree restricted to an area from northwest of Bairnsdale to northeast of Orbost in East Gippsland, Victoria (Figure 1). Previously regarded as a hybrid with *E. macrorhyncha* F.Muell. ex Benth. as one of its parents, *E. mackintii* was described as a distinct species in 1990 when morphological and chemical analysis found no evidence to support a hybrid origin (Kottek *et al.* 1990). The taxon has been listed as Vulnerable under the Victorian Government *Flora and Fauna Guarantee Act 1988* since May 2021 (DEWLP 2021a). Until the cessation of native forest logging in January 2024, forestry operations were considered the main risk to *E. mackintii* due to its occurrence in mixed species forest subject to timber harvesting (DEWLP 2022). Other hazards such as wildfires may impact the species, and successive disturbances could reduce recruitment and exacerbate population decline. The species is most at risk in lowland coastal plains where it is a minor species, with individuals scattered amongst other eucalypts. The range of *E. mackintii* is predominantly forested, interspersed with localised clearings and historical logging coupes scattered throughout.

Plants of *E. mackintii* grow up to 30 m tall in coarse sandy loams or clay soils in foothills (Figure 2a). Individuals can respond to fire damage by resprouting from epicormic buds (Figure 2b). Herbarium data indicate sporadic flowering and fruiting over a year (Supplementary Table S1, available online). During surveys for this study, little flowering was observed between June and October 2021 whereas flowering trees were common in December 2021 (Figure 2c).

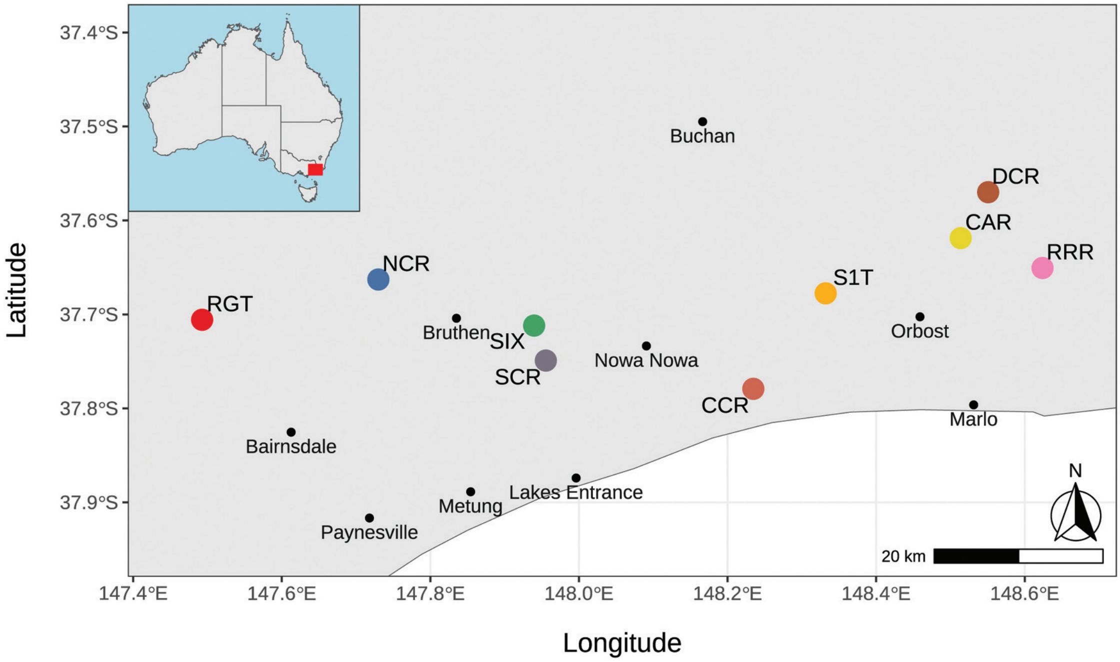

Figure 1 Locations of sampled populations.

Fruit is a hemispherical to globose capsule (Figure 2d). Rough, orange-brown bark occurs on the trunk and extends to the small branches. Leaves are lanceolate to falcate with an oblique base and slightly glossy and blue-green, giving a distinctive hue to the canopy. Juvenile leaves are oblong to ovate, with an oblique base and distinctive apex (Slee *et al.* 2020).

Adult plants of *Eucalyptus mackintii* can be distinguished from *E. macrorhyncha* by the lack of a distinct beak on the operculum and a fruit disc that is neither broad nor ascending as in *E. macrorhyncha*. Seedlings of *E. mackintii* are indistinguishable from *E. macrorhyncha* (Kottek *et al.* 1990) so it is not possible to determine whether the extensive seed germination seen after fire is from *E. mackintii*, a sympatric eucalypt, or a combination of species.

Sample collection

Field surveys for this species targeted locations across the species' range from northwest of Bairnsdale to northeast of Orbost based on National Herbarium of Victoria specimens or observation records from Atlas of Living Australia (Australasian Virtual Herbarium 2022). We found this species to grow in patches and only in areas with poor, generally sandy soils but it was commonly observed across the range. The widespread bushfire in 2019/2020 affected a large area of the species' range, so both burnt and unburnt locations were sampled. In burnt locations, juvenile to adult leaves were collected from resprouting trees where fruit was present on the ground to assist in identification.

Leaf samples of *E. mackintii* were collected ~40 m apart (approximately 2 canopy heights) to minimise the

Figure 2 (a) Habitat of *Eucalyptus mackintii*, (b) re-growth after fire, (c) flowers, and (d) fruit.

Table 1. Location details for *Eucalyptus mackintii* sample sites.

Site	Latitude	Longitude	Location	Burnt
RGT	-37.706	147.493	Rain Gauge Track	no
NCR	-37.663	147.730	Nicholson Creek Rd	yes
SIX	-37.712	147.940	Six Mile Track	no
SCR	-37.749	147.955	Siberia Crossing Rd	no
CCR	-37.779	148.235	Circle Rd	no
S1T	-37.678	148.332	Stoney #1 Track	no
CAR	-37.619	148.514	Caulson Rd	yes
DCR	-37.570	148.551	Dynamite Creek Rd	yes
RRR	-37.649	148.621	Rocky River Rd	no

chances of sampling siblings as most eucalypt seeds fall within the distance of the canopy height of parent trees (Booth 2017). Samples were made from 4 to 6 individuals at 12 populations. Preliminary results indicated that collections at three populations were likely to be a mix of species, so those sites were removed from the dataset prior to analysis leaving nine populations representing the species across its geographic range (Table 1, Figure 2). This highlights the challenges in management of this species; identification can be difficult, particularly after fires when diagnostic characters can be challenging if not impossible to find.

DNA extraction, amplification and sequencing

Approximately 15 mg from each of the 65 silica-dried leaf samples (including four technical repeats) were subsequently sent to the commercial genotyping service, Diversity Arrays Technology ('DArT', Canberra, Australia) for DNA extraction and DArTseq^{TM} analysis, a reduced representation sequencing method proven for use in eucalypts (Sansaloni *et al.* 2010; Kilian *et al.* 2012; Cruz *et al.* 2013). 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 HpaII and uniquely barcoded adaptors. Following PCR and quantification, the samples were standardised and pooled for sequencing in a single lane of an Illumina HiSeq2500. 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

The results from DArTseq contained 64 samples and 54,065 loci. DNA sequence data received from DArTseq analysis were filtered using the R packages dartRverse v.0.51 (Gruber *et al.* 2018; Mijangos *et al.* 2022) and Poppr (Kamvar *et al.* 2014, 2015) in R v.4.3.3 (R Core Team 2024). The data were filtered to a locus call rate of 0.9 and individual call rate of 0.9, a reproducibility score of 0.95, a Hardy-Weinberg equilibrium with a 5% level of significance, a minor allele count of 2, removal of monomorphic loci, and finally filtered on Hamming distance with a threshold of 0.2 to remove potential paralogues. After filtering, as outlined above, including removal of technical repeats and populations with misidentified individuals, the SNP data set contained 6471 loci and 44 individuals across nine populations.

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}) based on the estimator from Weir & Cockerham (1984), using 1000 bootstraps to estimate probability of F_{ST} values to be different from zero, were assessed using the dartRverse (Gruber *et al.* 2018; Mijangos *et al.* 2022), adegenet (Jombart & Ahmed 2011), hierfstat (Goudet & Jombart 2020) and Poppr (Kamvar *et al.* 2014, 2015) packages in R. Overall species genetic differentiation (F_{STp}), corrected for sampling error, was calculated using hierfstat (Goudet & Jombart 2020). Analysis of molecular variance (AMOVA) was tested using the Poppr (Kamvar *et al.* 2014, 2015) package in R. Isolation by distance (IBD) and fixed difference analyses were assessed using R package dartRverse (Gruber *et al.* 2018; Mijangos *et al.* 2022). Population genetic structure was explored using Structure v.2.3.4 (Pritchard *et al.* 2000) using K-values from 2 to 11. Ten independent runs were undertaken for each K value with a burnin of 100,000 and 200,000 MCMC iterations. The R packages pophelper (Francis 2017) and ggplot2 (Wickham 2016) were used to visualise results and select the most probable K based on the ΔK metric (Evanno *et al.* 2005). The Evanno method

Table 2. Genetic diversity characteristics of the populations, standard error in brackets.

Site code	Number of individuals	Expected heterozygosity	Observed heterozygosity	Inbreeding coefficient
RGT	5	0.156 (0.002)	0.142 (0.003)	0.148 (0.005)
NCR	5	0.159 (0.002)	0.144 (0.003)	0.155 (0.005)
SIX	5	0.162 (0.002)	0.145 (0.002)	0.162 (0.005)
SCR	5	0.156 (0.002)	0.142 (0.002)	0.151 (0.005)
CCR	6	0.160 (0.002)	0.144 (0.002)	0.148 (0.005)
S1T	4	0.156 (0.002)	0.144 (0.003)	0.160 (0.005)
CAR	4	0.161 (0.002)	0.145 (0.003)	0.173 (0.005)
DCR	5	0.166 (0.002)	0.145 (0.002)	0.174 (0.005)
RRR	5	0.163 (0.002)	0.144 (0.002)	0.170 (0.005)

can underestimate the optimal value of K and as such we present results for all K values where a peak was identified (Janes *et al.* 2017).

Results

The analysis of *Eucalyptus mackintii* found overall observed nuclear genetic diversity across all populations to be 0.189. Individual population genetic diversity values are shown in Table 2. The observed heterozygosity values were consistent across populations ranging from 0.142 (RGT, SCR) to 0.145 (SIX, CAR, DCR), as were inbreeding coefficients ranging from 0.148 (RGT, CCR) to 0.174 (DCR).

Principal component analysis showed genetic differentiation with population divergence indicated among some sampled populations although the amount of variation explained was low with only 3.22% on the first axis and 3.08% on the second axis (Figure 3). Population CCR is separated along the first axis and population NCR shows a slight degree of separation along the second axis. Clustering results identified three peaks using the ΔK metric (Evanno *et al.* 2005), the highest peak at K = 2, a second peak at K = 9 and a third smaller peak at K = 4 (Figure S1). The two genetic clusters (Figure 4a) showed similar distributions of each cluster across populations. The four genetic clusters reflected the PCA results, with populations CCR and NCR each having a unique genetic cluster present. The genetic cluster in NCR is also present to a smaller degree in a few individuals of RGT, reflecting the overlap seen in the PCA plot. The other two genetic clusters occur across all populations (Figure 4b). The nine genetic clusters were more aligned with populations, with geographically close populations SIX/SCR and CAR/RRR each sharing large portions of a genetic cluster (Figure 4c). Overall admixture is high amongst populations, again suggesting high levels of gene flow or common ancestry. Populations RGT and CCR show slightly less admixture than other populations, supporting the PCA results.

Overall corrected species genetic differentiation (F_{STp}) was found to be 0.0247. Supporting the results of the PCA, populations have very low levels of genetic differentiation. Measures of pairwise population F_{ST} (Table 3, lower triangle) were all found to be significant ($p<0.05$) and ranged from a low of 0.007 between populations DCR and CAR to a high of 0.045 between populations NCR and CCR which were also the populations most differentiated in the PCA. Despite its location geographically midway but most southerly in the species' range, population CCR is most differentiated from all populations with the paired values generally higher than between the other populations. The number of fixed differences between populations ranged from 0 to a high of 3 between populations (SCR and S1T and CAR) (Table 3, upper triangle). Investigation into IBD using a Mantel test found that the relationship between genetic and geographic distance was not significant (Mantel statistic = 0.237, Significance 0.13).

Discussion

The objective of this study was to acquire baseline information on the level and distribution of genetic variation in *Eucalyptus mackintii* and identify the implications for management of a species subject to disturbances including fire. The SNP dataset provides practical insight into gene flow within the taxon and suggests high levels of genetic connectivity across the

Table 3. Fixed differences between populations of *Eucalyptus mackintii* (upper triangle), Pairwise genetic distance amongst populations (F_{ST}) (lower triangle). Higher values indicate greater differentiation.

Population	RGT	NCR	SIX	SCR	CCR	S1T	CAR	DCR	RRR
RGT	-	1	0	0	0	1	0	0	0
NCR	0.029	-	0	0	1	2	1	0	1
SIX	0.022	0.03	-	0	0	1	2	0	0
SCR	0.025	0.032	0.014	-	2	3	3	0	0
CCR	0.037	0.045	0.032	0.038	-	0	1	0	0
S1T	0.022	0.03	0.019	0.022	0.031	-	-	0	0
CAR	0.026	0.028	0.023	0.016	0.034	0.014	-	0	0
DCR	0.027	0.023	0.02	0.026	0.028	0.012	0.007	-	0
RRR	0.021	0.034	0.022	0.021	0.041	0.017	0.012	0.012	-

species range. However, the landscape patterns revealed require some qualification as the sampled trees are likely to be several decades old, so the conditions under which seedlings germinated and that maturing plants were subject to may differ from contemporary conditions. Quantification of genetic diversity in contemporary seedling recruits is needed to confirm whether those genetic patterns are being maintained. The landscape genetic patterns among populations of *E. mackintii* are a useful guide for management. Including genetic information in management strategies has a role in ensuring that the genetic integrity of a rare species can be maintained (Ellstrand & Rieseberg 2016). Given the low percentage variation accounted for by the PCA, we anticipate that other unexplored factors such as environmental conditions and/or species demographic

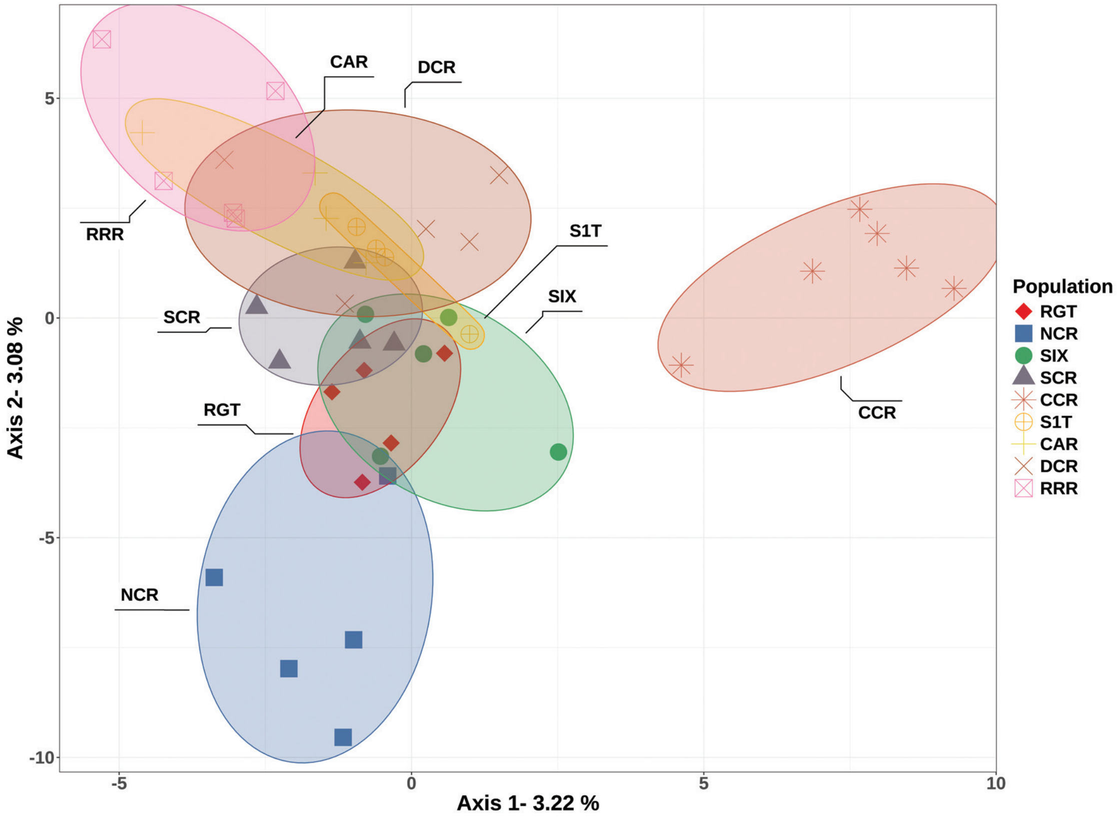

Figure 3 Principal component analysis of genetic differentiation.

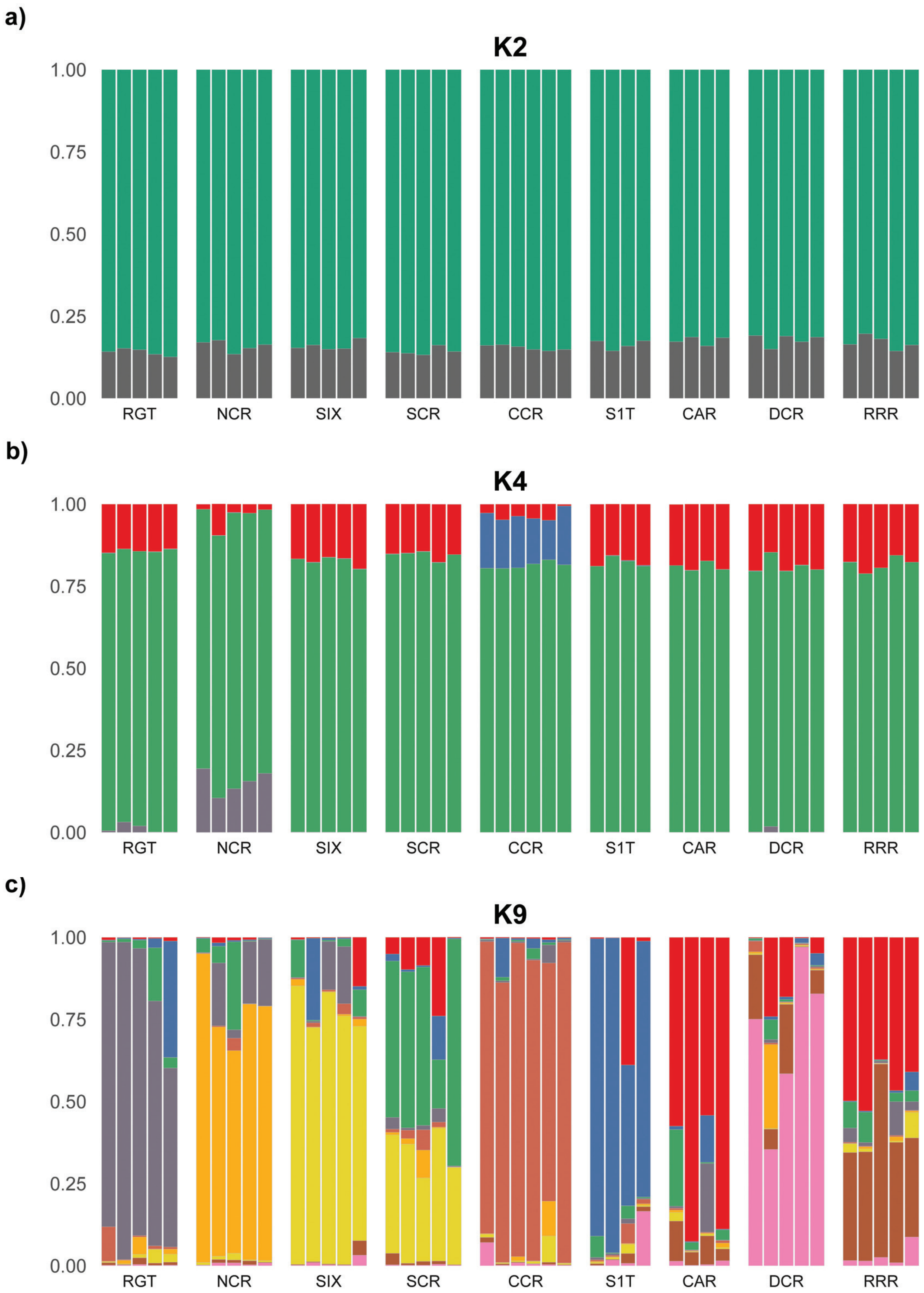

Figure 4 Genetic clustering results from *Eucalyptus mackintii*. Structure analysis for two clusters (a), four clusters (b) and nine clusters (c). Each individual is represented by a vertical bar which is apportioned into its kinship to each of the identified genetic clusters.

history have also influenced the genetic patterns observed.

Several eucalypt species exhibit low genetic structuring and high levels of gene flow, reflecting strong genetic connectivity across their respective landscapes. *Eucalyptus albens* Benth. and *E. sideroxylon* A.Cunn. ex Woolls were both found to have very low F_{ST} values, 0.018 and 0.017 respectively (Murray *et al.* 2019). Genetic differentiation was low between sites across the range of *E. mackintii* (0.0247) which is indicative of a high level of gene flow enabling genetic connectivity. Higher F_{ST} values have been found in eleven *Eucalyptus*, with values found between 0.03 in *E. pauciflora* to 0.127 in *E. cunninghamii* Sweet (Gauli *et al*. 2014; Supple *et al.* 2018; Rutherford *et al*. 2018). Despite the low levels of differentiation found in *E. mackintii*, there were some signals of genetic structuring amongst populations. Our results highlighted population CCR and to a lesser extent NCR as being somewhat genetically distinct, a characteristic not readily apparent from the spatial distribution of sample locations and which may be due to their local demographic histories. While gene flow appears high and genetic differentiation is low, it is still important to conserve the genetic diversity that is present across the range. The genetic diversity results found in *E. mackintii*, observed heterozygosity of 0.189, are similar to that found in eleven *Eucalyptus* species ranging from 0.133 in *E. stricta* Sieber ex Spreng. and *E. laophila* L.A.S.Johnson & Blaxell to 0.246 in *E. langleyi* L.A.S.Johnson & Blaxell (Rutherford *et al*. 2018). With an estimated generation time of 100–150 years and individual longevity of 150–250 years (DELWP 2021b), sampled trees are likely to be several decades old. Therefore, our results are not measures of contemporary genetic patterns meaning that the impact of any recent habitat modifications or environmental change on genetic connectivity may not be apparent for several generations.

Understanding the spatial distribution of a species is essential for effective management, protection, and conservation. While qualified and trained botanists are able to identify species, on-ground managers may have a harder time when there are multiple species that co-occur and have a very similar morphology. There are over 700 species of *Eucalyptus* in Australia and morphological similarity can make it challenging to accurately identify them to the species level, and particular groups can be especially problematic to non-experts (Slee *et al.* 2020). The misidentification of some samples collected as *E. mackintii* underscores the difficulty of identifying this species when it grows sympatrically with one or more other eucalypts, particularly after fire when a number of characteristics may not be available. Adding to this challenge is the propensity for *Eucalyptus* species to hybridise with their congeners, which can produce plants that are variable along a spectrum between parent plants, potentially adding to identification confusion (Potts & Reid 1985; Griffin *et al.* 1988; Neaylon *et al.* 2001; Field *et al.* 2009). At this stage, hybridisation between *E. mackintii* and sympatric stringybarks is not known but hybridity or introgression could be examined genetically by including all sympatric eucalypt species if the inclusion of seed from misidentified trees is likely to affect the conservation value of seed resources.

The resprouting of mature *Eucalyptus mackintii* following the 2019/2020 wildfires shows that the species has some resilience to fire. Resprouting is expected to result in earlier flowering than seedlings, which should assist in providing a more rapid replenishment of the soil seedbank and facilitate new seed collections. Despite these expectations, ongoing field monitoring is an important conservation requirement to enable effective assessment of the recovery of fire-affected populations of *E. mackintii* and the forest ecosystem more generally over time (Gallagher *et al.* 2021). Short fire intervals have been implicated in a failure in the ability of fire-tolerant trees to continually respond by resprouting (Fairman *et al.* 2019) although other studies have shown resilience to repeated canopy fires in vegetation dominated by eucalypts that resprout from epicormic buds (Collins 2020). To support seed production and ensure viable seed collection, fire management strategies should consider maintaining intervals that allow plants to recover and reach reproductive maturity and produce viable seed.

High levels of genetic diversity generally improve the potential of a species to persist under changing conditions (Nicotra *et al.* 2010) and should be captured as representatively as possible in any germplasm collections planned for conservation management. The Victorian Conservation Seedbank currently holds two seed collections for *E. mackintii*. The older collection made in 2007 is from location DCR, north of Orbost. The collection had an initial inferred viability of 100% (cut test) and germination rate of 80% and the high viability was retained in subsequent germination trials (VCS, unpublished data). In contrast, the 2021 seed collection sourced from Bruthen-Nowa Nowa Rd, the same location as an initial genetic collection, had only 10% inferred viability (cut test) and only 12% germination of filled seed. The initial genetic analysis of samples indicated that the leaf collections from this location were taken from more than one eucalypt species, thus samples from that location were removed from all analyses. The seed collection from the Bruthen-Nowa Nowa Rd location requires assessment to confirm the identity of the species held, or should be discarded and collected anew to remove the possibility of inadvertent sampling of non-target eucalypts.

In the limited number of studies available it was found that *ex situ* seedbank collections do not effectively capture the genetic variation of wild populations (Wei & Jiang 2021). Recommendations for optimal seed collecting suggest that when range-wide population structure exists, the spatial distribution of sampling is crucial although the importance is reduced as gene flow amongst populations increases (Hoban & Schlarbaum 2014). Sampling seed from approximately 200 to 300 individuals in total across a species' geographic range may suffice, however, a robust minimum collection for *ex situ* gene conservation will often be 1000 individuals or more to compensate for expected losses over time (Hoban 2019). To maximise the genetic diversity in *ex situ* collections, it would be prudent to collect from multiple locations across the species' range and across different years. This is particularly important for sites where seed quality is found to be low because this may vary from year to year. Each seed collection should be stored separately to provide for future genotype selection. Seed collections are needed to fill the geographic gaps in current collections. These include new collections in the western part of the species' range, such as the Rain Gauge Track (RGT) or Nicholson Creek Rd (NCR) in Alfred-Nicholson State Forest, locations within Colquhoun State Forest SCR, and on the southern (CCR) and eastern limits of the species' range (RRR). Such collections would enhance the genetic diversity of the VCS *ex situ* collections.

The conservation requirements of *Eucalyptus mackintii* may need to be re-assessed if a current ban on commercial forestry in native forest is permanent, thus removing the primary threatening process for the species. The species remains at risk due to climate change, with fire activity projected to increase in southeastern Australia. An increase in fire frequency and intensity may compromise the species' capacity to resprout successfully and replenish the soil seed bank. The greatest challenge to managing this species is likely accurate identification. Due to the risks associated with its narrow range and identification challenges, it is recommended that *ex situ* seed collections are held that represent the full genetic diversity that is present across the range, and that any collections be made by those specifically trained in their identification to ensure that the propagules are collected from the correct species.

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 completed with assistance from Parks Victoria. David Cantrill, Elizabeth James, and Todd McLay conceived the study and obtained funding. We thank Neville Walsh and Todd McLay for assistance with sampling.

Supplementary information

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

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

Table S1 Monthly distribution of herbarium specimens, showing counts of total specimens and those bearing buds, flowers or fruit.

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