

Evaluating conservation strategies for the endangered daisy *Schoenia filifolia* subsp. *subulifolia* (Asteraceae): fitness consequences of genetic rescue and hybridisation with a widespread subspecies

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Handling Editor:

Susan Hoebee

Received: 21 January 2022

Accepted: 4 July 2022

Published: 27 July 2022

Cite this:

Monks L et al. (2022)
Australian Journal of Botany, **70**(5), 344–357.
doi: [10.1071/BT22006](https://doi.org/10.1071/BT22006)

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ABSTRACT

Context. To establish translocated populations of threatened plants with the genetic resources to adapt to changing environmental conditions, the source of propagation material is an important consideration. **Aim.** We investigated the fitness consequences of genetic rescue and admixture for the threatened annual daisy *Schoenia filifolia* subsp. *subulifolia*, and the common *S. filifolia* subsp. *filifolia*, to inform seed-sourcing strategies for translocations of the threatened subspecies. **Methods.** We evaluated genetic diversity of two populations of *S. filifolia* subsp. *subulifolia* and four populations of *S. filifolia* subsp. *filifolia* by using microsatellite markers. We grew seedlings from each study population and cross-pollinated inflorescences within and among populations of the same subspecies, and between subspecies. We evaluated the fitness consequences of each cross by using seed set, seed weight and seed viability. **Key results.** There was a lower genetic diversity in the small (<50 plants, $N_{ar} = 3.28$, $H_e = 0.42$) compared to the large (>10 000 plants, $N_{ar} = 4.42$, $H_e = 0.51$) population of *S. filifolia* subsp. *subulifolia*, although none of the measures was significantly different, and seed fitness was slightly, although not significantly, reduced in interpopulation crosses compared with the small population. Genetic diversity was similar between the threatened and widespread subspecies; however, the subspecies were genetically divergent ($F_{st} = 0.242$ – 0.294) and cross-pollination between subspecies produced negligible amounts of seeds (<3% seed set). **Conclusions.** Although genetic rescue or admixture of *S. filifolia* subsp. *subulifolia* would not necessarily result in greatly increased levels of genetic diversity or seed fitness, we still consider it a potential option. Negligible seed set in crosses between subspecies indicates that deliberate hybridisation is not a possibility. **Implications.** Studies of fitness consequences of admixture or genetic rescue are rare yet critical to assessing the benefits of different translocation strategies.

Keywords: admixture, Asteraceae, demographic rescue, environmental change, genetic diversity, genetic rescue, reintroduction, seed fitness, threatened plants, translocation.

Introduction

Many causes of plant population extinction operate at a large scale, making it challenging, and in some cases impossible, to effectively manage threatened plant species *in situ*. Increasingly, conservation initiatives such as translocation are needed to ensure the persistence of a population or species. Translocations are defined as the intentional transfer of plants or plant regenerative material to a new location and are used to augment numbers of individuals in small and declining populations, to reintroduce plants to extinct populations, and to introduce plants to new safe locations (Commander et al. 2018).

The aim of translocation is to establish self-sustaining populations with sufficient genetic diversity to enable adaptive evolutionary change (Guerrant 1996). The levels of genetic

diversity in the founding plants will therefore affect the likelihood of adaptation and, so, the long-term persistence of the translocated population (Montalvo and Ellstrand 2001). However, sourcing propagation material to establish translocated populations with sufficient genetic diversity can often be challenging. Small, isolated populations of threatened plants may be affected by inbreeding, genetic drift and reduced geneflow, which can negatively influence the levels of genetic diversity and fitness of propagules (Broadhurst *et al.* 2008; Weeks *et al.* 2011; Frankham *et al.* 2017). Additionally, these small population sizes can limit the amount of plant material (i.e. seeds, root stock, cuttings) available for propagation (Guerrant 1996). Where new populations are to be established (introduction translocations), or populations re-established (reintroduction translocations), genetic diversity can potentially be maximised through the carefully considered admixture of several populations (e.g. van Rossum *et al.* 2020). For the augmentation of small, isolated, and inbred populations, genetic rescue, the introduction of new genetic material (Tallmon *et al.* 2004; Whiteley *et al.* 2015; Ralls *et al.* 2018), may benefit species persistence.

Admixture involves the relatively even mixing of plant material from isolated populations and establishment of these plants at a new location to facilitate hybridisation and increase genetic diversity (Shi *et al.* 2018). Selection pressure could be strong at the new location. In contrast, genetic rescue involves the addition of a small number of plants from other populations, into small populations that are inbred, have low or no geneflow from other populations, and is not attempted where selection pressure is strong (Tallmon *et al.* 2004). The addition of new genetic material into an inbred population for genetic rescue, and the hybridisation that occurs in admixture, can benefit threatened plants by increasing genetic diversity, population size, and offspring fitness (e.g. growth rate) through heterosis or adaptive evolution, thereby reducing extinction risk (Pickup *et al.* 2013; Whiteley *et al.* 2015; Shi *et al.* 2018). However, there are concerns that the use of genetic rescue for augmenting populations or admixture to establish new populations can have adverse consequences. Genetic rescue may lower the fitness of the population being augmented by swamping the gene pool of the locally adapted plants with hybrids between the local and introduced plants, which are less adapted to the local conditions (Hufford and Mazer 2003; Whiteley *et al.* 2015). Admixture or genetic rescue can result in a population with reduced fitness because of outbreeding depression when the new genetic material is introduced from a genetically differentiated, ecologically dissimilar or geographically distant population (Hufford and Mazer 2003; Edmands 2007; Shi *et al.* 2018). Additionally, there are doubts as to how long the fitness benefits of heterosis last, with maximum fitness benefits predicted for the F1 and F2 generations before a decline in benefits for subsequent generations (Bell *et al.* 2019). Other concerns are that

environmental conditions and threatening processes could play a larger, more imminent role in population decline than does a lack of geneflow, and these may be overlooked in the rush to undertake a translocation (Bouzat *et al.* 2009; Whiteley *et al.* 2015; Bell *et al.* 2019). Instead, proponents argue that the simple addition of immigrants to a population (i.e. demographic rescue) may have the same effect in decreasing probability of population extinction (Hufbauer *et al.* 2015; Bell *et al.* 2019) regardless of their contribution to genetic diversity and the gene pool.

The magnitude of the change in the level of fitness following admixture or genetic rescue can be associated with geographic distance between populations (Waser and Price 1989). That is, fitness measures can be lower in crosses between plants from the same or nearby populations (inbreeding depression) and between widely geographically separated populations (outbreeding depression), than in crosses with plants from populations over an intermediate geographic distance (Waser and Price 1989; Hufford *et al.* 2012). Cross-pollination experiments among different populations have demonstrated this effect, with greater germination and survival of F1 seedlings of *Styloidium hispidum* from intermediate-distance crosses, than from within-population, and long-distance crosses (Hufford *et al.* 2012). Similarly, improved seed set and seedling survival of *Jacquemontia reclinata* were observed over increasing geographic crossing distance (Maschinski *et al.* 2013) and higher seed viability; faster seed germination and increased seedling recruitment were recorded for *Zostera marina* from an intermediate crossing distance than from the near and far crossing distances (Billingham *et al.* 2007). These findings suggest that there is an optimal intermediate outcrossing distance that avoids both inbreeding depression and outbreeding depression that can inform genetic rescue of small populations or admixture to establish translocated populations.

As well as the biological concerns about the mixing of gene pools, cultural and legislative concerns have contributed to minimising attempts at genetic rescue (Frankham 2015; Ralls *et al.* 2018) and, until recently, limited the use of admixture to establish or re-establish populations (Maschinski *et al.* 2012). To counteract some of these concerns, risk frameworks have been developed to guide decisions on when and how to use genetic rescue or admixture to minimise potential negative consequences (Byrne *et al.* 2011; Frankham *et al.* 2011; Weeks *et al.* 2011; Frankham 2015). These frameworks indicate that the risk of outbreeding depression is low if the taxonomy is adequately resolved, there are no fixed chromosomal differences between the populations, gene flow has occurred within the past 500 years, and the populations are adapted to similar environments (Frankham *et al.* 2011; Frankham 2015). Additionally, the fitness benefits of genetic rescue have been demonstrated to occur to the F3 generation and beyond in numerous plant species (Pickup *et al.* 2013; Frankham 2016), which has helped allay concerns.

Some threatened plant species may exist only as a single population, or genetically, geographically, or ecologically divergent populations may be the only source of propagation material (Hedrick and Garcia-Dorado 2016; Kronenberger *et al.* 2017). In these cases, there is a question as to whether positive benefits of admixture or genetic rescue from more genetically divergent populations, or even closely related taxa such as subspecies, offset the risks of outbreeding depression (Baskett and Gomulkiewicz 2011; Hedrick and Garcia-Dorado 2016; Kronenberger *et al.* 2017). Empirical testing of the benefits of admixture or genetic rescue between genetically divergent populations will assist in assessing the trade-off between risks and benefits, but such experiments should be undertaken *ex situ* to allow for contained assessment of the consequences of such actions (Harrison *et al.* 2016).

Schoenia filifolia (Turcz.) Paul G. Wilson is an annual herb from the Asteraceae family that grows in patches across the south-west of Western Australia. The species consists of the following three subspecies: subsp. *subulifolia*, subsp. *arenicola* and subsp. *filifolia*. The first subspecies, *S. filifolia* subsp. *subulifolia*, has a conservation listing of endangered (Smith and Jones 2018). Translocations of this subspecies are an important component of its conservation. However, seed is available only from two extant populations, one of which is large (>10 000 individuals) and the other very small (<50) and declining. To assess the benefits and risks of admixture to augment the small population or translocation to establish a new population, we investigated interpopulation hybridisation in *S. filifolia* subsp. *subulifolia* and in the more common widespread *S. filifolia* subsp. *filifolia*, and crosses between the subspecies, to inform the development of a translocation strategy for *S. filifolia* subsp. *subulifolia*. We coupled this investigation with an assessment of patterns of genetic diversity across populations of subsp. *subulifolia* and subsp. *filifolia*. Specifically, we asked the following questions: (1) what are the levels of genetic diversity and genetic divergence between and among the study populations of both subspecies; (2) what are the consequence of cross-pollination among and within populations of each subspecies on the early fitness measures of seed set, seed weight and seed germinability; and (3) can cross-pollination between subspecies occur and what are the early fitness consequences? Answers to these questions will inform whether genetic rescue of the small population of subsp. *subulifolia* is likely to be beneficial, whether admixture to establish new populations or re-establish extinct populations of subsp. *subulifolia* will be an effective conservation strategy, and which source population or populations (intra- or interspecific) will maximise genetic diversity and therefore evolutionary potential, while minimising the negative consequences of outbreeding depression. We discuss the implication of our findings for conservation of other endangered annual herbs.

Materials and methods

The species

Schoenia filifolia, from the family Asteraceae, is an annual erect herb growing up to 0.5 m in height. Multiple flowers are held in a capitula surrounded by bright yellow involucre bracts (Wilson 1992). Flowers are hermaphroditic and flowering occurs between July and November. The species is distributed across the south-west of Western Australia (Fig. 1).

Three subspecies are described. The first, *S. filifolia* subsp. *subulifolia* is distinguished from the other two subspecies by having a larger capitula and a longer ray, and a hemispherical involucre. Flowers of subspecies *subulifolia* are self-incompatible and predominantly insect pollinated, although wind pollination may occur over short distances (Png 2012). The subspecies is currently known from three extant populations (although only two populations were known at the time this study was undertaken) in a small area around the inland town of Mingenew (<https://florabase.dpaw.wa.gov.au/>; Fig. 1; Table 1). Subspecies *arenicola* has not been seen since 1977 and, consequently, is not included in this study. Subspecies *filifolia* has a widespread distribution from Cue in the north, inland to Coolgardie and south to Kondinin (<https://florabase.dpaw.wa.gov.au/>; Fig. 1) and is not listed as a taxon of conservation concern (Table 1). Like pollination of subsp. *subulifolia*, that of subsp. *filifolia* is likely to be insect- or wind-mediated, although this has not been confirmed.

Sampling

Four populations of subsp. *filifolia* were chosen for sampling because they represented populations from across the range of the subspecies (Fig. 1). Two populations of subsp. *subulifolia* were chosen for sampling as they were the only two extant populations known when the study commenced (Fig. 1). The six populations were visited during flowering in September 2016. Up to 24 plants were sampled from each population (all above-ground parts) and stored as individuals in envelopes. Samples were freeze-dried and then stored on silica until required. A representative specimen was also collected from five of the sampled populations, identification was verified, and the specimens were lodged at the Western Australian Herbarium (PERTH 08938008, PERTH 08938016, PERTH 08938024, PERTH 08938032, PERTH 08937990). The Lockier population of subsp. *subulifolia* was previously verified (PERTH 07772041, <https://florabase.dpaw.wa.gov.au/>). In November 2016, seeds were collected from the four populations of subsp. *filifolia* from >25 plants across each population and then held at 15°C and 15% relative humidity until required. Seeds of two populations of subsp. *subulifolia*, already held at the Western Australian Seed Bank, Kensington, were

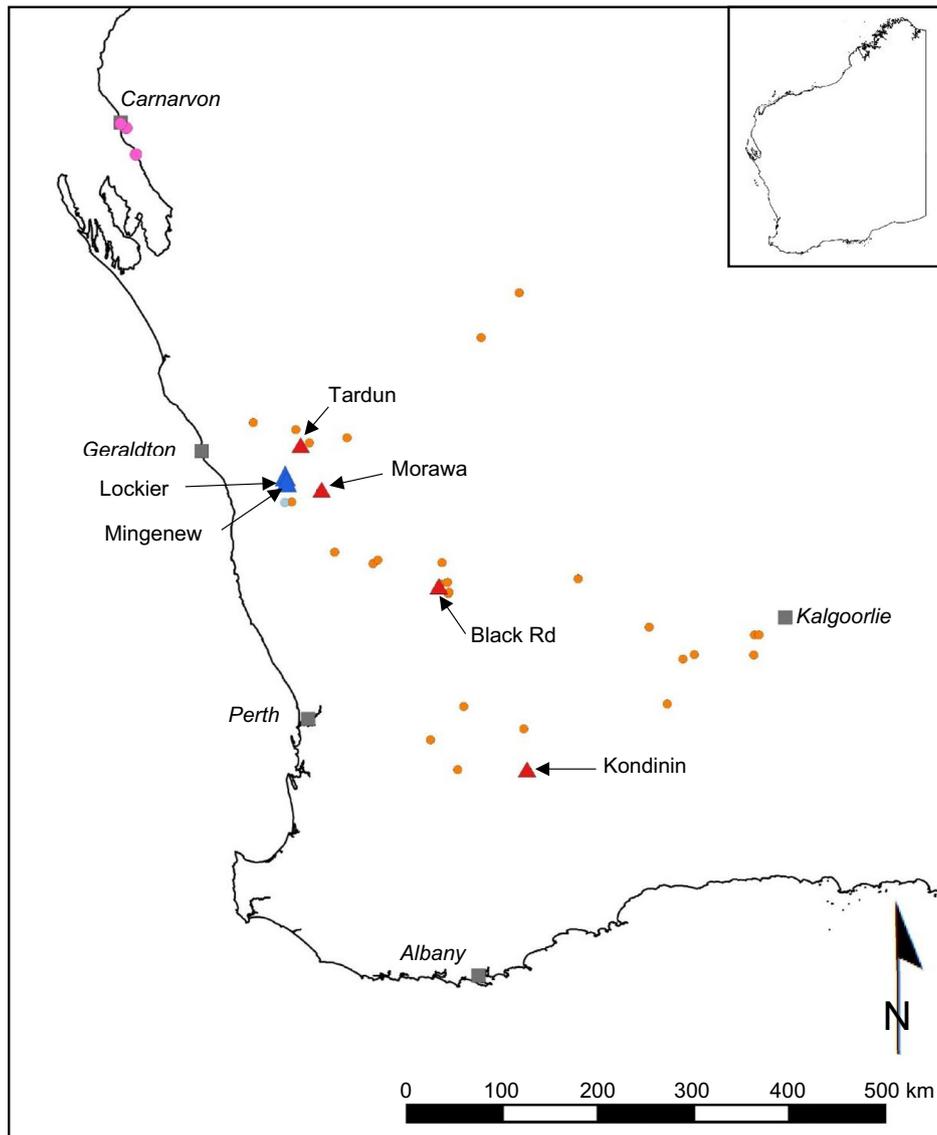


Fig. 1. Map of distribution of *Schoenia filifolia* subsp. *subulifolia* (blue symbols), *S. filifolia* subsp. *filifolia* (orange and red symbols) and *S. filifolia* subsp. *arenicola* (pink symbols, near Carnarvon) in Western Australia. Source populations for this study are indicated by triangles.

used for this study. These seeds were collected in October 2007 (Lockier) and October 2015 (Mingenew) and stored at -18°C and 15% relative humidity.

Genetic structure and genetic diversity

DNA was extracted from freeze-dried leaf material (approximately 50 mg) following the Doyle (1991) CTAB extraction method, with the addition of polyvinylpyrrolidone (PVP-40) and sodium sulfite to the extraction buffer (Byrne *et al.* 2001) and a second chloroform extraction step. DNA from one individual was sent to the Monash University Malaysia genomics facility for dye-based sequencing on an Illumina MiSeq desktop sequencer (Illumina, San Diego, California,

USA). Unique loci were identified, and primers designed for 138 potential loci by using the default parameters in QDD v3.1 (Megléc *et al.* 2010).

Eight individuals were randomly selected to trial amplification and polymorphism of microsatellite regions. Forty-eight of the primer pairs were tested and of these three primers showed no amplification, and 32 primers showed no variation or did not consistently amplify across all populations. From 13 remaining primers, seven were chosen (SCF01, SCF04, SCF08, SCF16, SCF20, SCF23, SCF35, Supplementary Table S1), because they reliably amplified across all populations.

The G5 label set was used to label forward primers with a fluorescent tag (either VIC, PET, NED or FAM). Microsatellite

Table 1. Differentiating floral feature, conservation listing and habitat for the three subspecies of *Schoenia filifolia*.

Subspecies	Floral features that distinguish the subspecies from other subspecies	Conservation listing	Range	Climate	Habitat
<i>subulifolia</i>	Hemispherical involucre	Threatened–Endangered	Narrow, 35 km ²	Average annual rainfall 399 mm. Wettest months May–August. Temperature range: max 36°C (in January), min 7°C (in August).	Grows in slight water-gaining depressions, or on slopes to creek-lines in brown clay-loam soils. Grows under <i>Eucalyptus</i> woodlands.
<i>filifolia</i>	Turbinate involucre	Not listed	Widespread, approximately 180 000 km ²	Average annual rainfall 230–399 mm. Wettest months January–March and May–August. Temperature range: max 33–38°C (in January), min 5–7°C (in July – August).	Grown on slope or low dunes adjacent to salt-lakes in grey, brown or red clay-loam soils. Grows in tall open shrub land.
<i>arenicola</i>	Cylindrical involucre	Poorly known (Data deficient)	Narrow, 100 km ²	Average annual rainfall 222 mm. Wettest months May–July. Temperature range: max 32.5°C (in February), min 11°C (in July).	Grows on subcoastal sand ridges in red sand-clay soils. Grows in heath.

Conservation listing from Smith and Jones (2018).

regions were then amplified by polymerase chain reaction (PCR) in multiplexes of two or three sets, by using a Qiagen Multiplex kit (Qiagen, Hilden, Germany). Each multiplex reaction contained 1 µL of DNA, 3.75 µL of Qiagen mix, 0.75 µL primer mix and 2 µL water (from Qiagen kit). Primer mix contained VIC, PET, NED, or FAM. The PCR amplification program was as follows: 95°C for 15 min, followed by 35 cycles of 94°C for 30 s, 60°C for 90 s, 72°C for 60 s, and a final extension of 60°C for 30 min. Two or three randomly selected individuals were added twice to each plate to serve as plate-level negative controls. The PCR product was diluted with sterile deionised water at a rate of 1:20 and then 1 µL of diluted PCR product was added to 12 µL of Hi-Di-formamide/0.125 µL GeneScan LIZ 500 (–250) size Standard (Applied Biosystems, Waltham, MA, USA). Fragments were viewed with automated fluorescent scanning detection using an Applied Biosystems 3730 DNA Analyser. Genotypes were scored in GeneMapper V5 (Applied Biosystems). The allele bins were assigned manually, checked automatically and, where required, the alleles were manually adjusted.

GENEPOP ver. 4.0 (Raymond and Rousset 1995) was used to test for Hardy–Weinberg equilibrium (HWE) across the seven loci with exact tests by the Markov-chain method. Tests for linkage disequilibrium (LD) between loci were performed in GENEPOP ver. 4. MICRO-CHECKER (van Oosterhout et al. 2004), using a Bonferroni adjusted 95% confidence interval and 10 000 repetitions, was used to look for evidence for large allele drop-out, scoring error due to stutter and null alleles. We also estimated the frequency of null alleles within a population, considering the possibility of inbreeding using INEST (Chybicki and Burczyk 2009).

GenAEx v6.5 (Peakall and Smouse 2006, 2012) was used to calculate the mean number of individuals genotyped, averaged over all loci (N), and the measures of microsatellite diversity, including expected (H_e) and observed (H_o) heterozygosity and the fixation index (F_{is}). Because of the differences in sample sizes among populations, HP-Rare (Kalinowski 2005) was used to calculate allelic richness (N_{ar}). Analysis of genetic diversity measures (N_{ar} , H_e , H_o and F_{is}) compared between subspecies using a nested one-way ANOVA and among populations within each subspecies by using a one-way ANOVA in R v3.6.2. Differences between population pairs were assessed using a Tukey HSD test (using the ‘TukeyHSD’ function in R v3.6.2).

Pairwise genetic differentiation among populations was estimated using unbiased F_{st} , D_{st} and $G'_{st H}$ (Hedrick 2005; Meirmans and Hedrick 2011) in GenAEx 6.5 based on the G-statistic option, with significance being tested using 999 permutations. Relationships among populations were analysed using principal coordinate analysis (PCoA) in GenAEx based on a standardised covariance matrix using pairwise genetic-distance measures. Genetic structure was inferred using Bayesian model-based clustering in STRUCTURE v2.3.3, by using the admixture model and correlated allele frequencies (Pritchard et al. 2000). The analyses used Markov-chain Monte Carlo (MCMC) parameters with a burn-in period of 100 000 and 100 000 iterations, with 10 replicate runs for each value of K from 1 to 7. The most appropriate K value was found using STRUCTURE HARVESTER web v0.6.94 (Earl and vonHoldt 2012), as described by Evanno et al. (2005). The optimal alignment of 10 replicates at the optimal K was determined using CLUMPP 1.1.2 (Jakobsson and Rosenberg 2007), and a similarity coefficient, h' , was calculated to assess

the similarity of individual replicates. A hierarchical approach was used to infer the number of clusters from the full dataset.

Glasshouse cross-pollination

In May 2017, seeds of subsp. *subulifolia* were removed from storage and equilibrated to room temperature over 24 h. To maximise germination, seeds of the six study populations were heat-shocked at 80°C for 14 days. Following heat-shock treatment, the seeds from each population were divided into three equal portions (replicates) and then plated onto individual petri dishes containing 7.5 g/L agar and incubated at 15°C. When the radicle had emerged at least 2 mm, the seed was scored as viable and was removed from the agar and transferred into soil mix in an individual pot (90 mm × 90 mm × 180 mm). Soil mix consisted of two parts composted pine bark, two parts coarse river sand and one part coco peat and was steam sterilised at 80°C for 2.5 h, then cooled prior to being used. Each potted seedling was labelled with source population information and an individual number and given 0.35 g of slow-release fertiliser. Seedlings were grown under nursery conditions until flowering (between 63 and 135 days).

When flowering commenced, plant height, widest crown width and crown width perpendicular to widest width, were measured for each plant. One inflorescence, where flowers had not yet opened, was selected on each plant, and covered with an organza mesh bag that excluded insect pollinators. Plants for crosses were chosen by selecting plants from the required cross type that were at the same stage of flowering. Crosses were reciprocal, with each plant donating and receiving pollen from another plant within the same cross type. Cross types and number of plants for each cross type are shown in Table S2. Flowering time overlapped between subsp. *subulifolia* and the Tardun, Morawa and Black Rd populations of subsp. *filifolia*. However, flowering time was much later in the Kondinin population of subsp. *filifolia* and did not overlap with flowering of subsp. *subulifolia* and, subsequently, we were unable to undertake any crosses between these populations. Flowering time of the Kondinin population, although overlapping with Tardun, Morawa and Black Rd populations, was delayed and resulted in fewer crosses between Kondinin and these populations.

Once flowering commenced, cross-pollination was undertaken every second day by gently rubbing the inflorescence of each plant against the selected cross. Flowerheads were re-bagged between cross-pollination events. Cross-pollinations occurred until 2 days after all flowers in the inflorescence had opened. Self-pollinations were undertaken on a subset of plants, using the same technique as for cross-pollination, but between inflorescences on the same plant. The plants were watered until seeds ripened. At seed release (inflorescences dried, and seeds loose within the bracts), the seeds of individual plants were collected and placed into a paper bag.

Thereafter, all the above-ground shoot material was collected and placed into a paper bag.

Shoot material was oven-dried at 60°C for 48 h, then weighed. Inflorescences from cross-pollinations and self-pollinations were dissected under a microscope and seeds were sorted into whole seeds (firm, plump and dark brown seed) and non-viable seed (soft, shrivelled, and white/cream seed). Any flower parts and non-viable seed were weighed, and weight added to whole plant dry weight. Whole seeds were also weighed. The viability of whole seeds was assessed using a germination test, following the same technique as described above. After 22 weeks, the viability of seeds that remained ungerminated, but still whole and firm, was assessed using a stain. Seed were soaked in a 1% w/v solution of 2,3,5-triphenyl tetrazolium chloride incubated at 30°C, in the dark, for 48 h. Seed were cut to assess staining. Seed that had stained red were classed as viable, seed that did not stain (remained white), or where only a small proportion of the seed stained, were classed as non-viable.

For the plants grown for the study (P generation) percentage germination, plant height, mean crown width, crown volume (calculated as height × crown width × perpendicular crown width) plant dry weight and number of seeds per inflorescence were compared between subspecies by using a nested one-way ANOVA (using 'aov' function in R v3.6.2), where population was the nested factor. Percentage germination data were arcsin-transformed and crown volume was log-transformed prior to analysis. A one-way ANOVA was used to compare among populations within each subspecies for the same variables. For each analysis, when the *P*-value was significant (*P* < 0.05), mean differences between population pairs were assessed using a Tukey HSD test (using the 'TukeyHSD' function in R v3.6.2).

For the cross-pollinations (F1 generation), the percentage of whole seeds, weight of whole seeds, and percentage of viable seeds, were compared for the different cross-types. These variables were first compared for intrapopulation crosses within each subspecies; interpopulation crosses within subsp. *filifolia*, and between subspecies crosses, by using one-way ANOVA (using 'aov' function in R v3.6.2). Second, the variables were compared for each subspecies among the three cross-categories (all intrapopulation crosses, compared with all interpopulation crosses within each subspecies and compared with all between the subspecies crosses) by using nested one-way ANOVA with cross-type as the nested factor. In each case, percentage data were arcsin-transformed prior to analysis and when the *P*-value was significant (*P* < 0.05), mean differences between cross pairs were assessed using a Tukey HSD test (using the 'TukeyHSD' function in R v3.6.2).

For the self-pollinations, the percentage of whole seeds, weight of whole seeds and percentage of viable seeds were compared among the populations within each subspecies by using one-way ANOVA (using 'aov' function in R v3.6.2).

These same measures were also compared between self-pollination crosses and intrapopulation crosses within subspecies, again by using one-way ANOVA (using 'aov' function in R v3.6.2). In each case, percentage data were arcsin-transformed prior to analysis.

Results

Genetic structure and genetic diversity

Sixty-one alleles were detected across seven loci. After adjusting for multiple comparisons ($n = 33$), significant departure from HWE ($P < 0.05$) in the form of heterozygote deficiency was observed at locus SCF01 in Tardun and Black Rd, locus SCF16 in Mingenew, Tardun and Morawa, locus SCF08 in Tardun and Morawa, and locus SCF20 in Morawa and Tardun. At no locus was there evidence of null alleles within any population and there was no evidence of large allele drop-out. When considering inbreeding using INEST, no locus had a frequency of null alleles of >0 across all populations. After adjusting for multiple comparisons ($n = 21$), no locus pairs were in linkage disequilibrium across any population.

There was significant differentiation among the *S. filifolia* populations, with high values for overall genetic fixation and allelic differentiation ($F_{st} = 0.407$ [$P = 0.001$]; $D_{st} = 0.607$;

$[P < 0.001]$, $G_{stH} = 0.759$ [$P < 0.001$]). Between populations of the two subspecies, pairwise measures of D_{st} and G_{stH} were high ($D_{st} \geq 0.691$, $G_{stH} \geq 0.762$, Table 2). Within subspecies, pairwise measures of D_{st} and G_{stH} were low for subsp. *subulifolia* ($D_{st} = 0.023$; $G_{stH} = 0.035$, Table 2) and moderate for subsp. *filifolia* (D_{st} between 0.214 and 0.272; G_{stH} between 0.274 and 0.340, Table 2). The PCoA clearly distinguished two discrete groups, with the two populations of subsp. *subulifolia* in one group, and the four populations of subsp. *filifolia* in the second group (Fig. 2). The first axis explained 44.8% of the variation in genetic composition and the second axis 15.3% of the variation (Fig. 2). STRUCTURE analysis confirmed the presence of two clearly differentiated groups, with the two populations of subsp. *subulifolia* occurring in one cluster and the four populations of subsp. *filifolia* occurring in the second cluster (Fig. 3). These results infer limited geneflow between the subspecies. These two clusters were supported by very high similarity among 10 replicates ($h' = 0.998$).

Comparisons of genetic diversity measures showed no differences between subspecies for N_{ar} , H_o and H_e . However, F_{is} was significantly lower in subsp. *subulifolia* than in subsp. *filifolia* ($P = 0.037$; Table 3). Within subspecies, the two populations of subsp. *subulifolia* were not significantly different from each other for any genetic-diversity measure, although there was a trend towards lower genetic diversity in the small Lockier population. The four populations of

Table 2. Pairwise F_{st} , Jost's D_{st} and Hedrick's G'_{stH} for two populations of *Schoenia filifolia* subsp. *subulifolia* and four populations of *S. filifolia* subsp. *filifolia*.

Subspecies	Population	Lockier	Mingenew	Tardun	Morawa	Black Rd
F_{st}						
<i>subulifolia</i>	Lockier	–				
	Mingenew	0.032	–			
<i>filifolia</i>	Tardun	0.288	0.249	–		
	Morawa	0.279	0.242	0.093	–	
	Black Rd	0.294	0.255	0.110	0.110	–
D_{st}						
<i>subulifolia</i>	Lockier	–				
	Mingenew	0.023	–			
<i>filifolia</i>	Tardun	0.759	0.738	–		
	Morawa	0.704	0.691	0.214	–	
	Black Rd	0.774	0.753	0.272	0.264	–
G'_{stH}						
<i>subulifolia</i>	Lockier	–				
	Mingenew	0.035	–			
<i>filifolia</i>	Tardun	0.823	0.799	–		
	Morawa	0.780	0.762	0.274	–	
	Black Rd	0.836	0.813	0.340	0.333	–

Between-subspecies comparisons are highlighted in bold. Kondinin population of *S. filifolia* subsp. *filifolia* was excluded from this analysis because all individuals had the same multilocus genotype.

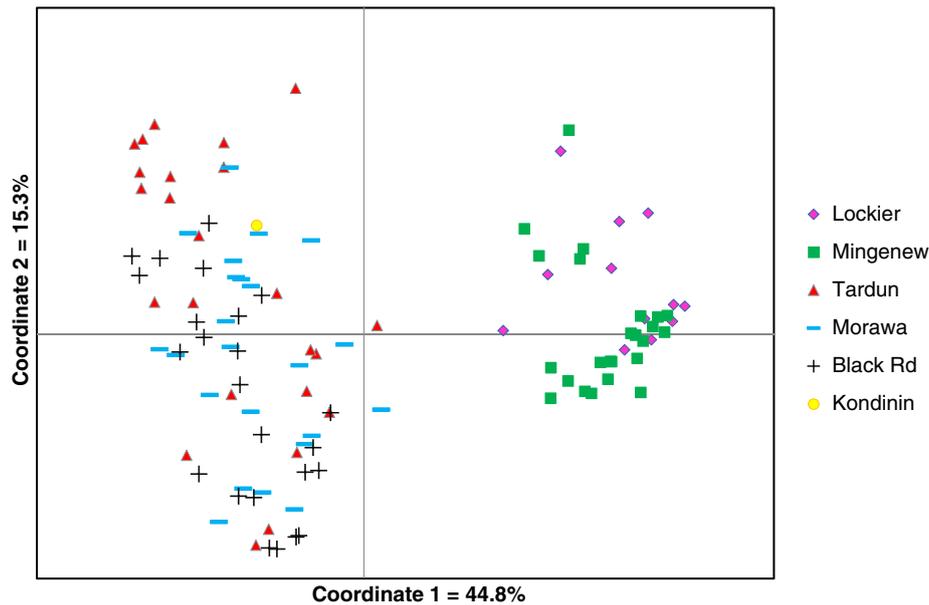


Fig. 2. Principal coordinates analysis of pairwise F_{st} values for two populations of *Schoenia filifolia* subsp. *subulifolia* (Lockier, Mingenew) and four populations of *S. filifolia* subsp. *filifolia* (Tardun, Morawa, Black Rd, Kondinin).

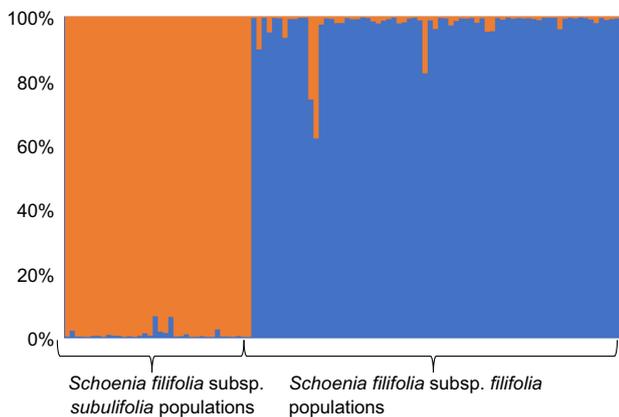


Fig. 3. Structure analysis for *Schoenia filifolia*, showing two distinct subspecies.

subsp. *filifolia* were also not significantly different from each other for any genetic-diversity measure. There was no variation for the Kondinin population at any of the seven loci between any of the 24 samples, which may be due to apomixis, and, subsequently, this population was excluded from analysis for H_o , H_e and F_{is} .

Cross-pollination study

Parental generation

All fitness parameters, except height, were significantly different between subsp. *subulifolia* and subsp. *filifolia* (germination, $P < 0.0001$; crown $P = 0.0018$; log crown volume, $P < 0.0001$; dry weight, $P = 0.0004$; total number

of seed per inflorescence, $P < 0.0001$; Table 4). Within subsp. *subulifolia*, plants from Mingenew were significantly larger than were plants from Lockier (height $P = 0.0044$, log crown volume $P = 0.0139$, dry weight $P = 0.0015$); however, germination was significantly lower ($P = 0.00875$; Table 4). All parameters within subsp. *filifolia* were significantly different among populations (initial viability $P = 0.00598$, height $P < 0.0001$, crown width $P < 0.0001$, log crown volume $P < 0.0001$, dry weight $P < 0.0001$), with plants from Tardun and Morawa generally taller and having more substantial crown volume than plants from Black Rd, and Black Rd plants larger than plants from Kondinin (Table 4). Germination was significantly lower at Kondinin than at Black Rd ($P = 0.034$) and Tardun ($P = 0.007$) and the number of seeds per inflorescence at Kondinin was significantly lower than that at Tardun ($P < 0.0001$), Morawa ($P < 0.0001$) and Black Rd ($P < 0.0001$).

Crosses

Seed set, seed weight and seed viability from self-pollination did not differ significantly among populations within the same subspecies. Inflorescences that were self-pollinated had less than 0.01% seed set in the six populations studied (Table 5) and this level of seed set was significantly less than that produced from intrapopulation crosses for subsp. *subulifolia* ($P > 0.0001$) and subsp. *filifolia* ($P > 0.0001$). Seed weight and seed viability of self-pollinated seed were also significantly less than those of intrapopulation crosses for subsp. *subulifolia* ($P = 0.01$;

Table 3. The number of individuals genotyped, mean number of individuals genotyped averaged over all loci (N), and measures of microsatellite diversity for two populations of *Schoenia filifolia* subsp. *subulifolia* and four populations of *S. filifolia* subsp. *filifolia*.

Subspecies	Population	Population size	Number of individuals genotyped	N	N_{ar}	H_o	H_e	F_{is}
<i>subulifolia</i>	Lockier	<50	12	11.57 (0.43)	3.28 (0.69)	0.36 (0.10)	0.42 (0.10)	0.09 (0.13)
	Mingenew	>10 000	24	23.71 (0.18)	4.42 (0.97)	0.38 (0.08)	0.51 (0.11)	0.19 (0.11)
<i>filifolia</i>	Tardun	100–500	24	22.29 (1.71)	4.05 (0.82)	0.29 (0.08)	0.55 (0.09)	0.52 (0.12)
	Morawa	100–500	24	21.71 (0.52)	3.83 (0.72)	0.26 (0.10)	0.54 (0.10)	0.55 (0.12)
	Black Rd	100–500	23	23.00 (0.38)	3.41 (0.42)	0.41 (0.04)	0.55 (0.04)	0.22 (0.11)
	Kondinin	~100	24	24.00 (0.00)	1.00 (0.00)	0.00	0.00	–

Genetic-diversity parameters include the mean number of alleles per locus (N), the rarefied number of alleles (N_{ar}), observed (H_o) and expected (H_e) heterozygosity, and the fixation index (F_{is}). Standard errors are in parentheses. There was no significant difference between any population pair within each subspecies. F_{is} was unable to be calculated for Kondinin, because all individuals had the same multilocus genotype.

Table 4. Germination, canopy dimensions at flowering, dry weight, and number of seeds per inflorescence of P (parental) generation plants of *Schoenia filifolia* subsp. *subulifolia* and *S. filifolia* subsp. *filifolia*.

Subspecies	Population	Germination (%)	Height (m)	Mean crown width (m)	Crown volume (m ³)	Plant dry weight (g)	Total number of seeds/inflorescence
<i>subulifolia</i>	Lockier	98.1 (0.1)a	0.224 (0.009)a	0.235 (0.007)a	0.013 (0.001)a	2.525 (0.111)a	153.7 (8.2)a
	Mingenew	76.6 (3.8)b	0.268 (0.011)b	0.248 (0.007)a	0.015 (0.001)b	3.095 (0.136)b	153.2 (7.7)a
	Mean	85.2 (5.7)*	0.245 (0.008)	0.241 (0.005)*	0.014 (0.0007)*	2.805 (0.091)*	153.5 (5.6)*
<i>filifolia</i>	Tardun	65.9 (2.2)a	0.254 (0.006)a	0.261 (0.007)a	0.018 (0.001)a	3.486 (0.126)a	91.7 (3.7)a
	Morawa	51.6 (2.9)bc	0.269 (0.007)a	0.259 (0.007)a	0.019 (0.001)a	4.301 (0.183)b	92.6 (4.0)a
	Black Rd	61.4 (1.7)ab	0.215 (0.007)b	0.191 (0.007)b	0.009 (0.001)b	2.482 (0.193)c	70.0 (3.2)b
	Kondinin	47.3 (4.1)c	0.124 (0.003)c	0.091 (0.004)c	0.001 (0.000)c	1.698 (0.144)d	28.0 (2.4)c
	Mean	56.6 (2.6)*	0.232 (0.005)	0.221 (0.006)*	0.014 (0.0007)*	3.280 (0.106)*	78.1 (2.4)*

Data are means and standard errors in parentheses; overall means is weighted. Within each subspecies, values followed by different letters are significantly different (statistical test for germination and crown volume on arcsin- or log-transformed data). Means followed by an asterisk are significantly different at $P = 0.05$.

Table 5. Mean percentages of whole seed produced per inflorescence, mean weight of whole seed per inflorescence and percentage viability of whole seed for selfed crosses within populations of *Schoenia filifolia*.

Population	Mean % whole seed per inflorescence	Mean seed weight of whole seed	Mean % viability of whole seed
Lockier	0.00025 (0.0001)	0.021 (0.009)	27 (10)
Mingenew	0.00005 (0.00003)	0.009 (0.005)	2 (2)
Tardun	0.00005 (0.00003)	0.005 (0.002)	13 (13)
Morawa	0.00024 (0.00012)	0.020 (0.006)	7 (5)
Black Rd	0.00013 (0.00008)	0.006 (0.002)	72 (15)
Kondinin	0.00112 (0.00106)	0.007 (0.006)	53 (47)

Standard errors are in parentheses. No population pairs were significantly different for any parameters.

$P > 0.0001$) and subsp. *filifolia* ($P = 0.0004$; $P = 0.04$; Tables 5, 6).

Intrapopulation crosses of subsp. *subulifolia* resulted in greater seed set, seed weight and seed viability in the smaller

Lockier population compared to the larger Mingeneew population ($P = 0.002$; $P = 0.02$; $P = 0.02$; Table 6). Within subsp. *filifolia*, there were significant differences among populations for seed set ($P < 0.0001$) and seed weight ($P = 0.002$), but not seed viability, with Kondinin producing no viable seed from intrapopulation crosses, and Black Rd having significantly reduced seed set compared with Tardun ($P > 0.0001$) and Morawa ($P = 0.002$), and significantly reduced seed weight compared with Tardun ($P = 0.002$; Table 6).

Seed set from the subsp. *subulifolia* interpopulation cross was 13.6% (Table 6). The six subsp. *filifolia* interpopulation crosses resulted in seed set ranging from 0 to 29.1%, with a significant difference between the combination of populations crossed ($P < 0.0001$, Table 6). Many of the differences lay with crosses involving the Kondinin population, with no whole seed produced between this population when crossed with Morawa, and only very small amounts when crossed with Tardun (5.7%) and Black Rd (0.4%) (Table 6). However, there were no significant differences between the various subsp. *filifolia*

Table 6. Mean percentage of whole seeds produced per inflorescence, mean weight of whole seeds per inflorescence, and percentage viability of whole seeds for crosses within and among populations of *Schoenia filifolia* subsp. *subulifolia* and *S. filifolia* subsp. *filifolia* (between-subspecies crosses are in bold).

Population	Mean percentage of whole seeds produced per inflorescence					
	Lockier	Mingenew	Tardun	Morawa	Black Rd	Kondinin
Lockier	22.7 (4.7)A					
Mingenew	13.6 (3.0)	6.5 (2.4)B				
Tardun	1.2 (0.8)	1.3 (0.6)	30.6 (3.0)A			
Morawa	3.0 (1.6)	2.1 (1.2)	21.4 (2.4)a	21.3 (3.1)A		
Black Rd	1.1 (0.7)	0.6 (0.4)	29.1 (5.1)a	22.9 (3.7)a	7.2 (2.4)B	
Kondinin	N/A	N/A	5.7 (4.2)b	0.0 (0.0)c	0.4 (0.3)c	0.0 (0.0)B

Population	Mean weight of whole seeds per inflorescence					
	Lockier	Mingenew	Tardun	Morawa	Black Rd	Kondinin
Lockier	0.106 (0.020)A					
Mingenew	0.053 (0.011)	0.037 (0.010)B				
Tardun	0.012 (0.010)	0.013 (0.005)	0.047 (0.006)A			
Morawa	0.011 (0.003)	0.022 (0.012)	0.031 (0.006)a	0.033 (0.004)A		
Black Rd	0.004 (0.002)	0.006 (0.001)	0.033 (0.007)a	0.025 (0.004)a	0.010 (0.004)B	
Kondinin	N/A	N/A	0.041 (0.041)a	N/A	0.001 (0.000)a	N/A

Population	Percentage viability of whole seeds					
	Lockier	Mingenew	Tardun	Morawa	Black Rd	Kondinin
Lockier	88.3 (4.0)A					
Mingenew	74.1 (6.0)	66.6 (8.5)B				
Tardun	67.5 (17.2)	67.9 (15.6)	53.1 (6.6)A			
Morawa	78.5 (13.6)	39.2 (15.6)	49.0 (6.9)a	59.5 (7.8)A		
Black Rd	97.8 (2.2)	75.0 (25.0)	56.5 (7.6)a	41.0 (7.9)a	32.7 (10.8)A	
Kondinin	N/A	N/A	24.1 (24.1)a	N/A	75.0 (25.0)a	N/A

Standard errors are in parentheses. N/A, no cross. Population pairs within subspecies that are significantly different are indicated by different upper-case (intrapopulation crosses) or lower-case (interpopulation within subspecies crosses) letters. Between subspecies, crosses had no significant difference between population pairs.

interpopulation cross combinations for seed weight or seed viability.

Between subspecies, crosses resulted in a very low seed set, ranging from 0.6% to 3% of flowers per inflorescence producing whole seed. Of the seed set from the between-subspecies crosses, viability ranged between 39.2% and 97.8% (Table 6). There was no difference in seed set, seed weight or seed viability between the different subspecies interpopulation crosses (Table 6).

Comparisons between the different cross-types showed no difference between intrapopulation and interpopulation within-subspecies crosses for any of the seed fitness measures for both subspecies. However, both seed set and seed weight were significantly different for subsp. *subulifolia* when intrapopulation and interpopulation within-subspecies crosses were compared with between-subspecies crosses ($P < 0.0001$, $P < 0.0001$, $P = 0.03$; Table 6). Seed set, in particular, was substantially higher in intrapopulation

(mean of 14.8%) and interpopulation within-subspecies (13.6%) compared with between-subspecies crosses (mean of 1.7%) (Table 6). Interestingly, there was no difference for seed viability between intrapopulation (80.6%) and interpopulation within-subspecies (74.1%) crosses compared with between-subspecies crosses (69.9%) in subsp. *subulifolia* (Table 6), so although there was only a very small number of seeds set for between-subspecies crosses, levels of viability in the small number of seeds was like that of the other cross-types. Differences were also seen in subsp. *filifolia* when intrapopulation and interpopulation within-subspecies crosses were compared with between-subspecies crosses, and these differences were not only for seed set ($P < 0.0001$, $P < 0.0001$) and seed weight ($P = 0.0002$, $P = 0.0058$), but also seed viability ($P = 0.03$, $P = 0.006$; Table 6). Seed set was substantially reduced for between-subspecies crosses (mean of 1.7%) compared with intrapopulation (mean of 18%) and interpopulation within-subspecies crosses (mean of 14.9%; Table 6).

However, between-subspecies crosses (69.9%) resulted in higher seed viability than did intrapopulation (52.6%) and interpopulation within-subspecies (48.9%) crosses for subsp. *filifolia* (Table 6), which contrasts with findings for subsp. *subulifolia*.

Discussion

Inbreeding, genetic drift and reduced geneflow can adversely affect the levels of genetic diversity and fitness of propagules in small, isolated populations of threatened plants (Broadhurst *et al.* 2008; Frankham 2012; Leffler *et al.* 2012; Frankham *et al.* 2017). Our investigation can be used to assess options for managing genetic diversity in the endangered *S. filifolia* subsp. *subulifolia*. These options include augmentation of the small population with different genetic material from the large population, admixture of the two known populations to establish a new population, and hybridisation between this subspecies and its sister taxon *S. filifolia* subsp. *filifolia*, for augmentation and/or establishing a new population. In contrast to expectations, we found that the small population of the subsp. *subulifolia* had similar levels of genetic diversity as, and increased seed-fitness measures compared with, the large population of the subspecies, although there was a trend towards a lower genetic diversity in the smaller population. Furthermore, levels of genetic diversity of both populations of subsp. *subulifolia* were comparable to that of the more widespread sister taxon, subsp. *filifolia*, and both subspecies showed high levels of outcrossing, with limited seed production from selfing. We also found that interpopulation cross-pollination within subsp. *subulifolia*, an option that could be used to augment the small population or establish a new admixed population, resulted in increased seed fitness only relative to the large population, and that between-subspecies cross-pollination resulted in minimal seed set and substantially reduced seed weight. Our findings suggest, first, that genetic rescue of the very small population of subsp. *subulifolia* or admixture to establish a new translocation would not necessarily result in large increases in the levels of genetic diversity, but is still a viable management option, and, second, that deliberate hybridisation between the two subspecies to increase genetic diversity is not an option, given the negligible number of seeds produced. Instead, demographic rescue of the small population (Lockier) of subsp. *subulifolia*, sourcing individuals from the large population (Mingenew), is likely to be the best management option to reduce extinction risk of the small Lockier population, given its limited seed availability. Admixture to establish a new population of subsp. *subulifolia* is also a practical management option to reduce extinction risk across the subspecies.

Admixture of several source populations to establish a translocated population or augmentation of a small population with propagules from a large genetically diverse population can increase genetic diversity and enhance establishment success and persistence of the new or augmented population (Weeks *et al.* 2011; Whiteley *et al.* 2015; Shi *et al.* 2018). For *S. filifolia* subsp. *subulifolia*, our data suggested that the case for admixture is not clear-cut. We found that in subsp. *subulifolia*, genetic diversity was not significantly reduced in the small declining Lockier population compared with the substantially larger population, although there was a trend towards lower levels of genetic diversity in this small population. There was very low genetic differentiation between the two populations, and seed-fitness measures were significantly higher in the smaller population than in the large population. However, fitness of seeds resulting from crosses between these two populations was not significantly reduced relative to intrapopulation crosses for the small population, although there was a trend towards lower seed-fitness measures in the interpopulation crosses. Although this does suggest that there is no major fitness advantage in crossing these populations, there is the possibility of a slight increase in genetic diversity. This possibility, combined with the practicality of sourcing a greater number of seed to establish a translocation, makes genetic rescue of the small population and admixture to establish new populations, viable management options.

Our findings indicated that inbreeding depression and reduced genetic diversity are not significantly reduced in the small population of subsp. *subulifolia* compared with the larger populations of this and the other subspecies, and this could be due to the Lockier population once being much larger. Several dormancy mechanisms operate on the seed in this subspecies (Plummer *et al.* 2001), which means that the soil seed bank can persist for some time and could harbour genetic diversity that has not yet been lost from the population. Nevertheless, it is evident that there is a trend towards lower genetic diversity in the Lockier population and there could be significant negative demographic effects associated with its very small (<50 plants) population size and ongoing decline. Whereas reproductive output (i.e. seed set) and germination rate were higher than those of the larger population, other demographic processes such as recruitment failure or mass mortality, may be a greater driver of extinction risk of small populations than are genetic factors (Lande 1988). Indeed, the actual minimum viable population size of the subspecies is unknown but is likely to be more than 50 plants. Therefore, options for demographic rescue to significantly increase population size are still a high priority. This could be achieved by augmentation of the population with *ex situ*-stored seed or *ex situ*-grown seedlings from the small population in the first instance, or new material from the large population in the second instance. In addition, the establishment of a new

population through admixture is a practical and viable strategy. Although this may not result in substantially higher levels of genetic diversity in the translocated population, maintaining larger numbers of plants and more populations will contribute to a reduction in extinction risk (Maschinski and Albrecht 2017).

Although translocation is not currently needed to conserve *S. filifolia* subsp. *filifolia*, information from this study could be of benefit when planning translocations of the closely related subsp. *subulifolia*. Where admixture to establish a new translocated population is considered as a way of increasing genetic diversity, knowledge of the patterns and levels of genetic differentiation can be used to maximise genetic diversity within translocations or augmented populations and minimise potential of outbreeding depression (Weeks *et al.* 2011; Maschinski *et al.* 2013). Our study suggests that admixture of the Tardun, Morawa and Black Rd populations is likely to result in a population with an increased genetic diversity relative to the three source populations, with minimal risk of outbreeding depression because of the low level of genetic differentiation, and with generally equivalent seed-fitness measures to those resulting from intrapopulation cross-pollination. However, our findings suggested that Kondinin be excluded from this admixed population. Although possessing several alleles that could enhance genetic diversity in an admixed population, there was a near-complete lack of seed set from crosses involving this population, much variation in habitat and morphology of plants from this population compared with the other three populations, and all adult individuals had the same multilocus genotype. The latter result may be due to apomixis, a common phenomenon in Asteraceae (Bicknell and Koltunow 2004). However, if it is a new apomictic taxon, further investigation is needed.

Another approach that can help guide seed-sourcing decisions for admixture and translocation planning is to investigate an optimal outcrossing distance to avoid both inbreeding and outbreeding depression (Waser and Price 1989; Hufford *et al.* 2012). We suggest that knowledge of optimal outcrossing distance would benefit translocation planning, both in the seed sourcing strategy, and locating translocation sites to optimise gene flow with wild populations. However, in practice, we found no clear pattern of increased seed fitness over an intermediate geographic distance compared with short or wide geographic distances for the subsp. *filifolia* crosses. Although the small number of populations used is a potentially limiting factor, other studies with relatively few populations have shown clear indications of an optimal distance over a range of geographic distances where outcrossing results in increased progeny fitness (Waser and Price 1989; Billingham *et al.* 2007; Hufford *et al.* 2012; Ayre *et al.* 2019). The same rule does not appear to apply to subsp. *filifolia* and this information can be used to guide translocation planning for the threatened subsp. *subulifolia*.

A key question was whether hybridisation between the subspecies could occur, because it offers potential to increase the genetic diversity in translocated populations of *S. filifolia* subsp. *subulifolia*. Inter-taxon hybridisation is rarely performed intentionally because of the perceived risk of outbreeding depression (Edmands 2007; Baskett and Gomulkiewicz 2011; Hedrick and Garcia-Dorado 2016; Kronenberger *et al.* 2017) often highlighted in risk frameworks (Byrne *et al.* 2011; Frankham *et al.* 2011; Weeks *et al.* 2011; Frankham 2015). However, in rare cases, inter-taxon hybridisation has been used to increase genetic diversity in threatened animal and plant species (Johnson *et al.* 2010; Valdiani *et al.* 2012; Zecherle *et al.* 2021). Further, hybridisation among different taxa occurs widely in Asteraceae (Whitney *et al.* 2010), and so there may be options to increase genetic diversity in the threatened subsp. *subulifolia* through hybridisation with the sister taxon subsp. *filifolia*. Additionally, with only two populations of subsp. *subulifolia* being known at the time of this study, seed was considered a significant limiting factor for translocation, and so hybridisation between the subspecies could have been an avenue to increase seed availability. Unfortunately, artificial cross-pollination between the two subspecies resulted in minimal levels of seed set and, although this seed was viable, such a low rate of seed production would not be a practical way of helping establish a new viable population.

Previous observations by Wilson (1992) suggested that taxon boundaries within *S. filifolia* require further investigation and we also note that the very low level of seed production from crosses between these two subspecies, compared with within the subspecies, seems indicative of reproductive barriers that might be expected between different species. This highlights the importance of adequate taxonomic resolution when considering mixing divergent populations for augmenting or establishing translocated populations of threatened plants. As the results of our study clearly indicated two distinct taxa, consideration should be given to further taxonomic revision of the two subspecies, *S. filifolia* subsp. *subulifolia* and *S. filifolia* subsp. *filifolia*, possibly elevating both to species level, taking into consideration the high level of genetic differentiation between the two subspecies, and the substantially reduced seed set when these subspecies are crossed. *S. filifolia* subsp. *filifolia* also warrants further taxonomic evaluation because there is variation in morphology and some evidence of reproductive isolation between Kondinin and the other three study populations.

The results of our study can inform translocations of other herbaceous or annual species from Asteraceae. Although other studies of herbaceous Asteraceae species have shown increased fitness following inter-population hybridisation, (DeMauro 1993; Pickup *et al.* 2013; Gavin-Smyth *et al.* 2021), our results for *S. filifolia* subsp. *subulifolia* showed that this is not the case and that genetic rescue of the small

population (Lockier) or admixture to establish a new translocation is unlikely to result in substantially increased levels of genetic diversity or increased seed fitness. Our study highlighted that research into the levels of genetic diversity and genetic divergence among populations will be a valuable tool to support seed-sourcing decisions (Hufford and Mazer 2003), particularly before undertaking translocation actions such as admixture and genetic rescue. Where consideration is being given for sourcing new genetic material for translocations that maybe more distantly related (even between subspecies), these genetic studies will provide essential information, as will *ex situ* cross-pollination studies, to reduce the potential for outbreeding depression. In our case, genetic data coupled with cross-pollination studies suggested that demographic, rather than genetic, rescue is the best management option for *S. filifolia* subsp. *subulifolia*.

Supplementary material

Supplementary material is available [online](#).

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Data availability. The data that support this study will be shared upon reasonable request to the corresponding author.

Conflicts of interest. The authors declare no conflicts of interest.

Declaration of funding. Funding for this study was received from the Australian government's National Environmental Science Program Threatened Species Recovery Hub and Murdoch University.

Acknowledgements. We thank Alanna Chant for assistance with leaf and seed collection, Mike Hislop for specimen identification, Andrew Crawford for seed collection of *S. filifolia* subsp. *subulifolia* and assistance with seed germination and seedling propagation, the property owners for permission to sample from the *S. filifolia* subsp. *subulifolia* populations, Margaret Byrne for editing the manuscript and Matthew Williams for statistical advice.

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