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Pulling the wool over our eyes: deciphering genomic variation in *Acacia lucasii* (Fabaceae) and close allies

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Introduction

Several regions of Australia have high numbers of unique, restricted and rare flora species due to a combination of environmental factors including temperature and precipitation, soil texture, and topographic heterogeneity (Mokany *et al.* 2022). Southeastern Australia is species-rich and diverse owing to the complex profile of the Great Dividing Range that provides substantial geological, elevation, climatic and environmental complexity (Taylor 1994, Ollier 1995) which may have influenced the expansion or contraction of species' ranges during historic climatic oscillations (Bryant & Krosch 2016, Byrne 2008). The combination of landscape-wide environmental factors and environmental events play a role in shaping the species diversity and distributions seen in different plant groups common in the southeast (e.g. Rosetto *et al.* 2012, Nge *et al.* 2021, McMaster *et al.* 2024).

The complex patterns seen in the diversity and distributions of taxa make evaluation of the genetic variation, structure and historical gene flow across a species' range an important step in defining conservation units and can aid in the identification of cryptic taxa. Genetic evaluation prior to conservation actions enables the use of species-specific information to guide management. Genetically informed collection and use of germplasm *in situ* and in the establishment of seed orchards

Abstract

Acacia lucasii Blakely (woolly-bear wattle) occurs in only two remote, disjunct localities in southeast Australia. The entire species' range was burnt in the Black Summer megafires (2019–2020). This study investigates the genomic variation across the geographic range of *A. lucasii*, the taxonomically associated *A. costiniana* Tindale and a closely related population of uncertain status. High levels of genetic differentiation were found between all locations and taxa. *Acacia lucasii* populations had low genetic diversity and high levels of inbreeding, reflecting the isolation of populations that are likely remnants of a previously more widespread precursor affected by historic climatic oscillations resulting in contemporary geographic separation and divergence. Irrespective of taxon, the genetically distinct populations may be best conserved as individual conservation units.

Keywords: DArTseq; *Acacia*; species complex; range disjunction, restricted range

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or the design of translocations (Commander *et al.* 2018) lessens the risk of inappropriate genetic combinations (Coates *et al.* 2018). This approach should facilitate the creation of viable, self-sustaining populations with the genetic integrity to withstand and adapt to future environmental changes (Sgrò *et al.* 2011). Range-restricted species and/or those with small populations are particularly susceptible to abiotic stochastic events such as fires and climate change. Such species whose distribution and/or population numbers are severely compromised confound the assumptions of population genetic analysis; that is, sufficiently large population size, the occurrence of random mating, and the absence of mutation, genetic drift, or natural selection (Frankham *et al*. 2002) but are frequently conservation targets. For such range-restricted species, or species complexes where similar species occur in adjacent areas, genetic analysis can be of use in determining the boundaries between taxonomic units, populations, and conservation units, resolving genetic lineages where phylogenies may have been unsuccessful in describing relationships (Coates *et al.* 2018).

The catastrophic Australian megafires in the 2019–2020 bushfire season burnt over 10 million hectares of the landscape and affected some part of the geographic range of between 36–69% of all Australian plant species, including large areas of southeastern Australia (Gallagher *et al.* 2021). Rapid risk assessment determined that 688 Victorian flora species listed on the state *Flora and Fauna Guarantee Act 1988* (Vic) had some portion of their range burnt (DELWP 2022). Plant taxa considered to be most at risk of impacts and extinction from the fires were assessed and prioritised continent-wide, based on their risk of post-fire decline against 11 criteria based on key species traits and impact area (Gallagher *et al.* 2021, Auld *et al.* 2022). A total of 486 flora species were recognised federally as requiring actions to assess impacts and support recovery (DAWE 2020). Among the immediate to medium-term actions proposed, germplasm collection and carefully planned translocations were some of the actions identified as most needed for plant species (DAWE 2020).

The genus *Acacia* Mill. is comprised of 1,082 species with 1,073 mostly endemic species found in Australia where they grow in a wide range of habitats on the mainland, Tasmania, and nearby islands, ranging from coastal to subalpine, tropical, and arid (Kodela & Maslin 2025). Despite the continent-wide distribution of the genus, many species have restricted ranges and disjunct distributions which can elevate their extinction risk (e.g. *Acacia whibleyana* R.S.Cowan & Maslin (Blyth *et al.* 2020), *A. purpureapetala* F.M.Bailey (van der Merwe *et al.* 2021)) often in combination with reduced fecundity and clonality (e.g. *Acacia anomala* C.A.Gardner ex Court (Coates 1988), *A. daviesii* Bartolome (Bartolome *et al.* 2002), *A. sporadica* N.G.Walsh (Walsh 2004)). Many threatened taxa are listed under state and federal legislation due to small population size, range restriction, or occurrence at a single or few disjunct locations (Category B-D, IUCN/SPC 2022). *Acacia lucasii* Blakely (woolly-bear wattle) is a species known from only two remote, disjunct localities in southeastern Australia, namely the Nunniong Plateau in Alpine National Park, Victoria (Vic) and 170 km away in the Upper Turross area in Wadbilliga National Park, New South Wales (NSW) (VicFlora 2025, Maslin 2012a).

Acacia lucasii is considered Critically Endangered under the *Flora and Fauna Guarantee Act 1988* (Vic) due to its restricted geographic range in Victoria but has no formal listing in either NSW or federally. As an obligate seeder, fire is likely to stimulate the regeneration of *A. lucasii* seeds stored in the soil seed bank, however, the small number of populations and disjunction between the two localities flag susceptibility to stochastic events and inbreeding as inherent vulnerabilities for the species. The entire range of *A. lucasii* was burnt in the Black Summer megafires and thus the species was one of 31 *Acacia* species that were prioritised for post-fire assessments and recovery actions. Another localised taxon, *A. costiniana* Tindale from the Tinderry Mountains NSW, is taxonomically associated with *A. lucasii*. In addition, herbarium specimens collected from the Nalbaugh Plateau in southeast NSW between 1985 and 2021 determined as either *A. lucasii* or *A. costiniana* may constitute a distinct taxon, based on morphological differences (D. Albrecht, pers. observ.), and is herein referred to as *Acacia* aff. *lucasii*. Known occurrences of the three entities are within protected areas (VicFlora 2025; Maslin 2012a) but among the post-fire response actions proposed for *A. lucasii* are germplasm collection and planned translocations (DAWE 2020), both of which would benefit from the availability of genetic information to guide species-appropriate use of germplasm. In this study, we investigate genomic variation across the geographic range of *A. lucasii* and compare *A. lucasii* to taxonomically associated *A. costiniana* and *A.* aff. *lucasii* to guide conservation actions. We aimed to determine the genetic identity of the putative taxon *A.* aff. *lucasii*, thereby allowing for both future taxonomic revisions and a reliable basis for future species management.

Methods

Study species

Acacia lucasii grows as an understorey shrub to 4 m high in eucalypt woodland or forest and in heath on exposed rocky granite or quartzite slopes including scree slopes at elevations from 900–1300 m. The species is very distinctive due to its rusty-brown, woolly new growth and pods, with flowering recorded from September to April (VicFlora 2025, Maslin 2012a). It occurs in two disjunct areas where it is extremely localised, the Nunniong Plateau in the Alpine National Park (NP), Vic, and Wadbilliga NP in south-eastern NSW (Figure 1). The morphology of *A. lucasii* varies between locations, with populations in Victoria differing from those in NSW in the size of the gland on the phyllode margin and its distance to the pulvinus. The Extent of Occurrence (EOO) is estimated at 16 km^2 based on accepted, post-1970 records from the Victorian Biodiversity Atlas (DELWP 2021). Early maturity is a reproductive trait of *Acacia* species/*lucasii* (Gibson *et al.* 2011) and surveys within 2–3 years post-fire recorded flowering seedlings (see Figure 2).

Acacia lucasii is most closely allied to *A. costiniana*, a taxon restricted to the Tinderry Mountains in southern NSW (Figure 1), where it occurs on granitic slopes and sheltered gullies in dry sclerophyll forest and heath communities at an elevation of approximately 1200 m (Maslin 2012b). *Acacia costiniana* is distinguished from *A. lucasii* by its arching branches and green phyllodes, the latter being generally smaller, distinctly undulate and having a sparse covering of shorter, straighter appressed hairs (Figure 2, Maslin 2012b). *Acacia* aff.

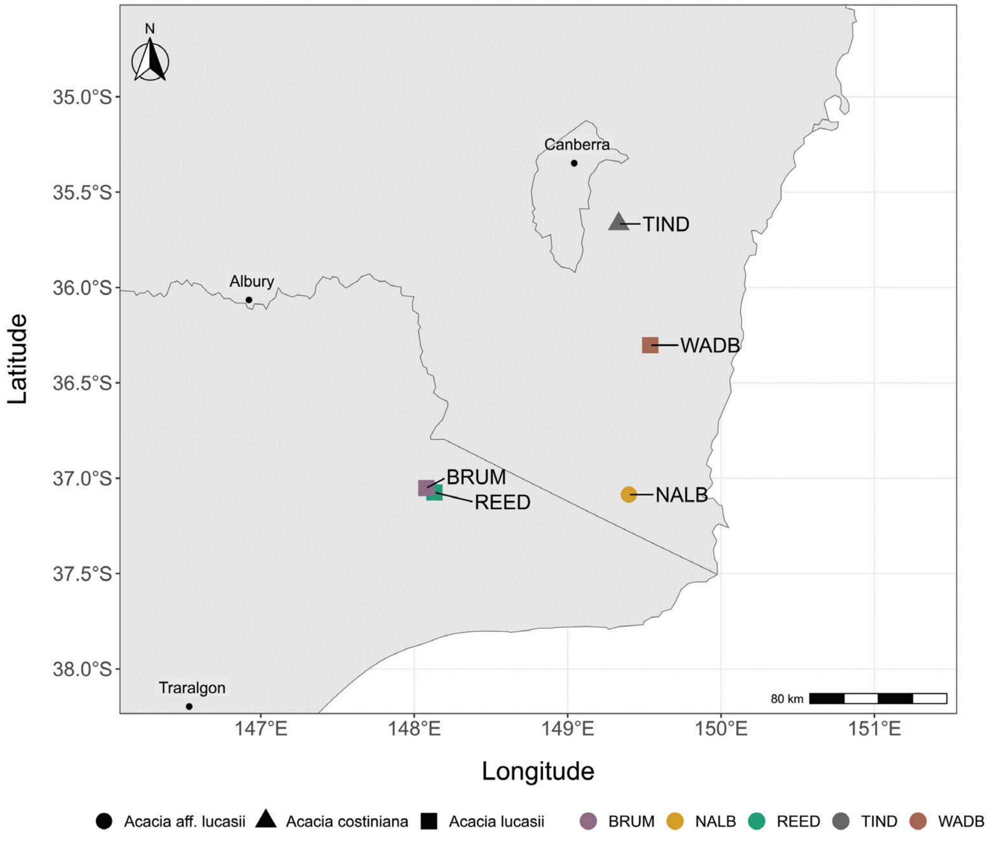

Figure 1. Map of *Acacia* species and populations sampled for this study.

Figure 2. Photograph of *Acacia lucasii* (image courtesy of authors).

lucasii is found at elevations of approximately 1000 m on the Nalbaugh Plateau in South East Forests NP in southern NSW and differs from *A. lucasii* in having pendulous or sub-pendulous branchlets, usually more undulate phyllodes clothed with non-crisped hairs, glabrous corollas and pods with sparser non-crisped hairs.

Sampling

Field surveys to locate populations of *Acacia lucasii* were made in December 2021, September 2022, and March 2023. Targeted locations were based on specimen records from the National Herbarium of Victoria and National Herbarium of NSW. All sampled populations were confirmed as burnt in the Black Summer megafire and standing plants were juveniles that appeared to have recruited post-fire. Samples of *A. lucasii* were collected from the two sub-populations on the Nunniong Plateau in Alpine NP, Brumby Point (BRUM) and Reedy Track (REED) which are located on separate ridge lines (Table 1, Figure 1). Both populations were approximately 100 individuals in size. Samples were also collected from a single sub-population of approximately 100 individuals covering 300 m on Wadbilliga Road in Wadbilliga NP (WADB; Table 1). Although the overall extent of *A. lucasii* in Wadbilliga NP is larger compared to the Nunniong Plateau populations, sampling was restricted to sub-population WADB because the additional 3–4 subpopulations were inaccessible due to bushfire, then flood damage at the time of sampling. This included sub-populations located along the northern end of Bumberry Creek Firetrail and at Conways Gap. For comparison, *A. costiniana* was sampled from adult plants in Tinderry Nature Reserve (TIND) which was unburnt in the Black Summer fires, and seedlings of the informal taxon *A.* aff. *lucasii* were sampled from the Nalbaugh Plateau in South East Forests NP (NALB) where all adults had been consumed by fire (Figure 1, Table 1). At each location, five leaves were collected from five individuals spaced at minimum intervals of 5–10 m, placed in labelled envelopes and dried on silica gel.

DNA extraction, amplification and sequencing

Approximately 15 mg from each of the 25 silica-dried leaf samples (plus 3 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 *Acacia* (Kilian *et al.* 2012; van der Merwe *et al.* 2021). DNA was extracted at DArT™ using the NucleoMag Plant 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

Table 1. Location and sampling details for the *Acacia* samples used in this study including taxon details.

Taxon	Site	Latitude	Longitude	Location
Acacia lucasii	BRUM	-37.0519	148.0789	Brumby Point Alpine NP, Victoria
	REED	-37.0747	148.1319	Reedy Track Alpine NP, Victoria
	WADB	-36.3023	149.5385	Wadbilliga Rd Wadbilliga NP, New South Wales
Acacia costiniana	TIND	-35.6667	149.3333	Tinderry NR, New South Wales
Acacia aff. *lucasii*	NALB	-37.0858	149.3992	Nalbaugh Plateau SE Forests NP, New South Wales

MseI and uniquely barcoded adaptors. Following PCR and quantification, 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

DArTSeq DNA sequence data contained 27 samples and 30,827 loci, with one failed sample (ALUC_2342 from BRUM). All analyses were conducted in R (R Core Team 2019) version 4.4.0 unless otherwise specified. Initial screening for clones was made by separating the dataset into broad taxonomic groups and using Euclidean genetic distance calculations using the R package dartR (Gruber *et al.* 2019). A threshold to select clonal samples was calculated as the average value between repeat pairs plus/minus three standard deviations of the repeat pair values. Any sample pairs above these values in the genetic distance measure were considered clonal samples. A kinship analysis using PLINK method of moment (MoM) for the Identity-By-Descent analysis in SNPRelate (snpgdsIBDMoM; Zheng *et al.* 2012) was used to verify monoclonal individuals. Two datasets were used for data analyses as outlined below.

Dataset 1 comprising all samples of *A. lucasii, A. costiniana* and A. *aff. lucasii,* was used to investigate genetic diversity and differentiation within and among the taxa. Data were filtered using the R packages dartR (Gruber *et al.* 2018), Poppr (Kamvar *et al.* 2014, 2015) and SNPRelate (Zheng *et al.* 2012) in R (R Core Team 2019). To enhance data quality yet retain SNPs across broader phylogenetic groups, the data were filtered for secondaries, read depth (5–150), a reproducibility threshold of 0.95, loci call rate of 0.65, individual call rate of 0.3, minor allele frequency (MAF) set at 8% and for Hamming distance to remove possible paralogues and monomorphic loci which were removed using the R package dartR (Gruber *et al.* 2019). One *A. costiniana* individual from TIND was removed in these steps to give the final Dataset 1 comprised of 2,722 SNPs across 23 individuals.

A phylogenetic network was constructed with the NeighborNet method using a Euclidian Distance matrix, which is not as sensitive to missing data as other options, in the R package (ver. 0.1.0, see http://rdrr.io/github/ IVS-UZH/RSplitsTree/) and visualised in R using ggplot2 (ver. 3.4.2; Wickham 2016) and tanggle (ver. 1.0.0, see http://rdrr.io/github/KlausVigo/tanggle/). Unlike bifurcating trees, phylogenetic networks can capture complex evolutionary histories (e.g., reticulation events) and thus may be more informative when assessing relationships among closely related species (Huson and Bryant 2006).

To visualise the major axes of variation between clusters of individuals, populations and species, principal component analysis (PCA) was undertaken, implemented in the adegenet package (Jombart 2008; Jombart & Ahmed 2011) in R (R Core Team 2019). Pairwise population differentiation (F_{ST}) and isolation by distance (IBD) was assessed using dartR (Gruber *et al.* 2018), analysis of molecular variance (AMOVA) was tested using Poppr (Kamvar *et al.* 2014, 2015) and geographic distance between populations was calculated using GenAlEx 6.503 (Peakall & Smouse 2006, 2012).

A Bayesian analysis implemented in STRUCTURE v. 2.3.4 (Pritchard *et al.* 2000) was used to identify K genetic clusters in the dataset without any information regarding taxon assignment or geographic proximity. The upper K limit was set to six on the assumption that there could be three potential species but five potential genetic groupings based on population disjunctions. Ten independent runs were undertaken for each K value with a burn-in of 100,000 and 200,000 MCMC iterations. The R package 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).

Dataset 2 was used to examine levels of genetic diversity in populations of *Acacia lucasii* only. It comprised samples from the three *A. lucasii* populations and contained 17,731 SNPs across 14 individuals prior to filtering. This second dataset was filtered as described above with the loci call rate set at 0.60, individual call rate of 0.35 and MAF set to 7% to produce the final *A. lucasii* Dataset 2 comprised of 1,205 SNPs across the 14 individuals. For both datasets, global heterozygosity statistics were calculated and expected and observed heterozygosity, private alleles and inbreeding coefficients were assessed using the adegenet (Jombart & Ahmed 2011), hierfstat (Goudet & Jombart 2020) and

Table 2. Pairwise geographic distance in km (top triangle) and pairwise F_{ST} values between each population sampled in this study (bottom triangle), calculated for Dataset 1 (23 individuals, 2,722 SNPs).

Taxon		***A. lucasii***			***A. costiniana***	***A.* aff. *lucasii***
	Site	BRUM	REED	WADB	TIND	NALB
A. lucasii	BRUM	-	6	177	192	147
	REED	0.151	-	173	189	141
	WADB	0.377	0.367	-	65	77
A. costiniana	TIND	0.444	0.461	0.411	-	136
A. aff. *lucasii*	NALB	0.585	0.546	0.427	0.567	-

Poppr (Kamvar *et al.* 2014, 2015) R packages. IBD and AMOVA were performed as detailed above.

Results

Species level genetic differentiation and structure

Dataset 1 had an overall nuclear genetic diversity of 0.039, and overall genetic differentiation (F_{STp}) value of 0.438. The Victorian populations of *A. lucasii* (BRUM, REED) are the most closely located populations, separated by only 6 km but separated from the NSW *A. lucasii* population WADB by 173 and 177 km respectively (Table 2). Pairwise population differentiation (F_{ST}) between the *A. lucasii* populations were 0.151 between BRUM and REED, 0.367 between REED and WADB and 0.377 between BRUM and WADB (Table 2). Pairwise F_{ST} values were higher between the *A. lucasii* and *A. costiniana* populations, ranging from 0.411-0.461. The *A.* aff. *lucasii* population NALB is the most geographically isolated population (77–147 km from any population) and had the highest pairwise F_{ST} values with other populations, ranging from 0.427 to 0.585 (Table 2).

The NeighborNet phylogenetic network of Dataset 1 displays four groupings of individuals, and the differentiation between sampling locations and species

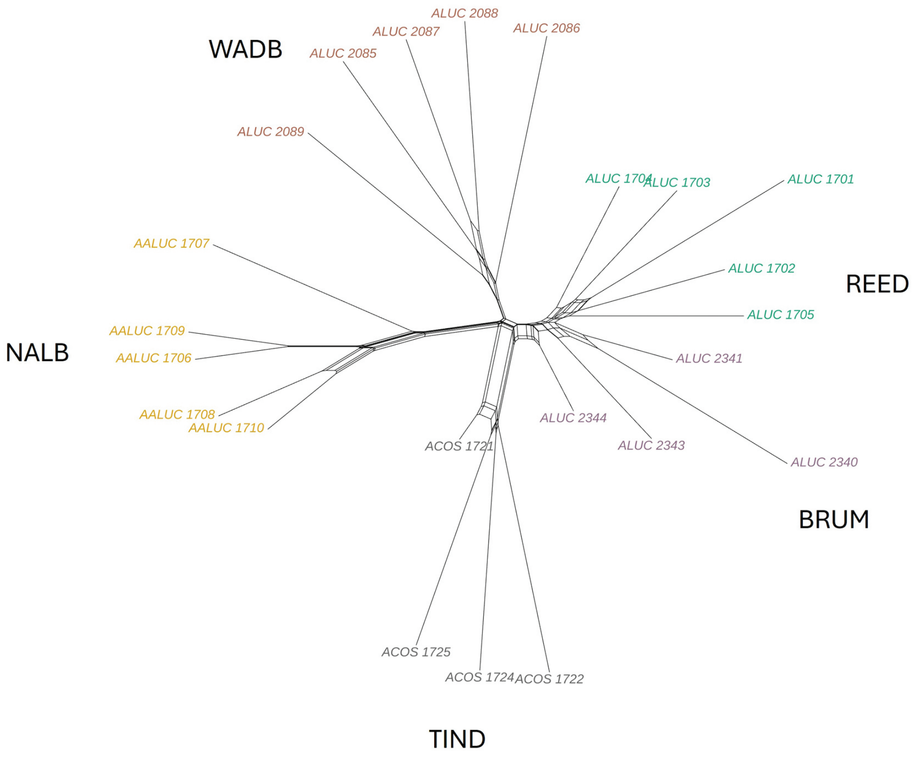

Figure 3. NeighborNet phylogenetic network based on a Euclidian Distance matrix, among the 23 samples of Dataset 1, with sample name coloured and labelled by location on outside of network. *Acacia lucasii* (BRUM, REED and WADB), *A. costiniana* (TIND), *A.* aff. *lucasii* (NALB).

Table 3. The results of AMOVA on for Dataset 1 of 23 individuals, 2,722 SNPs across the three taxa, and Dataset 2 of 14 individuals, 1,205 SNPs across the three *Acacia lucasii* populations. All results presented were statistically significant (p<0.001) at the 95% confidence level.

	Dataset 1			Dataset 2		
	Df	**Sum of Squares**	**Percentage of variation**	**Df**	**Sum of Squares**	**Percentage of variation**
Between populations	4	1686.7	41.4	2	439.4	29.5
Between samples within populations	18	1548.7	38.8	11	735.5	49.2
Within samples	23	401.4	19.8	14	167	21.4
Total	45	3636.8	100	27	1341.9	100

(Figure 3). *Acacia costiniana* and *A.* aff. *lucasii* had the longest splits indicative of greater genetic distance compared to the distance between populations of *A. lucasii*. The Victorian populations of *A. lucasii*, REED and BRUM, share a branch, with separation of the populations towards the tip. The NSW population of *A. lucasii*, WADB, clusters separately from the Victorian populations. The individuals from the *A. costiniana* population from TIND cluster together on a longer branch than the *A. lucasii* populations which could reflect the taxonomic divergence of this species. Finally, the *A.* aff. *lucasii* population also clusters on a long branch away from the other taxa, consistent both with this taxon's isolation and possible taxonomic divergence based on differences in morphology.

The principal component analysis of Dataset 1 (Figure 4) displays the taxonomic separation in the dataset with all samples of *A. lucasii* separated from *A.* aff. *lucasii* along the first axis and separation of *A. costiniana*, across the second axis. The first axis explains 15.47% of the variation in the dataset and despite the geographic distance between the three populations of *A. lucasii*, these cluster together in the bottom right side of the plot and are clearly separated from *A.* aff. *lucasii* (Figure 4a). The second axis explains 13.64% of the variation in the dataset which separates the population of *A. costiniana* from the three *A. lucasii* populations. The New South Wales population of *A. lucasii*, WADB is separated from the Victorian populations BRUM and REED across the third axis (11.48% of variation) reflecting the geographic distance between populations of this taxon (Figure 4b).

The Evanno ΔK method identified K = 5 clusters in the STRUCTURE analysis, however there was also a peak at K = 3 (see Supplementary Material 1, available online), thus both K = 3 and K = 5 charts are presented in Figure 5. At K = 3 the Victorian *A. lucasii* populations, REED and BRUM, belong to the same cluster while the NSW population of this taxon, WADB, shows admixture between the Victorian *A. lucasii* cluster and a second cluster (Figure 5b). The Nalbaugh Plateau population of *A.* aff. *lucasii* is primarily composed of the second cluster and shows admixture with a third cluster which is found primarily in *A. constiniana*. At K = 5, each cluster primarily represents one population (Figure 5a). Some admixture with the genetic cluster from the adjacent REED population is present in all individuals from the *A. lucasii* population BRUM. One individual from NALB has small amounts of admixture from the BRUM and the WADB clusters.

The AMOVA for Dataset 1 showed similar levels of genetic variation between the five populations (41.4%) and between the samples within the populations (38.8%) while only 19.8% was explained between the samples (Table 3, Dataset 1). There was a significant positive correlation between genetic distance and geographic distance ($r = 0.592$, $p = 0.042$).

Genetic diversity in *Acacia lucasii*

Nuclear genetic diversity for *A. lucasii* (Dataset 2) across all populations was found to be higher than the overall Dataset 1 at 0.054, with a corrected species genetic differentiation (F_{STp}) of 0.319. Genetic diversity values for the *A. lucasii* populations are shown in Table 4. Allelic richness (A_R) was highest in the NSW population WADB (1.30) and lowest in BRUM (1.24). The number of private alleles in *A. lucasii* populations ranged from a low of 166 (BRUM) to a high of 398 (WADB), suggesting that gene flow is restricted as detected in the previous genetic differentiation results. For all sampled populations, observed heterozygosity (H_O) was consistently lower than expected heterozygosity (H_E), resulting in

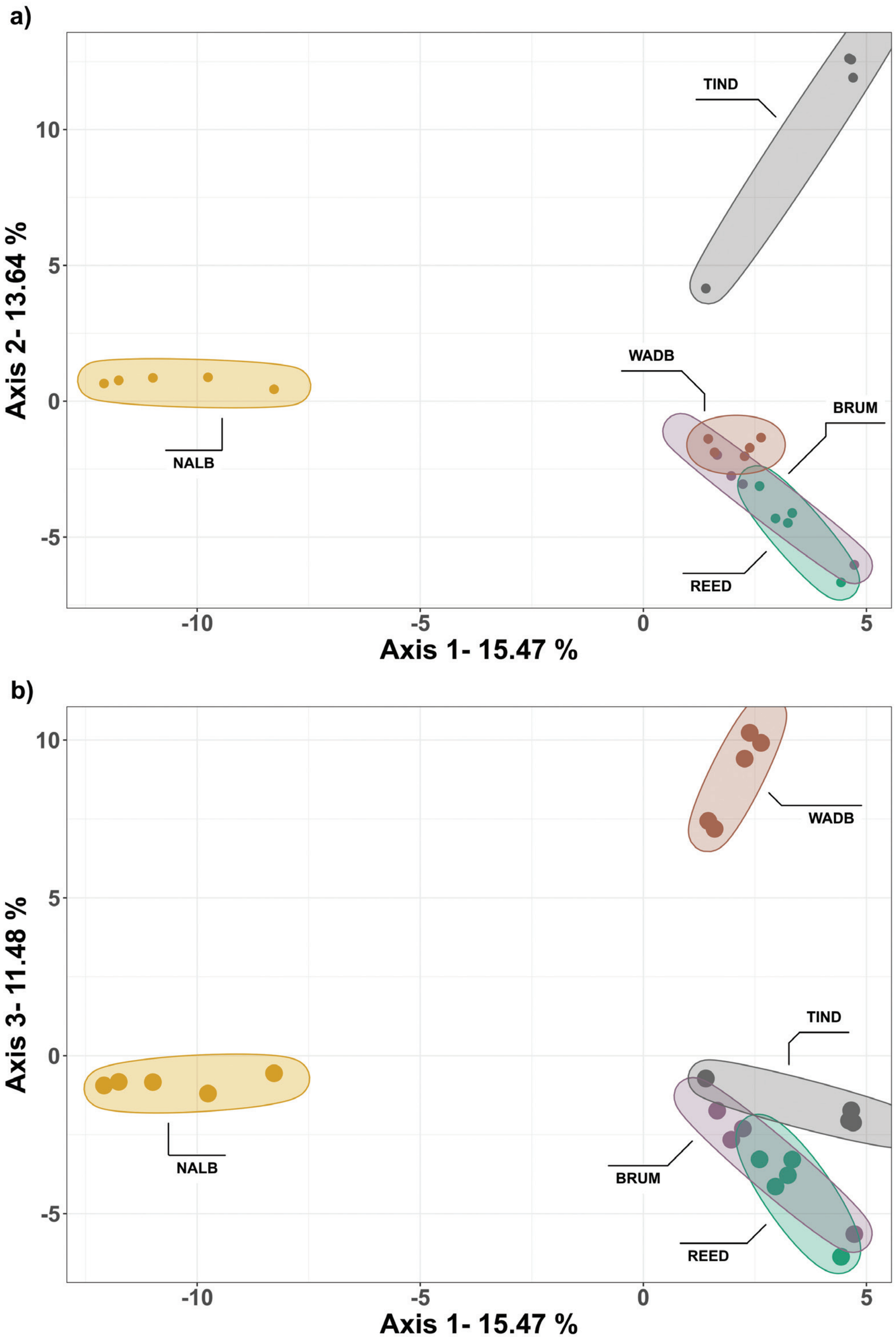

Figure 4. Plots of the principal component analysis of Dataset 1 (23 individuals, 2,722 SNPs), (a) the first and second axis, (b) the first and third axis. Individual samples are coloured by their population of origin with shapes representing taxa, *A. lucasii* = diamonds, *A. costiniana* = triangles, *A.* aff. *lucasii* = circles.

Table 4. Genetic diversity characteristics of the *Acacia lucasii* populations based on Dataset 2 with 1,205 SNPs and 14 individuals. Standard error is provided in brackets.

	No. individuals	No. Private alleles	Mean allelic richness (A_R)	Expected heterozygosity (H_E)	Observed heterozygosity (H_O)	Inbreeding coefficient (F_{IS})
BRUM	4	166	1.24 (0.01)	0.147 (0.005)	0.023 (0.002)	0.843
REED	5	233	1.29 (0.01)	0.179 (0.004)	0.036 (0.002)	0.798
WADB	5	398	1.30 (0.01)	0.192 (0.004)	0.104 (0.003)	0.461

inbreeding coefficients (F_{IS}) for *A. lucasii* that ranged from 0.461 (WADB) to 0.843 and 0.798 for the Victorian populations REED and BRUM, respectively (Table 4).

In contrast to Dataset 1, the AMOVA for Dataset 2 found highest levels of genetic variation between samples within the populations (49.2%), compared to that between populations (29.5%) and within all samples (21.4%, Table 3). A strong but not significant correlation between genetic distance and geographic distance (IBD) was detected ($r = 0.988$, $p = 0.166$).

Discussion

This study examined genomic variation in populations of the disjunct and threatened *Acacia lucasii* and to two regionally and closely related congeners, *A. costiniana* and *A.* aff. *lucasii*. The geographic ranges of the taxa do not overlap, and each occurs in highly isolated populations. Our results suggest that *A. lucasii* comprises two geographically disjunct genetic lineages that have a close affinity to two lineages represented by *A. costiniana* and *A.* aff. *lucasii*. The genetic evidence

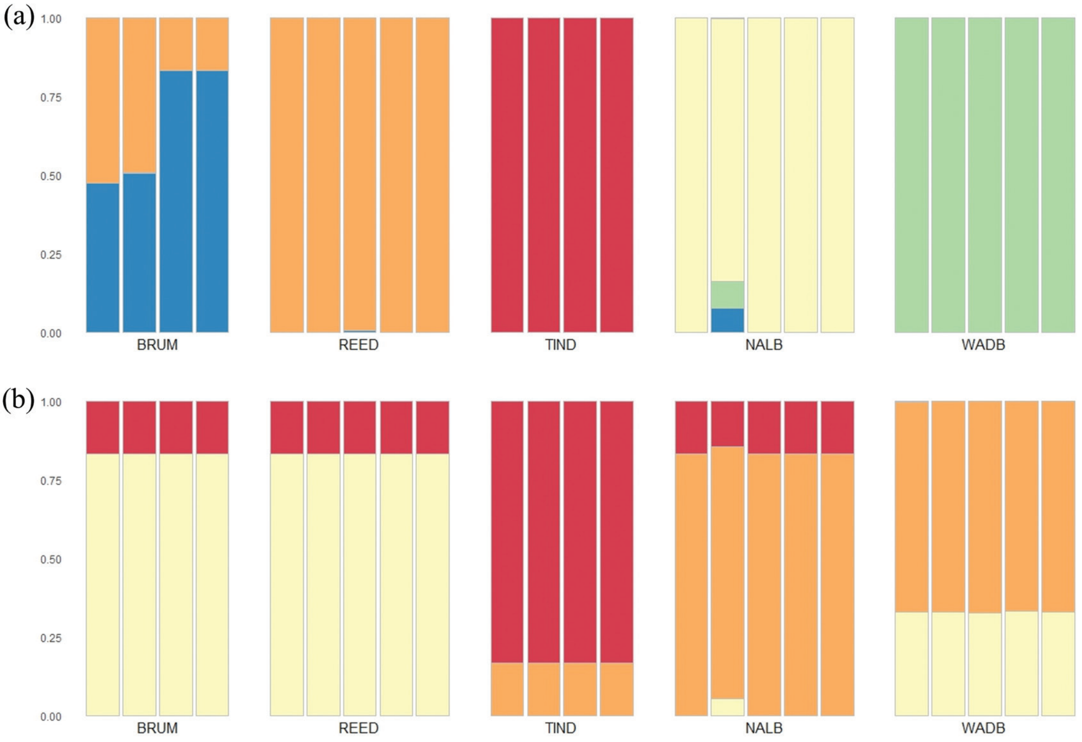

Figure 5. The results of the STRUCTURE analysis on Dataset 1 of 23 individuals and 2,722 SNPs with (a) the STUCTURE results displaying bar chart of individual Q-membership proportions to each of K = 5 clusters, with individuals clustered by population and populations labelled below (b) the STRUCTURE results displaying bar chart of individual Q-membership proportions to each of K = 3 clusters, with individuals clustered by population and populations labelled below.

provides support for delimitation of a minimum of three taxa (taxon 1 -TIND (*A. costiniana*); taxon 2 – BRUM, REED, WADB (*A. lucasii*); and taxon 3 – NALB (*A.* aff. *lucasii*)) or four taxa (taxon 1 -TIND (*A. costiniana*); taxon 2 -WADB (*A. lucasii*); taxon 3 -BRUM, REED (*A. lucasii*); and taxon 4 -NALB (*A.* aff. l*ucasii*)) subject to further examination of morphological differences. The inconsistency in conservation status across different jurisdictions for *A. lucasii* (Critically Endangered in Victoria but no conservation listing in NSW), and the likelihood of an unrecognised, distinct entity, *A.* aff. *lucasii*, means that conservation resources are not distributed evenly, despite the rarity of each genetic lineage. These results exemplify the argument that genomic analysis is essential for the taxonomic assessment of morphologically similar taxa prior to conservation listing (Cascini *et al.* 2025). The inclusion of an evolutionary perspective is a goal often cited in conservation management (Moritz & Potter 2013) but can be difficult to implement (Cook & Sgrò 2019). Deciding where to draw the line between divergence that reflects differentiation between disjunct intraspecies populations and the divergence that typifies interspecific differentiation is complex in cases such as this (Stankowski & Ravinet 2021), particularly when data sets are small and sampling may not have included the full known distribution of all taxa.

Effective conservation of rare species such as *A. lucasii* can be complicated by difficulties distinguishing the focal taxon from genetically similar lineages. The presence of cryptic genetic lineages may require management strategies that account for broader genetic relationships to ensure that conservation actions reflect underlying evolutionary patterns and preserve genetic diversity more comprehensively (Ahrens *et al.* 2017). We propose that results of this study tell a narrative that the extant populations of *A. lucasii*, *A. costiniana* and *A.* aff. *lucasii* are remnants of a previously more widespread precursor, whose range has expanded or contracted during historic climatic oscillations and complex geological events, resulting in contemporary geographic separation and divergence (Byrne 2008, Bryant & Krosch 2016). We found low levels of diversity (A_R, H_E, H_O) in each *A. lucasii* population, which fall within the range found in the critically endangered but more populous *A. purpureapetala* (van der Merwe *et al.* 2021) but are lower than those found in other studies of endangered *Acacia* species (Blyth *et al.* 2020, Rossetto *et al.* 2020). Low heterozygosity levels are considered detrimental for long term survival because adaptive capacity and fitness are likely to be compromised (Reed & Frankham 2003, Leimu *et al.* 2006). The results presented here therefore confirm a level of genetic risk for *A. lucasii*. The low heterozygosity at sites suggests biparental inbreeding in small, genetically depauperate populations leading to genetic bottlenecking, genetic drift and the fixation of alleles (Frankham *et al.* 2002). F_{ST} values indicate that gene flow is unlikely to have occurred in any recent timeframe except between the more geographically close Victorian populations of *A. lucasii* (REED and BRUM). Realistically, even the 6 km distance between these populations may be beyond ant- or bird- seed dispersal distance, at the limits of invertebrate or bird pollination distance, and long distance abiotic dispersal events could be rare (Whitney 2002, Stone *et al.* 2003, Gibson *et al.* 2011). Thus, gene flow between these populations may be associated with either undiscovered populations in inaccessible areas or represent a bygone time where the species' distribution may have been more contiguous in the landscape. High levels of genetic divergence (F_{ST}) can follow isolation, where small populations drift quickly, leading to fixed differences between populations. The apparent introgression of genetic clusters identified in the STRUCTURE analysis for K = 3 is also consistent with a scenario of retained sequence similarity from a more common precursor that underwent range contraction and fragmentation in concert with historical climate change in southeastern Australia. Fragmentation, both natural as landscape complexities have evolved, or human mediated, could have enhanced genetic drift now evident as the separate genetic lineages identified at K = 5.

Conservation implications

While the loss of populations and reduction in population sizes of *Acacia lucasii* or its ancestors are not necessarily recent or human-induced, it does not lessen the importance of minimising within-taxon loss of diversity, and concomitant adaptive capacity, or the risk of incremental species loss which may compromise the functional integrity of ecological communities

(Halley & Pimm 2023). The inconsistency in conservation status across different jurisdictions for *A. lucasii,* and the likelihood of an unrecognised but distinct entity, *A.* aff. *lucasii,* means that conservation resources are not distributed evenly despite the rarity of each genetic lineage. This supports the argument for genomic analysis to assist in the taxonomic assessment of morphologically similar taxa prior to conservation listing (Cascini *et al.* 2025). To reconcile the current taxonomy, sampling of the additional sub-populations of *A. lucasii* in Wadbilliga NP and the inclusion of an additional sub-population of *A. costiniana* from the northern Tinderry Ranges would be needed to confidently identify where populations lie on the speciation continuum. More intensive sampling across populations of all taxa would assess fine scale structuring, spatial autocorrelation and breeding patterns, which could be useful for seed collection protocols.

A precautionary approach may be to consider the conservation of each population as individual conservation units, or applying units to conserve the Nalbaugh entity *Acacia* aff. *lucasii* and the Victorian populations of *A. lucasii* separately from NSW populations of *A. lucasii*, as these represent genetically defined lineages (Mace 2004). This approach would be similar to that used for a group of rare, leafless *Bossiaea* species also from southeastern Australia where genomic analysis has enabled a revision of previous taxonomy based on morphology and led to changes in the approach to the conservation of the group as a whole (McMaster *et al.* 2024). Irrespective of taxonomic rank, the use of mixed germplasm sources for genetic rescue between conservation units is not recommended as it may be detrimental given the distinct lineages, geographic disjunctions and high differentiation. A manipulated exchange of germplasm by either broadcasting seed or planting seedlings from the opposite, closely situated Victorian populations BRUM and REED could boost genetic diversity and reduce the high degree of inbreeding within each of these populations and reduce the risk of extinction.

Although only *Acacia lucasii* and *A.* aff. *lucasii* were burnt by the Black Summer bushfires, all three study taxa are potentially threatened by inappropriate fire regimes and climate change because of their small geographic distribution and EOO, and inability to disperse or re-colonise from sources other than local. These obligate seeders rely on the establishment of a soil seed bank between fires and subsequent population re-establishment through germinating seed post-fire for their persistence in the landscape. Fire suppression such as management intervention to protect assets may inhibit regeneration due to competition from co-occurring species, whilst an increase in fire frequency and intensity may deplete the taxon's seed bank and thus its potential for recruitment post-fire (DELWP 2021). Early maturity is a reproductive trait of wattles (Gibson *et al.* 2011) and seedlings of *A. lucasii* were observed in flower at WADB within two years of the Black Summer fires, thus appearing to be responding positively. However, all sampled plants were juveniles that appeared to be new recruits post-fire from the soil seed bank. Even with several years of reproduction, the soil seedbank, at best, will only encompass the genetic diversity present in the standing stock. Assisted gene flow via population augmentation with non-local seed is feasible but potential genetic risks would need prior careful consideration. The occurrence in national parks affords some level of protection from external threats, however herbivory from animals, misapplication of fire intervals for prescribed burns, or repeated bushfires prior to the re-establishment of seedbanks remain as threats to the survival of each population in this study (Auld *et al.* 2022).

The small number of populations makes *ex situ* germplasm collections a feasible proposition to provide a rapid insurance population for *Acacia lucasii* and its close congeners. Given the apparent early reproductive maturity post-fire, *ex situ* seed banking is likely to be a cost-effective strategy to conserve genetic diversity and can be combined with population monitoring to understand post-fire population recovery. Multiple conservation seed collections from the NSW distribution of *A. lucasii* and of *A. costiniana* made prior to the Black Summer fires are held at the Australian Plantbank (Sydney) and National Seedbank (Canberra), and a small seed collection of *A. lucasii* from Brumby Point made in 2025 is held at the Victorian Conservation Seedbank (Melbourne). Additional sampling of seed from Reedy Track and repeated collections across multiple years for both populations of *A. lucasii* in Victoria would ensure that *ex situ* germplasm is as genetically representative as possible. Collections of *A.* aff. *lucasii* would complete the *ex situ* conservation holdings for each lineage of this group. The availability of *ex situ* seed collected and stored as separate maternal lines (Commander *et al.* 2018) allows for the possibility of translocations or population augmentation in the case of future catastrophic events that impact the viability of *in situ* populations.

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