A range-wide analysis of population structure and genomic variation within the critically endangered spiny daisy (Acanthocladium dockeri)

Elodie Brooks1 · Amy L Slender1 · Suong Cu1 · Martin F Breed1 · James CR Stangoulis1

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Abstract
Understanding population structure and genetic diversity is important for designing effective conservation strategies. As a critically endangered shrub, the six remaining extant populations of spiny daisy (Acanthocladium dockeri) are restricted to country roadsides in the mid-north of South Australia, where the species faces many ongoing abiotic and biotic threats to survival. Currently the spiny daisy is managed by selecting individuals from the extant populations and translocating them to establish insurance populations. However, there is little information available on the genetic differentiation between populations and diversity within source populations, which are essential components of planning translocations. To help fill this knowledge gap, we analysed population structure within and among all six of its known wild populations using 7,742 SNPs generated by a genotyping-by-sequencing approach. Results indicated that each population was strongly differentiated, had low levels of genetic diversity, and there was no evidence of inter-population gene flow. Individuals within each population were generally closely related, however, the Melrose population consisted entirely of clones. Our results suggest genetic rescue should be applied to wild spiny daisy populations to increase genetic diversity that will subsequently lead to greater intra-population fitness and adaptability. As a starting point, we suggest focussing on improving seed viability via inter-population crosses such as through hand pollination experiments to experimentally assess their sexual compatibility with the hope of increasing spiny daisy sexual reproduction and long-term reproductive fitness.

Keywords Acanthocladium dockeri · Endangered · Fragmentation · Genotyping by sequencing · Spiny Daisy

Introduction
Small, fragmented plant populations often have reduced genetic diversity, which risks elevating inbreeding and genetic drift within populations (Templeton et al. 1990; van Treuren et al. 1991; Heywood 1993; Furlan et al. 2012; Neaves et al. 2015). Inbreeding and genetic drift are particularly concerning for small populations because they can increase the prevalence of disease (O’Brien and Evermann 1988; Hajjar et al. 2008). Genetic erosion is the reduction in effective population size within a population over time; where infrequently occurring alleles are likely to be lost, further hindering population survival by reducing adaptive potential (van Treuren et al. 1991). In addition, populations that are at risk of habitat removal are likely to experience population genetic bottlenecks (Buza et al. 2000). Maintaining or even increasing gene flow between fragmented populations is therefore of high importance as it should help support long-term population fitness and increase evolutionary resilience within a species (Moritz 1999). Mapping genetic diversity within a species of conservation concern provides insights into its genetic risk, which is increasingly being used to guide conservation interventions (i.e. for translocations) (Williams et al. 2014; Rossetto et al. 2021). Such knowledge enables managers to prioritize sites for in situ conservation and facilitates identification of source material for the establishment of ex situ activities such as translocations (Young et al. 2002).

Acanthocladium dockeri (spiny daisy) is listed as critically endangered under the Commonwealth of Australia’s Environment Protection and Biodiversity Conservation Act 1999 and within the IUCN criteria (Sharp et al. 2010; Clarke et al. 2013; Bickerton et al. 2018). It is the only species within the monotypic genus Acanthocladium, meaning it is
an important representative of Australian botanical diversity (Jusaitis and Adams 2005; Clarke et al. 2013; Bickerton et al. 2018). Only six small populations are known to occur within the mid-north of South Australia and are separated by 4-110 km distances. These populations have been named in previous studies as Hart, Telowie, Thornlea, Melrose, Yangya and Rusty Cab (Fig. 1) (Jusaitis and Adams 2005; Clarke et al. 2013; Bickerton et al. 2018). These populations are susceptible to anthropogenic interference as they occur along narrow roadsides that are impacted by agricultural practices. They are exposed to chemical fertilisers and pesticides, herbivory and compete with weeds during the winter months (Clarke et al. 2013). Seed production in the wild is infrequent making recruitment via sexual reproduction rare (Jusaitis 2008). No seedlings have ever been observed in the field despite the presence of insect pollinators on spiny daisy flowers (Jusaitis and Adams 2005). The roughly 50 cm diameter shrubs produce suckers within their woody perennial root system, and this has helped these populations to endure (Clarke et al. 2013). Previous work has shown spiny daisies have low pollen viability and their flowers have a deformed pollen tube possibly contributing to prolonged vegetative reproduction that will eventually cause sterility (Jusaitis and Adams 2005; Jusaitis 2008).

Previous work has assessed the genetic diversity amongst four populations known at the time (Thornlea, Yangya, Hart and Rusty Cab) using allozymic markers (Jusaitis and Adams 2005). The authors found that each population represented a single, distinct genotype suggesting all individuals within each population were clones and there was no inter-population gene flow (Jusaitis and Adams 2005). Efforts to conserve the spiny daisy included regular site monitoring and maintenance (i.e. pest control and weeding), in addition to the establishment of at least one translocation site for each population (Sharp et al. 2010; Clarke et al. 2013). Subsequent conservation activities further led to the discovery of another two populations (Telowie and Melrose) (Clarke et al. 2013). Although informative, the analysis of allozyme markers only provides a limited representation of the allelic variation in a genome, in comparison to the use of more modern genome-wide techniques (Gaudeul et al. 2004; Narum et al. 2013). It is therefore necessary that population studies use high-resolution genomic methodologies to better inform management and conservation goals.

Next-generation sequencing (NGS) applications have more frequently been used for non-model organisms, improving conservation management strategies (Seeb et al. 2011; Narum et al. 2013). Genotyping by sequencing (GBS) is a popular method that uses NGS platforms to reduce genome complexity and characterise genomic variation across thousands of genome-wide single nucleotide polymorphisms (SNPs). GBS provides a better insight into population structure compared to more traditional methods such as allozymes or microsatellites as there is greater genomic resolution (Beissinger et al. 2013; Narum et al. 2013).

The aim of our study was to determine levels of genetic diversity within and among six extant populations of the spiny daisy using a genome-wide SNP dataset derived by GBS. We hope to use this information to help conservation management of this species. Based on previous findings, we predicted that each population would be genetically differentiated and contain low levels of genetic diversity. We addressed the following questions: (i) How strong is the genetic differentiation among populations? (ii) How variable is intra-populations genetic diversity? (iii) Is evidence of gene flow present among the extant populations?

Materials and methods

Study sites and sampling

All spiny daisy populations occur along roadsides within close proximity to country townships in the mid-north of South Australia (Fig. 1) (Jusaitis and Adams 2005; Clarke et al. 2013). Some of these sites are surrounded by several species of native vegetation identified in the Spiny Daisy Recovery Guide (Clarke et al. 2013). Thornlea, Yangya and Rusty Cab sites are situated East of Laura and are the closest geographically (4 km) to each other. The Hart site occurs within the Clare Valley, approximately 65 km south of the township Laura, between a main sealed road and an old railway reserve. The sites are located in semi-arid grasslands, adjacent to and sometimes partially on private property. The
Telowie site is located approximately 20 km north of Port Pirie and its closest population, Thornlea is 30 km to the south-east of Telowie. Melrose represents the most northernly site, occurring 31 km north-east of Telowie. Plant density of these populations have been monitored over time and was recorded in 2007 by the Department of Environment, Water and Natural Resources (DEWNR) (Appendix Table S1) (Clarke et al. 2013).

Young leaf tissue samples were collected from a total of 90 individuals (15 plants per population) among the six sites. Five leaves from each plant harvested were taken along a 45 m transect at each site. Along the transect, the plants were randomly selected at a three-metre interval and within three metres from the transect. This means that sampled individuals were never less than 3 m apart. In instances where plants were absent at a designated interval, the next closest individual along the transect was sampled and the altered distance was recorded. This occurred at the Hart site where the distance between the last individual sampled and its previous sample was 10 m. Transects were also altered if the distribution of the plant populations was not linear up to 45 m. This was the case with the Telowie population, in which the transect had to be separated into two directions. As most sites contained a small number of individuals, a sample size of 15 individuals was thought to provide an adequate representation of the genetic material within the population.

Young leaves were harvested from the tip of a branch, down to the fourth node as DNA quality is known to improve for younger tissue (Moreira and Oliveira 2011). Leaves were selected from one to three separate branches of the plant. The five leaves from each plant were individually stored and temporarily preserved in liquid nitrogen. Samples were then freeze-dried using the Beta 2–8 LSCplus ice condenser for a 2-day period to allow for the extraction of moisture within the leaf.

**DNA extraction and genotyping**

DNA was extracted from two to three leaves (6–7 mg) from each individual by Diversity Arrays Technology Pty Ltd (DArT) according to their inhouse protocol (Jaccoud et al. 2001; Kilian et al. 2012). Library preparation involved the restriction enzymes PstI and MseI for complexity reduction which targeted low-copy sequences (Wenzl et al. 2004; Melville et al. 2017; van Deventer et al. 2020). Sequencing was conducted using DArTseq™; a genome complexity reduction sequencing methodology, similar to RADseq (Restriction site Associated DNA sequencing) (Sansaloni et al. 2011; Kilian et al. 2012; Rodger et al. 2021). The procedure was performed using an Illumina short-read platform producing 300,000 reads of 75 base pairs in length which were then filtered and shortened based on sequence quality. The closest relative with a complete genome sequence is the sunflower (*Helianthus annuus*) with a genome size of 3.6 gigabases (Kane et al. 2011; Badouin et al. 2017). Assuming the spiny daisy genome has a similar size to that of the sunflower, we expected the genome coverage with this methodology to be approximately 0.65% of the spiny daisy genome. SNP calling and initial marker filtering based on sequencing read depth and accuracy were performed using standard procedures developed by DArT (Kilian et al. 2012).

**Marker quality control**

Filtering of less informative SNPs and SNPs introducing bias was conducted in R v 4.0.2 (2020.06.22) (RStudio Team. 2020) using the DARTR package (Gruber et al. 2018). The sequencing procedure was replicated so that each locus was scored based on genotype repeatability. We applied a reproducibility filter to stringently remove loci which contained inadequate repeatability scores (<99%). Failure to call SNPs due to reduced DNA quality causes missing genotypes which can severely bias some analyses. Loci containing >1% missing data were removed. Secondary SNPs occur when multiple SNPs occur within the same sequencing read. These cause linkage disequilibrium, another source of bias for some analysis and were therefore removed. To improve data quality and computational efficiency, a minor allele frequency threshold of 5% was applied (The International HapMap 2005; Würschum et al. 2013).

**Population genetic structure and differentiation**

Genetic structure within the final dataset was examined in R v 4.0.2 using various approaches. Firstly, a Discriminant Analysis of Principle Components (DAPC) within the R package Adegenet was computed to identify genetic clusters and examine the validity of individual assignment within groups. This analysis employs a k-means clustering algorithm which infers the optimal number of k based on the selection of clusters to maximise between-group variation. The most optimal model was defined upon the value which corresponded to the lowest BIC (Bayesian information criterion). A Principal Coordinates Analysis (PCoA) was then used to visualise genomic variation (Jombart 2008). Lastly, to investigate the extent of gene flow between different geographical locations, individual admixture coefficients were produced in the R package LEA, a Bayesian clustering program similar to that of STRUCTURE (Frichot et al. 2015). Shared ancestry among individuals were examined using models of K ranging between 1 and 10 with each
Population structure between all sampling localities was supported by several genetic analyses. The DAPC showed that the most likely model of populations structure was $K = 6$, based on the lowest Bayesian information criterion (Appendix Fig. S1). The PCoA showed a similar result to the DAPC (Fig. 2). The PCoA demonstrated that individuals from each population clustered together, but there was variation between the level of differentiation among populations (Fig. 2). Axis 1 (21.1% of variation) separated the two furthest populations, Melrose (furthest north) and Hart (furthest south), from all the other populations while axis 2 (19.2% of variation) separated Hart and Rusty Cab from all other populations (Fig. 2). Thornlea, Yangya and Telowie were the most genetically similar and Yangya showed the most variation between its individuals.

The proportion of shared ancestry between the six populations produced by the Bayesian clustering program LEA was very small (Fig. 3). Alternative models of $K$ also displayed little admixture between populations (Appendix Fig. S2).

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All populations contained similar levels of high differentiation according to pairwise $F_{ST}$ estimates (Table 1). The mean pairwise $F_{ST}$ estimates between populations was $0.53 \pm 0.03$ SD (Table 1). Melrose produced slightly higher pairwise $F_{ST}$ estimates with all other populations (mean = $0.57 \pm 0.007$ SD).

AMOVA results displayed significantly greater variation within individuals (63%) than among populations (37%). No variation was detected among individuals (0%) ($p = 0.01$; Table 2). The proportion of fixed differences between the populations was similar across population pairs (mean = $5.1 \pm 0.6\%$) (Fig. 4). Pairwise comparisons involving Melrose had the highest proportion of fixed differences.
The summary statistics of pairwise relatedness outcomes indicated high relatedness within each population (mean \(r = 0.89 \pm 0.03\) SD) (Table 3). Melrose contained the highest relatedness (population mean \(r = 0.96\)), and Rusty Cab demonstrated the lowest relatedness (population mean \(r = 0.85\)) between individuals (Table 3). Although all \(F_{IS}\) results produced a similar range of values (mean = -0.82 ± 0.04 SD) (Table 3) these values were not significant (\(p = 1\)). Four of the populations contained only two clones of the 15 individuals sampled. Telowie had slightly more clones (5), but Melrose consisted entirely of clones (Table 3).

**Genetic diversity and isolation-by-distance**

Observed heterozygosity (mean = 0.26 ± 0.03 SD) appeared to be considerably higher than expected heterozygosity (mean = 0.13 ± 0.02 SD) and this pattern was found across all the populations (Fig. 5). Thornlea contained the highest value of expected and observed heterozygosity (0.15; 0.28) and Melrose contained the lowest value of expected heterozygosity and observed heterozygosity (0.09; 0.19). There was no significant trend in isolation by distance despite there being a positive correlation between geographic and genetic distance between pairs of populations (\(r = 0.48\), \(p = 0.16\)).

**Table 1** Pairwise \(F_{ST}\) estimates across the six sampling localities based on the 7,742 SNPs analysed.

|       | Melrose | Yangya | Hart    | Telowie | Thornlea |
|-------|---------|--------|---------|---------|-----------|
| Yangya| 0.574   |        |         |         |           |
| Hart  | 0.561   | 0.527  |         |         |           |
| Telowie| 0.576   | 0.520  | 0.528   |         |           |
| Thornlea| 0.558   | 0.498  | 0.514   | 0.500   |           |
| Rusty Cab| 0.568   | 0.508  | 0.515   | 0.512   | 0.500     |

**Table 2** Summary AMOVA table displaying distribution of genetic variation within and between populations (\(p = 0.01\)).

| Source             | df | SS            | MS            | Estimated Variance | Variance explained (%) |
|--------------------|----|---------------|---------------|--------------------|------------------------|
| Among populations  | 5  | 88683.178     | 17736.636     | 589.423            | 37%                    |
| Among individuals  | 84 | 4530.933      | 53.940        | 0.000              | 0%                     |
| Within individuals | 90 | 89204.000     | 991.156       | 991.156            | 63%                    |
| Total              | 179| 182418.111    | 1580.579      | 1580.579           | 100%                   |

(mean = 5.8 ± 0.2%) (Fig. 4). The lowest proportion of fixed differences was between Thornlea and Yangya (4.18%).

**Relatedness within populations**

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Discussion

This study confirms that strong genetic structure is present among all currently known extant populations of spiny daisy with little to no evidence of inter-population gene flow. This indicates that each site represents a unique subset of genetic variation of this species. The inclusion of Telowie and Melrose populations has increased the amount of known genetic diversity that exists within the species by approximately 33%, however the lack in connectivity among these isolated populations reinforces the extinction risk faced by this species (Ellstrand and Elam 1993; Young et al. 1996; Spielman et al. 2004; Frankham and Wicken 2006). Although relatedness within each population was high, only Melrose represented a single genet. All other populations contained a small number of clones. Our findings therefore differ to that of previous genetic studies that described each population to consist entirely of clones (Jusaitis and Adams 2005). Levels of observed heterozygosity were greater than expected heterozygosity, likely as a result of high levels of clonal reproduction and/or mechanisms preventing sexual reproduction. Our study helps improve the knowledge of the distribution of limited genetic diversity within this critically endangered species and demonstrates that the spiny daisy has likely experienced strong genetic bottlenecks (Rodger et al. 2021), probably due to large-scale habitat disturbance (Jusaitis and Adams 2005; Clarke et al. 2013; Brown and Hodgkin 2015). The varying levels of genetic diversity detected among populations and subtle differences in population structure provide a deeper insight towards prioritization strategies for conservation management (Gardiner et al. 2017).

The presence of sporophytic self-incompatibility (SI) is present in many Asteraceae species and serves as an evolutionary mechanism to promote outcrossing within populations and restrict the build-up of deleterious alleles which is often associated with inbreeding in small, isolated populations (Zagorski et al. 1983; Les et al. 1991; Ferrer and Good-Avila 2007). Given the absence of excess homozygosity and sexual reproduction, high relatedness within the spiny daisy populations may reflect increased clonality, whereby the majority of the population represents descendants from a single lineage (Young and Brown 1999). Compatibility of the pollen grain is typically determined by the diploid genotype at the S locus of the paternal individual, inhibiting fertilization between individuals who contain the same S-alleles. The mating system within these spiny daisy populations may be attributed to this mating system (Frankel and Galun 1977; DeMauro 1993). Species which exhibit self-incompatibility often demonstrate limited reproductive performance, particularly via inhibition of the stigma, pollen tube growth and reduced seed set (Lloyd 1968; Oloumi and Rezanejhad 2009).

The co-occurrence between SI and clonality is common among small populations as it helps compensate for limited seed production and reduces selection pressure which favours the breakdown of SI, thus preventing inbreeding (Vallejo-Marin 2007; Franklin-Tong 2008; DeMauro 1993) demonstrated that the partial-SI within the lakeside daisy (Hymenoxys acaulis) can vary depending on the degree of relatedness between mates (DeMauro 1993). She found that although selfing resulted in incompatibility, mating’s between full siblings and between parent and F1 progeny were able to produce seed (DeMauro 1993). A similar incidence may have occurred within the spiny daisy. Jusaitis and Adams (2005) reported that seed collected and artificially raised from Hart contained similar genetic composition to that of their genetically identical parents; however the offspring differed among 2–5 loci and these changes did not contain new alleles (Jusaitis and Adams 2005). The increased resolution of data obtained within this study shows that members within Hart are not all identical, therefore, it is possible that individuals which produce seed may in fact contain different S alleles allowing them to interbreed, although this would be rare due to low genetic diversity (DeMauro 1993). A lack in observed seedling recruitment within the wild due to either SI, inadequate environmental conditions or reduced pollinator activity could each contribute towards an absence of historical sexual reproduction (Jusaitis and Adams 2005).

Although there was no evidence of inter-population gene flow, Thornlea, Rusty Cab and Yangya sites contained lower levels of pairwise genetic differentiation, higher levels of genetic diversity and fewer numbers of clones than Melrose and Telowie. A possible explanation for this is that these populations have been isolated for fewer generations and/or have experienced occasional sexual reproduction in the past, which would reduce genetic drift (Young et al. 2002; Milton et al. 2004). As these three sites occur proximal to each other, and face exposure to similar environmental conditions.
conditions, increased genetic diversity may reflect historic opportunistic gene flow followed by genetic drift upon being isolated (Milton et al. 2004). Increased spatial separation among populations may have led to increased rates of clonality within populations as a result of limited mate availability. Changes in reproductive strategy from a sexual to vegetative mode in response to patchy geographic distribution has been observed in other Asteraceae species in Australia (Young et al. 2002; Blyth et al. 2021). For example, populations of *Rutidosis leiolepis* that occur in higher altitudes have been reported to contain greater levels of clonality and population genetic differentiation than those at lower altitudes. Reductions in pollinator activity within these areas, altered length of flowering season, as well as increased disturbance events were considered possible reasons towards increased clonality (Young et al. 2002). Variation in the level of clonality observed among spiny daisy populations may therefore have been influenced by changes in historic connectivity. High intra-population relatedness with strong differentiation, particularly within Melrose and Telowie, indicate effects of genetic drift are likely due to higher rates of cloning (Campbell and Husband 2005).

Clonal reproduction serves as an advantageous strategy within challenging landscapes as it increases population size and maintains fitness within generations due to the repetition of heterozygous genotypes (Young et al. 1999; Stoeckel et al. 2006; Navascús et al. 2010). Clonality can allow for the sharing of resources (such as water, carbohydrates, minerals and photosynthate) among ramets and this competitive advantage can act as a vital tool for population colonisation (Alpert 1996; Pennings and Callaway 2000; Stuefer et al. 2004; Jusaitis and Adams 2005; Donahue and Lee 2008). Our study shows that the extent of clonality and relatedness varies among populations with Melrose consisting entirely of clones. It may be possible that this population has been founded more recently than the other populations, resulting in low genetic diversity in the form of somatic mutations (Stoeckel et al. 2006; de Meëüs et al. 2007; Wang et al. 2018). Asexual reproduction during unfavourable conditions such as when mates or pollinators are limited or during periods of increased environmental stress, can serve as a short-term, cost-effective mechanism to increase population size as it assures reproduction (Wang et al. 2018). The most clonal populations: Telowie and Melrose (respectively containing 33% and 100% clones), also represent the most northern sites. The Melrose population may be at a greatest risk of extinction as it might be at the edge of the climatic limit for this species and does not have the genetic variability to adapt to a changing environment (Dodd and Douhovnikoff 2016). Assisted migration can help retain genetic diversity, and this is important should the original site become further degraded or locally extinct (Clarke et al. 2013; Blyth et al. 2021). The translocation of individuals from all populations (via vegetative cuttings), including Melrose into areas more similar to the expected range of climatic suitability such as at Banrock Station has been carried out (Bickerton et al. 2018) and this serves as a solution towards preventing the loss of species genetic diversity, especially if the original populations become locally extinct. Increased growth rates, flowering and seed set within this population were experienced when plants gained access to improved habitat quality (increased watering and soil tillage), suggesting that the plants may be better suited towards environments which contain greater similarity to that of their historic distribution (Eckert 2002; Sharp et al. 2010; Bickerton and Tourenq 2015; Bickerton et al. 2018). Currently there is a study analysing the genetic diversity within these seedlings to determine whether sexual reproduction has occurred within the species. Further studies which examine the species ecological requirements and assess genetic composition within progeny will lead to opportunities that reintroduce gene flow among populations.

### Conservation implications and management recommendations

Our study helps improve the knowledge of genetic structure within the critically endangered spiny daisy, demonstrating that it is likely experiencing strong genetic drift probably due to processes that limit sexual reproduction (Sharp et al. 2010; Clarke et al. 2013; Bickerton et al. 2018; Rodger et al. 2021). However, as the evolutionary resilience of a species relies upon adequate levels of genetic diversity which can only be achieved through successful interbreeding, and our study therefore raises concerns about its adaptability and we call for updated conservation management interventions (Bjilisma et al. 2000; Brook et al. 2002; Young et al. 2002; Blambert et al. 2016). We urge the implementation of strategies which enhance reproductive fitness to facilitate sexual reproduction and encourage seed set as the initial step towards species recovery. Although preliminary hand pollination experiments between Hart and Thornlea have been trialled, limited seed viability has been a notable hurdle towards successful germination (Jusaitis and Adams 2005; Sharp et al. 2010; Clarke et al. 2013; Bickerton et al. 2018). Updated crosses among individuals from each population (propagated from vegetative cuttings) would help determine the severity of this problem and explore reproductive compatibility among populations.

Common garden trials enable conservation biologists to measure fitness in parental as well as outcrossed progeny, evaluating the effects and risks of outbreeding depression in a controlled setting (Ottewell et al. 2016; Martín-Forés
et al. 2018; Blyth et al. 2020, 2021; Van Rossum and Le Pajolec 2021). These trials have been increasingly used as an effective management approach to re-introduce genetic diversity within clonal, highly related and moderately differentiated in-situ populations as well as guide the design of ex-situ translocation populations (Ellström 2014; Ottewell et al. 2016; Blyth et al. 2021; Brunton et al. 2021; Gavin-Smyth et al. 2021). We advise the use of common garden experiments to validate the benefits of introducing novel genotypes within in-situ and ex-situ populations. In addition to habitat suitability, many studies have shown that the composition of genetic diversity of founder individuals acts as a primary driver towards successful early establishment of translocation sites (Schäfer et al. 2020; Van Rossum and Le Pajolec 2021). The increased probability of containing pre-adapted genotypes, as well as the reduced likelihood of bottleneck effects, should improve its evolutionary potential (Gamfeldt and Källström 2007; Breed et al. 2019). The variation of population genetic diversity detected within this study can direct ex-situ conservation efforts by determining sampling intensities within populations for the purpose of genetic rescue (Gardiner et al. 2017; Rodger et al. 2021). As each population represents a subset of unique genetic diversity, representatives from each locality should be used for outcrossing experiments as this will maximise genetic diversity within the progeny. For collection purposes, the intensity of sampling within sites needs to be evaluated depending on the level of genetic diversity within each site (Greenfield et al. 2016; Gardiner et al. 2017; Rossetto et al. 2021). Extensive sampling within clonal sites such as Melrose will help increase population size, however, it may pose an unnecessary use of resources e.g. if sampling is too intense, as it will likely under-represent the genetic diversity of the species (Broadhurst et al. 2008; Gardiner et al. 2017). Instead, pollen from one individual in Melrose could be used to fertilise several individuals in other populations.

**Conclusion**

Our study has shown that the spiny daisy is at high risk of extinction due to its limited genetic diversity which has likely been influenced by habitat loss. Each population contained (i) strong genetic structure, (ii) low levels of genetic diversity and (iii) little to no evidence of gene flow. Although excessive homozygosity was not detected, high relatedness among individuals suggests this species may be unlikely to increase its genetic diversity without intervention as populations remain isolated. Further studies which examine (i) reproductive compatibility between populations via hand pollination experiments and (ii) species ecological requirements will help provide insight into strategies to improve genetic diversity in addition to facilitating species restoration. Results from common garden trials will also help elucidate the risks and benefits of initiating gene flow among differentiated populations. Our study highlights the urgency of genetic rescue for the spiny daisy and is a guide to develop conservation management strategies which will increase evolutionary resilience within the species.

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**Declarations**

**Conflict of interest** The authors do not have any conflicts of interest to declare.

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