

## High levels of clonality in *Pomaderris oblongifolia* and *Pomaderris buchanensis* (Rhamnaceae): implications for conservation

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### Introduction

Conservation of highly fragmented range-restricted flora species is challenging as they are susceptible to genetic decline in addition to having a higher risk of extinction compared to more widespread species with continuous distributions (Ellstrand and Elam 1993; Broadhurst *et al.* 2017; IUCN SPC 2024). Two of the main components of the Common Assessment Method (CAM) are species' range size and population size. CAM is used in Australia to evaluate a species' risk of extinction against the International Union for Conservation of Nature (IUCN) Red List Categories (TSSC 2021; IUCN SPC 2024). Geographic range size is a strong predictor of a species' extinction risk because the impact of local loss of individuals and habitat is minimised if a species range is large, and the chance of range-wide impacts is reduced (Hamrick and Godt 1989; Gaston & Fuller 2009; LeBreton *et al.* 2019). A lack of genetic connectivity between disjunct populations can increase population genetic differentiation, which may increase the chance of local adaptation and reduce the adaptive ability of populations (Aitken & Whitlock 2013; Broadhurst *et al.* 2017; Hopley & Byrne 2019; Pennington *et al.* 2021).

The second component, estimating population size based on a census of the number of mature (adult) individuals, is integral to the

### Abstract

*Pomaderris oblongifolia* N.G.Walsh and *P. buchanensis* N.G.Walsh are two rare, range-restricted species from Victoria's Snowy River region composed of small, fragmented subpopulations. To assist conservation management, genetic variation was quantified and identified high levels of clonality in both species, as well as in the related, sometimes sympatric, species *P. aspera* Sieber ex DC. Genetic data for *P. buchanensis* were consistent with triploidy, a common trait in the genus. The close relationship between sympatric *P. buchanensis* and *P. aspera*, revealed by kinship analysis suggests that *P. buchanensis* may be a triploid variant derived directly from *P. aspera*. Studies to determine the breeding system, apomixis and ploidy levels would be beneficial for guiding the assembly of genetically representative *ex situ* germplasm collections.

**Keywords:** population fragmentation, rare species, SNPs

CAM, as breeding population size determines the ongoing regeneration of a species, the maintenance of genetic diversity across generations and the effects of demographic stochasticity (Lande 1993; Frankham 2005; TSSC 2021; IUCN SPC 2024). However, the use of population census data generated by direct observation for conservation assessments and activities can be problematic without considering the effects that reproductive biology may have on the genetic diversity of a species. Plants commonly violate the theoretical expectations of genetic diversity through mixed mating systems (selfing, outcrossing), asexual reproduction and polyploidy (Kireta *et al.* 2019; Rice *et al.* 2019).

Polyploidy can bestow potential survival advantages through heterosis, greater adaptability via a higher number of alleles per locus and the production of asexual seed by apomixis. However, they also need to persist through genomic instability and not be sterile triploids or higher oddploids (reviewed in Comai 2005). Ancient polyploidy, followed by subsequent re-diploidisation are responsible for the radiation and diversification of angiosperm flora (Comai 2005; Rice *et al.* 2019; Nge *et al.* 2024). Polyploid plants may have traits that provide a higher competitive advantage compared to diploid species (Chan *et al.* 2022). Asexual reproduction, such as root-suckering, is implicated in the persistence of marginal, rare and/or endangered species and as a mechanism to cope with sexual reproductive failure but does not promote dispersal (Vallejo-Marín *et al.*, 2010; Bradbury *et al.*, 2015; Millar and Byrne 2020). Apomixis may compensate for sexual failure, enabling dispersal via established seed dispersal pathways and is often associated with polyploids and implicated in the evolutionary success of species in complex environments (Chen *et al.* 2019; Delnevo *et al.* 2023). Even though asexual reproduction and polyploidy may enable rapid population growth, offer reproductive insurance and better colonising abilities, both mechanisms influence fecundity and genetic recombination, which can lead to populations comprising only a small number of genetically unique individuals (Warburton *et al.* 2000; Hojsgaard and Hörandl, 2019; Delnevo *et al.* 2023; Simmons *et al.* 2023). Thus, if asexual reproduction is present, deriving effective population size by direct observation alone may overestimate the number of genetically unique individuals present in a population

(Warburton *et al.* 2000; Simmons *et al.* 2023) and may underestimate the levels of genetic diversity, inbreeding and declining evolutionary potential in a species (Fisher 1930; Wright 1931; Young *et al.* 2002; James and McDougall 2014). Conservation *in situ* and *ex situ* therefore relies upon knowledge of levels of clonality, true breeding population size and the relatedness between individuals (Hoban 2019; Hoban and Strand 2015).

*Ex situ* conservation collections such as long-term storage of seeds play a strong role in the conservation of a given species and its genetic diversity (IUCN/SSC 2014). The function of many *ex situ* collections is to act as a genetically diverse insurance collection that can be used as a source of germplasm for population restoration (Martyn Yenson *et al.* 2021). In the absence of genetic information, generalised advice proposes that germplasm collections are made from at least 50 individuals across sites (or maximum number of individuals if less than 50; Martyn Yenson *et al.* 2021), as minimum viable population size is often estimated at 50 individuals (Jamieson and Allendorf 2012). Ideally, the diversity in *ex situ* collections should be comparable to that *in situ* as it provides the basis for a species' restoration. Uneven population sampling, low genetic diversity in cultivation, and ongoing cultivation can affect the genetic integrity of *ex situ* germplasm (Diaz-Martin *et al.* 2023) and lead to reduced fitness in cultivation (Forgiarini *et al.* 2023). While asexual reproduction via rhizomes or root-suckering may be visible, clonality via apomixis can be cryptic in the absence of genetic data as plants produce viable seed but the collection of seed from multiple plants does not ensure genetically variable collections as seeds maintain the parental genotype (Koltunow and Grossniklaus 2003; Martyn Yenson *et al.* 2021). Species-specific outcomes will be improved if germplasm collections can be designed around a taxon's landscape genetic patterns and fitness (Chen *et al.* 2019; Garner *et al.* 2020; Willi *et al.* 2021).

*Pomaderris oblongifolia* N.G. Walsh and *P. buchanensis* N.G. Walsh are closely related, highly restricted shrubs occurring in fragmented locations along the Snowy River in East Gippsland, Victoria. Both species were identified as high priority for urgent management intervention following the extensive fires in eastern Australia in 2019/2020 (Gallagher 2020). *Ex situ* conservation,

germplasm collection and genetic augmentation has been used in an effort to halt or reverse the decline of several threatened *Pomaderris* spp. (Roberts & Trueman 2016; Chen *et al.* 2019; DELWP 2022). However, seed has been difficult to obtain for many species of *Pomaderris* (McAuliffe *et al.* 2016; Roberts & Trueman 2016) including several triploid species (Chen *et al.* 2019). Polyploidy and/or asexual reproduction via apomixis have been recorded in a substantial number of *Pomaderris* taxa and have been linked with species richness in the genus (Harvey & Rattenbury 1985; Chen *et al.* 2019; Nge *et al.* 2024). In line with flow cytometry measurements by Chen *et al.* (2019), recent genomic work has found *P. oblongifolia* to be diploid ( $2n = 24$ ) (Nge *et al.* 2024). *Pomaderris buchanensis*, which was not assessed by flow cytometry, is suggested to be triploid with this genomic data (Nge *et al.* 2024). Apomixis has been reported for both diploid and higher ploidy levels in *Pomaderris* species and evidence exists for both diploid and tetraploids in the same *Pomaderris* species (Harvey & Rattenbury 1985; Chen *et al.* 2019). While seed production has not been observed in natural populations of *P. oblongifolia* nor *P. buchanensis*, given the isolation of populations, it is unlikely to be observed without targeted trips. Thus, mating systems, seed viability and natural seed banks are unknown for both taxa.

This research provides an assessment of genetic diversity in *P. oblongifolia* and *P. buchanensis* using a proprietary reduced representation sequencing method (DArTseq; Diversity Arrays Technology, Canberra, Australia) to identify localised genetic structure and connectivity among locations. Clonality within locations and relationships between taxa are examined using genomic data to improve conservation assessment and guide *ex situ* germplasm collections of both taxa. Future areas of research are identified to guide conservation of these species in the face of increasing environmental stress.

## Methods

### Study species

*Pomaderris oblongifolia* and *P. buchanensis* are shrubs endemic to the Snowy River in Victoria, southeastern Australia, and are listed as Endangered (EN) and Critically Endangered (CR), respectively, under the *Flora and*

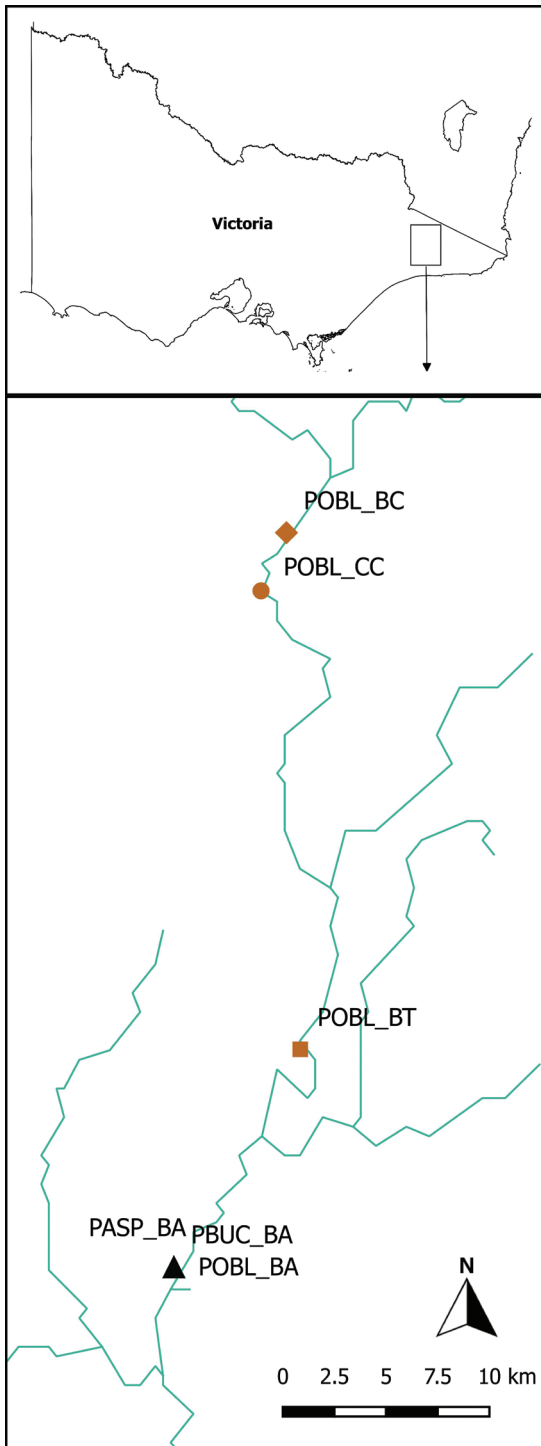
*Fauna Guarantee Act 1988* (Vic). The listings are due to small numbers of plants occurring in disjunct locations within the fire-prone habitat across a narrow length of the Snowy River.

*Pomaderris oblongifolia* is endemic to the gorge tract of the Snowy River in East Gippsland, Victoria, from McKillops Bridge, the Little River Gorge, and other locations downstream to the confluence of the Snowy and Buchan Rivers. *Pomaderris buchanensis* has a narrower distribution, being known historically from only four small, disjunct sites from just north of the Buchan River confluence to Long Point on the Snowy River. *Pomaderris buchanensis* was once considered a hybrid of *P. oblongifolia* and *P. aspera* and is distinguished from *P. oblongifolia* by leaf and floral characters (Walsh 2008). Both *P. oblongifolia* and *P. buchanensis* often co-occur with *P. aspera*, a widespread, common diploid species (Chen *et al.* 2019; Nge *et al.* 2024), which differs from the species of interest in being a tree with much larger leaves and floral features (Walsh 2022). Pollinators, seed dispersal and fire response are not specifically known for either *P. oblongifolia* or *P. buchanensis*, however, in other *Pomaderris* species, insects are implied as primary pollinators, and positive post-fire germination responses and limited unaided seed dispersal have been found (Patykowski *et al.* 2014; 2016). Most *Pomaderris* species are obligate seeders (Whelan 1995, Le Breton *et al.* 2019) but in some locations of low intensity fire, *P. oblongifolia*, has been observed to resprout basally (DELWP 2022).

The Victorian Conservation Seedbank (VCS) does not hold any seed collections for either target taxon. Plants are cultivated at Royal Botanic Gardens Victoria (RBGV), however, the small amount of seed collected from these cultivated plants is of unknown viability and has the potential to include hybrids due to many *Pomaderris* species being cultivated in close proximity and therefore not representative of the genetic variation of the species.

### Sampling

Surveys for *Pomaderris oblongifolia* and *P. buchanensis* targeted previously recorded locations in the Snowy River National Park based on National Herbarium of Victoria herbarium records, field trips and locations observed along the Snowy River by RBGV staff in January and October 2021. The landscape surveyed



**Figure 1.** Location of sampled populations of *Pomaderris* species in this study. *P. oblongifolia* (POBL), *P. buchanensis* (PBUC), *P. aspera* (PASP). Locations occur along the Snowy River in East Gippsland in the southeast corner of mainland Australia.

included steep, rocky banks and slopes, or rocky areas with crevices where plants were emerging post-fire. Survival and regeneration were confirmed for *P. oblongifolia* at four locations while *P. buchanensis* was found at two locations (MEL collections NGW 9170/PBUC\_BA and NGW 9178) where it co-occurred with *P. aspera*. Samples of *P. buchanensis* were collected at only one of these locations (Figure 1, Table 1). The entire field survey area had burnt at varying levels of intensity in the Black Summer bushfires (2019–2020), with some small patches of unburnt remnant vegetation. Most plants sampled were ‘sub-adult’ non-reproductive one year old plants. Twenty-three samples of *P. oblongifolia* were collected from the four locations along the Snowy River (Figure 1) and encompass the geographic range of the species: near the confluence with Boundary Creek (POBL\_BC, 5 samples, unburnt adults), near the confluence with Currie Creek (POBL\_CC, 5 samples, fire severity not recorded), at New Guinea Bend / Betts Creek (POBL\_BT, 5 samples, moderate burn, basal resprout) and near Basin Creek Falls (POBL\_BA, 8 samples, unburnt adults). Twenty-one samples of *P. buchanensis* were collected from a site near Basin Creek Falls (NGW 9170; PBUC\_BA) which was located on the opposite riverbank from POBL\_BA. The site was unburnt and consisted of c. 200 flowering plants approximately 150 cm high (DELWP 2022). Four samples of *P. aspera* co-occurring with *P. buchanensis* were collected at Snowy River Basin Creek (PASP\_BA). Sampling was spread across the distribution of the target taxon at each location, with samples collected approximately 5 m apart.

**DNA extraction, amplification and sequencing**

Approximately 15 mg from each of the 48 leaf samples plus one technical replicate were sent to a commercial genotyping service, Diversity Arrays Technology (‘DArT’, Canberra, Australia) for DNA extraction and DArTseq™ analysis which is a reduced representation sequencing method (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 per the manufacturer’s 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

**Table 1. Sites sampled for *Pomaderris* along the Snowy River in Victoria. *P. oblongifolia* (POBL), *P. buchanensis* (PBUC), *P. aspera* (PASP). Number of samples collected at each location with number of unique genotypes detected in parentheses. Accession number in the National Herbarium of Victoria (MEL) collection.**

Site Code	No. of samples (unique genotypes)	Location	MEL Accession no.
POBL_BA	8 (1)	Snowy River, 200 m from gauging station, upstream of Basin Creek (BA)	MEL 2494342A
PASP_BA	4 (1)	Snowy River, 200 m from gauging station, upstream of Basin Creek (BA)	MEL 2494339A
PBUC_BA	21 (1)	Snowy River, 200 m from gauging station, upstream of Basin Creek (BA)	MEL 2496564A
POBL_CC	5 (1)	Junction of Currie Creek and Snowy River (CC)	MEL 2494325A
POBL_BT	5 (1)	South of Betts Creek Track on New Guinea Bend (BT)	MEL 2496543A
POBL_BC	5 (3)	North of the junction with Boundary Creek (BC)	MEL 2496517A

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

All analyses were undertaken using packages implemented in R ver. 4.2.1 (R Core Team 2022) and RStudio ver. 2023.12.1+402.pro1 (R Studio Team 2023). Raw unfiltered DNA SNP data derived from DArTSeq™ analysis comprised 49 samples across the three taxa and 27686 loci including one technical replicate. Data for *P. oblongifolia*, *P. buchanensis* and *P. aspera* were analysed together to enable comparison between the taxa. Data were stringently filtered to a locus rate of 90% and individual call rate of 75%, a reproducibility score of 1, a minor allele frequency (MAF) of 8%, a Hardy-Weinberg equilibrium with a 5% level of significance, secondaries and monomorphs removed and finally filtered on Hamming distance to remove potential paralogues using the package dartR (Gruber *et al.* 2018). This resulted in a filtered dataset of 3939 SNPs across 49 samples.

To identify clonal genotypes, a pairwise genetic distance (Euclidian) was calculated between all samples using the R package dartR (Gruber *et al.* 2018). A 'sequencing error' was calculated by taking the pairwise genetic distance between the identical samples (technical replicate, value = 30.46). Pairwise samples with less than twice the average sequencing error (distance value  $\leq 60.92$ ) were considered ramets

of a clonal genotype. The mean distance between clonal samples (ramets) of 36.98 ( $\pm 5.15$  S.D.) was clearly distinct from the mean distance among all other stems ( $134.86 \pm 34.59$  S.D.), giving high confidence in clone identification.

The technical repeat was then removed, and data refiltered as described above, with MAF set to 5%. To verify the relationship of clonal stems, pairwise kinships were estimated on the filtered dataset of 3948 loci across 48 samples using PLINK method of moment (MoM) for the Identity-By-Descent (IBD) Analysis in SNPRelate (snpgrdsIBDMoM; Zheng *et al.* 2012). While a conservative method (McMaster *et al.* 2024), it is ideal for non-model species. Kinship values of 0.50 represent a monozygotic twin, with a kinship threshold of  $\phi=0.354$  used to define clones. Kinship values of  $\phi=0.25$  and higher indicate a first-degree relationship (full siblings, parent-child etc.) and  $\phi=0.125-0.25$  indicate a second-degree relationship (i.e., half-siblings, grandparent-grandchild etc). Unrelated pairs are expected to have values close to  $\phi=0$  (Manichaikul *et al.* 2010). Kinship values were presented separately for *P. oblongifolia* as the analysis found no relationship between this taxon and *P. buchanensis* and *P. aspera*.

Finally, the dataset was refined to include only one sample per clonal group and was comprised of 16136 SNPs across 8 samples. Data were then filtered as above with MAF set to 12.5%. Principal component analysis (PCA) was undertaken to identify genetic clusters and visualise the major axes of variation between clusters using adegenet (Jombart 2008; Jombart & Ahmed 2011). Individual observed heterozygosity and inbreeding coefficients were assessed using hierfstat (Goudet & Jombart 2020) and dartR (Gruber *et al.* 2018).

Isolation by distance and fixed differences between locations were assessed using dartR (Gruber *et al.* 2018). The R package ggplot2 (Wickham 2016) was used to visualise results.

## Results

Clonality was evident in all sampled locations of *Pomaderris oblongifolia*, *P. buchanensis* and *P. aspera* (Tables 2 & 3, Supplementary Table 1 & 2 available at [www.rbg.vic.gov.au/science/journal/](http://www.rbg.vic.gov.au/science/journal/)). Kinship analysis demonstrated that three locations of *P. oblongifolia* POBL\_BT, POBL\_CC and POBL\_BA are monoclonal with a unique genotype at each site (Tables 1 & 2). At the fourth population, POBL\_BC, three genotypes were identified with one recovered from three of the five samples collected. The location with both *P. aspera* and *P. buchanensis* had two genotypes, broadly corresponding to the morphological taxon identification at the time of sampling (Table 1). Importantly, *P. buchanensis* is closely related to *P. aspera* but not to *P. oblongifolia* (Tables 3 & 5; Supplementary Table 1 & 2).

For *P. oblongifolia*, the genotype at POBL\_BT and sample POBL\_0192 from Boundary Creek have a kinship value of 0.20 (Table 2) which identifies them as second-degree relatives such as half-sibs and close to the value of 0.25 which defines first degree relationships such as parent-offspring and full-sibs. Similarly, the genotype at POBL\_CC has a third-degree relationship with all three Boundary Creek POBL\_BC genotypes (Table 2). This result and the increasing genetic distance with POBL\_BC is consistent with POBL\_BC at the north of the species' distribution being a potential 'source' of populations founded further down the catchment and an indicator of historical connectivity (Figure 1).

At Basin Creek, the kinship values for *P. aspera* indicate that the samples collected are monoclonal and that one individual, PBUC\_0290, collected as *P. buchanensis*, is in fact a clonal genotype of the *P. aspera* samples collected from this location (Table 3). The remaining 20 samples of *P. buchanensis* were also identified as being monoclonal. It should be noted that the average kinship values are lower between individuals PBUC\_0301 and PBUC\_0302 (bold) and all other samples, and while lower than the other values, they are still above the clone threshold. A single kinship pair value of  $\phi=0.33$  between PBUC\_0289 and PBUC\_0301 was found suggesting that these

individuals have a first-degree relationship, such as parent-offspring or full sibling rather than being clonal, however this value is marginal. Of note, is the first-degree relationship between the *P. aspera* and *P. buchanensis* genotypes, indicating a parent-offspring or full sibling relationship between the taxa. The exceptions are the kinship values for samples PBUC\_0301 ( $\phi=0.09-0.10$ ) and PBUC\_0302 ( $\phi=0.13-0.14$ ) which suggest a second-degree relationship (i.e. half-siblings, grandparent-grandchild etc).

The final dataset, representing one sample per clonal group, contained eight samples and 3049 loci after filtering across four *P. oblongifolia* locations and the *P. buchanensis*-*P. aspera* location. Overall, nuclear genetic diversity was 0.232, with observed heterozygosity levels ranging from 0.182 to 0.255 (Table 4). Inbreeding ( $F_{is}$ ) values ranged 0.346 for PASP\_BA to 0.533 for PBUC\_BA (Table 4).

Pairwise comparisons of fixed differences ranged from 410–426 for *P. oblongifolia* individuals within the same population (POBL\_BC; POBL\_0190, POBL\_0191, POBL\_192; Table 5). Fixed differences between some *P. oblongifolia* populations were lower. For example, the fixed differences between POBL\_CC and POBL\_BC ranged from 319–398 and POBL\_0192 from Boundary Creek (BC) and POBL\_0090 from Betts Track (BT) had even lower fixed differences, reflecting the close relationship identified in the kinship analysis (Tables 2 & 5). Fixed differences were highest between PASP\_BA, PBUC\_BA and all *P. oblongifolia* ranging from 709–1013 and were lowest (2) between PASP\_BA and PBUC\_BA (Table 5).

The principal components analysis showed high levels of differentiation among samples, with all *P. oblongifolia* samples and the *P. buchanensis*-*P. aspera* samples separated across the first axis which accounts for 43.32% of the variation (Figure 2). The *P. aspera* sample PASP\_0280 and *P. buchanensis* sample PBUC\_0294 are closely clustered in the negative space of axis 1. The *P. oblongifolia* samples are spread across the second axis (14.32% of the variation) with clustering of individuals that are more closely related (Figure 2). The proximate placement of POBL\_BC individual (POBL\_0192) with the genotype from POBL\_BT (POBL\_0090) and POBL\_BC individual (POBL\_0191), with the POBL\_CC (POBL\_0050) genotype again reflects the close relationship between these sites (Figure 2, Table 2).

Table 2. Pairwise kinship values for *Pomaderris oblongifolia* samples within and among populations. Darker shading indicates more closely related individuals. Underline indicates samples used to represent the unique individuals in genetic diversity statistics.

Pop	Sample	BA							CC							BC							BT					
		POBL	POBL	POBL	POBL	POBL	POBL	POBL	POBL	POBL	POBL	POBL	POBL	POBL	POBL	POBL	POBL	POBL	POBL	POBL	POBL	POBL	POBL	POBL	POBL	POBL	POBL	POBL
	0271																											
	0278	0.44																										
	0277	0.44	0.44																									
BA	0276	0.43	0.43	0.44																								
POBL	0275	0.42	0.43	0.42	0.42																							
	0274	0.43	0.43	0.43	0.42	0.43																						
	0273	0.44	0.43	0.44	0.43	0.42																						
	0272	0.41	0.41	0.40	0.41	0.40	0.41																					
	0046	0	0	0	0	0	0																					
CC	0047	0	0	0	0	0	0	0.45																				
POBL	0048	0	0	0	0	0	0	0.45	0.45																			
	0049	0	0	0	0	0	0	0.45	0.45	0.44																		
	0050	0	0	0	0	0	0	0.44	0.44	0.44	0.44																	
	0190	0	0	0	0	0	0	0.02	0.02	0.02	0.02	0.02																
BC	0191	0	0	0	0	0	0	0.07	0.07	0.07	0.07	0.03																
POBL	0192	0	0	0	0	0	0	0	0	0	0	0.00	0.03															
	0188	0	0	0	0	0	0	0.02	0.03	0.02	0.02	0.45	0.03	0.01														
	0189	0	0	0	0	0	0	0.02	0.02	0.02	0.02	0.44	0.03	0.00	0.44													
	0088	0	0	0	0	0	0	0	0	0	0	0	0.0	0.20	0	0												
BT	0089	0	0	0	0	0	0	0	0	0	0	0	0	0.21	0	0	0.44											
POBL	0090	0	0	0	0	0	0	0	0	0	0	0	0	0.19	0	0	0.42	0.42										
	0091	0	0	0	0	0	0	0	0	0	0	0	0	0.20	0	0	0.44	0.44	0.42									
	0092	0	0	0	0	0	0	0	0	0	0	0	0	0.20	0	0	0.44	0.43	0.41	0.44								

**Table 3. Pairwise kinship values for *Pomaderris buchanensis* and *P. aspera* samples from the Basin Creek population. Darker shading indicates more closely related individuals. Underline indicates samples used to represent the unique individuals in genetic diversity statistics.**

	PASP										PBUC														
	0279	0282	0281	0280	0303	0295	0287	0302	0294	0286	0301	0293	0285	0300	0292	0284	0299	0291	0283	0298	0290	0297	0289	0296	0288
<b>PASP</b>	0.49	0.50	0.50	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
		0.49	0.50	0.50	0.19	0.19	0.22	0.23	0.23	0.23	0.48	0.48	0.48	0.47	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48
			0.49	0.50	0.19	0.20	0.22	0.23	0.23	0.23	0.47	0.48	0.48	0.47	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48
				0.49	0.19	0.20	0.22	0.23	0.23	0.23	0.47	0.48	0.48	0.47	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48
					0.19	0.20	0.22	0.23	0.23	0.23	0.47	0.48	0.48	0.47	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48
					0.19	0.20	0.22	0.23	0.23	0.23	0.47	0.48	0.48	0.47	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48
					0.22	0.23	0.22	0.23	0.23	0.23	0.47	0.48	0.48	0.47	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48
					0.13	0.14	0.14	0.14	0.14	0.40	0.41	0.43	0.43	0.43	0.43	0.43	0.43	0.43	0.43	0.43	0.43	0.43	0.43	0.43	0.43
					0.21	0.23	0.22	0.22	0.22	0.48	0.48	0.50	0.43	0.43	0.43	0.43	0.43	0.43	0.43	0.43	0.43	0.43	0.43	0.43	0.43
					0.22	0.23	0.23	0.23	0.23	0.47	0.48	0.50	0.43	0.43	0.43	0.43	0.43	0.43	0.43	0.43	0.43	0.43	0.43	0.43	0.43
					0.09	0.10	0.10	0.10	0.10	0.38	0.39	0.41	0.36	0.41	0.41	0.41	0.41	0.41	0.41	0.41	0.41	0.41	0.41	0.41	0.41
					0.22	0.23	0.22	0.23	0.23	0.47	0.48	0.50	0.43	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
					0.22	0.23	0.22	0.23	0.23	0.47	0.48	0.50	0.43	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
					0.21	0.22	0.22	0.22	0.22	0.47	0.48	0.50	0.43	0.49	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
					0.20	0.21	0.21	0.21	0.21	0.46	0.47	0.48	0.42	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48
					0.20	0.21	0.21	0.22	0.23	0.46	0.47	0.48	0.42	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48
					0.22	0.23	0.22	0.23	0.23	0.47	0.48	0.50	0.43	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
					0.22	0.23	0.23	0.23	0.23	0.47	0.48	0.50	0.43	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
					0.22	0.23	0.23	0.23	0.23	0.47	0.48	0.50	0.43	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
					0.22	0.23	0.23	0.23	0.23	0.47	0.48	0.50	0.43	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
					0.21	0.22	0.22	0.22	0.22	0.47	0.48	0.49	0.43	0.49	0.49	0.49	0.49	0.49	0.49	0.49	0.49	0.49	0.49	0.49	0.49
					0.49	0.50	0.50	0.50	0.50	0.20	0.20	0.23	0.14	0.23	0.23	0.23	0.21	0.23	0.23	0.23	0.22	0.22	0.22	0.22	
					0.21	0.22	0.22	0.22	0.22	0.45	0.47	0.48	0.42	0.48	0.48	0.47	0.48	0.48	0.48	0.48	0.47	0.47	0.47	0.47	
					0.27	0.27	0.27	0.27	0.27	0.39	0.40	0.42	0.35	0.41	0.42	0.42	0.40	0.41	0.42	0.42	0.41	0.41	0.41	0.41	
					0.22	0.23	0.23	0.23	0.23	0.46	0.47	0.49	0.42	0.48	0.48	0.47	0.47	0.49	0.49	0.49	0.48	0.48	0.47	0.47	
					0.22	0.23	0.23	0.23	0.23	0.47	0.48	0.50	0.43	0.50	0.50	0.50	0.49	0.50	0.50	0.50	0.49	0.48	0.48	0.49	

**Table 4. Genetic diversity characteristics of *Pomaderris oblongifolia*, *P. buchanensis* and *P. aspera* individuals.**

Taxon	Population	Individual	Observed heterozygosity ( $H_o$ )	Inbreeding coefficient ( $F_{is}$ )
PASP	BA	0280	0.244	0.376
PBUC	BA	0294	0.182	0.533
POBL	BA	0271	0.217	0.445
		0050	0.229	0.414
	BT	0090	0.243	0.377
	BC	0190	0.242	0.379
		0191	0.240	0.385
		0192	0.255	0.346

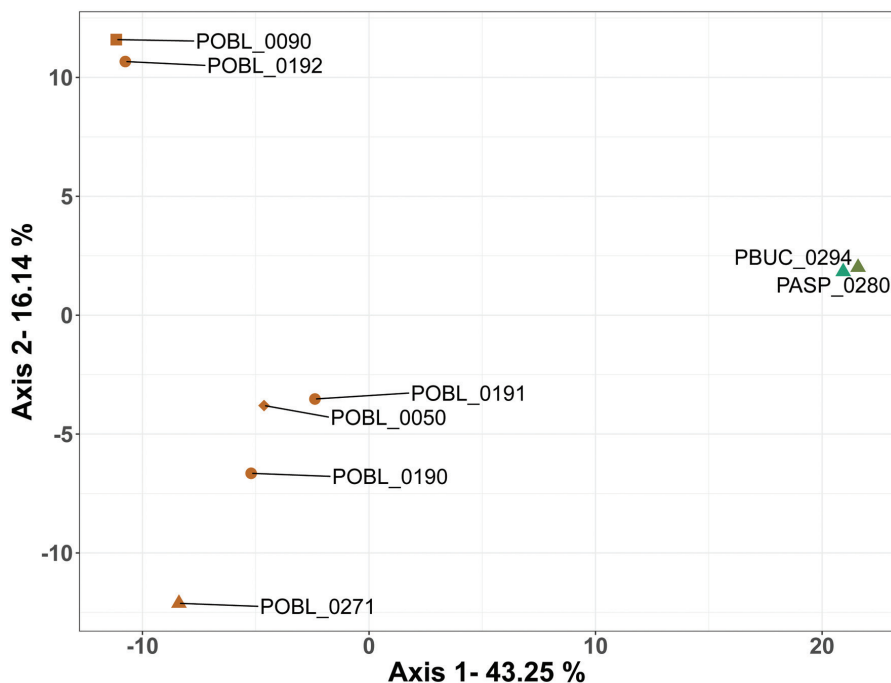
**Table 5. Matrix of fixed differences among individuals of *Pomaderris oblongifolia*, *P. buchanensis* and *P. aspera*.**

		BA	BA	CC	BT	BC			BA
		PASP_0280	PBUC_0294	POBL_0050	POBL_0090	POBL_0190	POBL_0191	POBL_0192	POBL_0271
BA	PASP_0280	0							
BA	PBUC_0294	2	0						
CC	POBL_0050	709	751	0					
BT	POBL_0090	897	961	440	0				
BC	POBL_0190	731	777	376	461	0			
	POBL_0191	604	648	319	462	410	0		
	POBL_0192	844	912	398	109	426	419	0	
BA	POBL_0271	944	1013	553	541	518	547	501	0

### Discussion

The two major findings of this study were that clonality was evident in all sampled locations of *Pomaderris oblongifolia*, *P. buchanensis* and *P. aspera*, and that *P. buchanensis* has a close relationship with *P. aspera* but not with *P. oblongifolia*. The use of SNPs generated by DArTseq allowed an evaluation of genomic diversity within *P. oblongifolia* and *P. buchanensis*. The apparent recovery of *P. oblongifolia* and *P. buchanensis* following the widespread bushfires in 2019/2020 is evidenced

in the recruitment of seedlings at affected locations. The discovery of extensive clonality in both species stresses the importance of understanding the levels and patterns of genetic diversity across a species range for *in situ* and *ex situ* conservation. These results raise uncertainty about the level of sexual reproduction and highlight the knowledge gaps in the breeding system.



**Figure 2.** Principal component analysis of genetic differentiation in *Pomaderris oblongifolia* (squares, POBL), *P. buchanensis* (triangle, PBUC) and *P. aspera* (circles, PASP) on the Snowy River.

## Population biology of the rare Snowy River *Pomaderris*

The identification of six *Pomaderris oblongifolia* genotypes from 23 samples and the occurrence of three monoclonal sites significantly reduces the number of individuals for conservation assessment purposes (TSSC 2021; IUCN SPC 2022). The presence of three genotypes within the *P. oblongifolia* Boundary Creek location (POBL\_BC) suggests that some level of sexual reproduction has occurred in the past. Plants at this site were recorded as unburnt mature plants, occurring at a lower density and over a much wider area (100 individuals across 5000 m<sup>2</sup>) compared to other locations (50–100 individuals across 2500 m<sup>2</sup>) which may indicate different disturbance histories. The high levels of clonality observed in *P. oblongifolia* reduce the number of genetically unique individuals to considerably less (20–60%) than the population census counts.

Even more critical is the result for *P. buchanensis*, with only one genetically unique individual recovered from 21 samples; if the same genetic patterns exist in the other known locations, this could mean only two to four unique genotypes of *P. buchanensis* exist for the species. The close relationship between samples collected as *P. buchanensis* and *P. aspera* at Basin Creek also raises questions around the delimitation of these species. The similarity between *P. buchanensis* and *P. aspera* has important conservation implications. *Pomaderris aspera* is widespread across the east coast of Australia and has a close affinity to both *P. oblongifolia* and *P. buchanensis* with the three species thought to have speciated less than 5 Mya (Nge *et al.* 2021). *Pomaderris aspera* is known to co-occur with *P. oblongifolia* and *P. buchanensis* at a few locations along the Snowy River (Walsh 2008). The kinship analyses in this study identified monoclonality in both the *P. buchanensis* and *P. aspera* samples from the Basin Creek. A first-degree relationship (parent-offspring or full sibling) was also identified between the *P. buchanensis* and *P. aspera* samples but the *P. buchanensis* samples did not have the same level of genetic similarity to *P. oblongifolia* (Supplementary Table 1). Based on this data, there is no evidence that *P. buchanensis* is a contemporary hybrid between *P. aspera* and *P. oblongifolia* but does not discount both species being implicated in the evolution of *P. buchanensis* in more ancient time scales, given their close phylogenetic relatedness.

## Life history characteristics that may cause low genetic diversity

Diversification in *Pomaderris* is thought to consist largely of *in situ* speciation within individual biomes, potentially driven by high levels of polyploidy, apomixis, an ability to resprout and sexual production of seed, all of which allow for rapid colonisation of new habitat (Nge *et al.* 2021; 2024). No seed set has been observed in *P. buchanensis* either in the wild or in cultivation which adds to the recent evidence that the species is a triploid (Nge *et al.* 2024), a characteristic found in other rare *Pomaderris* (*P. pallida*, *P. reperta*; Chen *et al.* 2019). While polyploidy may provide advantageous traits for survival, triploids may be partially or fully sterile due to chromosome mismatching (Comai 2005; Chen *et al.* 2019), an obstacle that can be overcome to some extent by asexual reproduction. The misidentification of one *P. aspera* individual as *P. buchanensis* (PBUC\_0290) highlights their morphological similarity, supports their close phylogenetic relatedness (Nge *et al.* 2021; 2024) and offers an insight into the evolutionary history of *P. buchanensis*. Previous studies have indicated that *P. oblongifolia* and *P. aspera* are diploid, but intraspecific ploidy variation has been found in other species of *Pomaderris* (Chen *et al.* 2019). Triploids are often found in mixed-ploidy populations (Husband 2004; Wallace *et al.* 2017). A limitation of this study was the absence of samples from an additional *P. buchanensis* location and broader sampling of *P. aspera* from the Snowy River. Further surveys, more thorough sampling, and the inclusion of samples of all three taxa (*P. buchanensis*, *P. aspera* and *P. oblongifolia*) from the Snowy River in future genetic studies, coupled with flow cytometry across multiple individuals at multiple sites, are needed to characterise ploidy levels. Better sampling and other sequencing approaches would also allow for genomic estimation of ploidy using nQuire as described in Nge *et al.* (2024) or ploidyNGS (Augusto Corrêa Dos Santos *et al.* 2017). In combination, these techniques could elucidate the evolutionary relationship between the taxa and whether *P. buchanensis* is a triploid variant derived directly from *P. aspera*.

While patterns of low genetic diversity and population structure could result from a dramatic loss of individuals in a species with low genetic variation, asexual reproduction is a more likely explanation given

the life history characteristics previously recorded for *Pomaderris*. Basal resprouting has been observed in *P. oblongifolia*, however vegetative reproduction via root-suckering has not been observed in either that taxon or *P. buchanensis*. Apomixis is a possible explanation for the clonality and low genetic diversity identified in our data that reportedly occurs in diploid *Pomaderris* species in southeastern Australia (Chen *et al.* 2019) as well as many polyploid species (Bicknell and Koltunow 2004; Hojsgaard & Hörandl 2019; reviewed in Hörandl *et al.* 2024). Given the level of clonality and the apparent recruitment from seed after fire, an understanding of the realised mating system in *P. oblongifolia* and *P. buchanensis* is needed to inform appropriate conservation actions. If apomixis is the most likely explanation, its presence needs to be confirmed with embryological studies and the evaluation of endosperm:embryo ploidy levels if seed can be obtained. If present, apomixis must be quantified across the distribution of both taxa to ensure that the likelihood of collecting apomictic seed from wild populations is understood. If seed is collected for *ex situ* conservation purposes prior to reproductive biology studies, it is particularly important to follow best practice by storing seed lots as separate maternal lines (Commander *et al.* 2018) as separate plants may be clonemates producing genetically identical seeds. If possible, germinants from any reproductive biology studies should be included in future genetic studies.

### Conservation of both taxa

*In situ* seedbanks play an important role in conserving the genetic diversity of a species in fire prone environments and sexually-produced seed enhances the genotypic diversity at a location. The length of time to flowering has not been recorded post-fire for either *P. oblongifolia* or *P. buchanensis* but is needed for planning fire regimes with time intervals long enough for several years of seed to accumulate in the seedbank or to assess the potential seedbank following wildfires. Re-assessment of the genetic structure of both narrow-ranged endemic *Pomaderris* species in this study over time is advisable to follow the genetic demographics and guide germplasm sampling to effectively maintain the genetic representation of wild populations in *ex situ* collections. It is prudent to propagate individuals

vegetatively from formally identified, wild collected and genotyped individuals which would enable replication in *ex situ* experiments to characterise the breeding system. Given the small percentage of plants analysed at each site, additional, dense sampling is advisable to confirm levels of clonality. The discovery of additional new genotypes will be of conservation value, particularly if *ex situ* collections are to be used for seed production.

Evaluation of the extinction risk of *P. oblongifolia* and *P. buchanensis* against IUCN criteria, based on the combination of the census of mature genetic individuals through population sub-sampling and geographic range size, shows that both taxa warrant listing under the CR threat category. To ensure that the conservation management applied to *P. oblongifolia* and *P. buchanensis* is appropriate, it is vital to determine their breeding systems. Episodic sexual recruitment is known to occur in plants capable of asexual reproduction but can be difficult to confirm directly (Eriksson 1989) as apomictic seedlings in natural populations are likely to be indistinguishable from sexually produced seed in the absence of genetic testing. Breeding system studies would also be important for resolving the similarity between *P. buchanensis* and *P. aspera*. Equally, if *P. buchanensis* is a variant derived directly from *P. aspera*, it may be a candidate for removal from the FFG/EPBC listing and managed with *P. aspera* as a taxon of least concern.

### Acknowledgements

This work was funded by the Victorian Government Bushfire Biodiversity Response and Recovery Program. Samples were collected under DELWP permit 10008918 by Royal Botanic Gardens Victoria employees Neville Walsh, Andre Messina and Todd Mclay, with assistance from Parks Victoria. David Cantrill, Elizabeth James and Todd Mclay conceived the study and obtained funding.

### Supplementary information

1. Filtered matrix of pairwise genetic distance, on the filtered dataset of 3939 loci across 49 samples, including technical replicate (italicised).
2. Pairwise kinship values ( $\phi$ ) on the filtered dataset of 3948 loci across 48 samples using the KING method of moments for identity-by-descent (IBD).

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