Morphological and genetic variation in the rare daisy *Olearia pannosa* subsp. *cardiophylla* (Asteraceae)

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Abstract

Olearia pannosa Hook. subsp. *cardiophylla* (F.Muell.) D.A.Cooke is rare and vulnerable, occurring in small populations in only four reserves within the state of Victoria. Variation within and among populations of this taxon was assessed morphometrically and by random amplified polymorphic DNA (RAPD) analysis. Morphological analysis, including herbarium samples from South Australia, confirmed the distinction of this subspecies from *O. pannosa* subsp. *pannosa* and validated the characters Cooke (1986) used to distinguish the two subspecies.

Genetic variability within subsp. *cardiophylla* was detected within local populations, among populations and between geographic regions in Victoria. The disjunction between populations of northern (Rushworth and Wedderburn) and southern (Brisbane Ranges and Anglesea) regions confirmed by RAPD analysis was supported by morphometric analysis, although Rushworth individuals appear to be more distinct genetically than morphologically. Gene flow between geographic regions appears to be restricted. An assessment of fruit condition and seed viability determined that between 7.5% and 21.2% of all fruits contained viable seed.

Key words: *Olearia pannosa*, Asteraceae, rare taxa, genetic variation, RAPDs

Introduction

Olearia pannosa Hook. (Asteraceae) is a long-lived perennial with plants possibly able to live more than 100 years (Cropper 1993). The species is a sprawling undershrub to 1.5m high with erect or prostrate woody stems. Leaves are alternate, broad-ovate to elliptic and stems and the underside of leaves are densely tomentose, the last of these features presumably prompting the specific epithet '*pannosa*' and the common name 'Velvet Daisy-bush'. Inflorescences are 35 to 75mm in diameter, terminal and solitary. Ray florets have white ligules and the corolla of the disc florets is yellow.

First described by Hooker (1851), *Olearia pannosa* has undergone several taxonomic changes. Following an initial amendment to the description by Lindley and Paxton (1852), the species was subsequently divided into two and placed in separate genera, *Olearia pannosa* and *Eurybia cardiophylla* (Mueller 1853). Simultaneously, Sonder (1853) changed the genus completely, and recognised two species, *Steetzia pannosa* and *S. muelleria*. It is unknown whether the two authors collaborated prior to publishing their descriptions in the same journal volume. Despite Sonder's nomenclature, Mueller described *Olearia pannosa* as *Eurybia pannosa* in 1865 (Mueller 1865a), placing the two species back into the same genus. In the same year, Mueller described *Eurybia pannosa* as *Aster pannosus* (Mueller 1865b) and once again recognized the two species as distinct at the generic level. The taxonomy was then untouched until 1986, when D.A. Cooke described the species as *Olearia pannosa*, and recognized two subspecies: *Olearia pannosa* subsp. *pannosa* and *Olearia pannosa* subsp. *cardiophylla* (F.Muell.) D.A.Cooke, based on leaf shape and tomentum of lower leaf surfaces and peduncles.

Olearia pannosa is endemic to South Australia and Victoria. In South Australia, populations occur on the Eyre Peninsula, Yorke Peninsula, around Adelaide and

Kangaroo Island, and the two subspecies *pannosa* and *cardiophylla* (F.Muell.) D.A.Cooke are also recognised. Victorian specimens are referred to *Olearia pannosa* subsp. *cardiophylla,* although Walsh and Lander (1999) suggested that this subspecies required recognition at the rank of species. Distributions of the two subspecies are presented in Figure 1.

In Victoria, *Olearia pannosa* subsp. *cardiophylla* is rare and listed as threatened under the Victorian Flora and Fauna Guarantee Act 1988 (Anon 2000), and an action statement has been prepared (Hills *et al.* 2003). However, the conservation status is not recognized at national level, and the species is not listed under the Commonwealth 'Environment Protection and Biodiversity Conservation Act' 1999 (Hills *et al.* 2003).

Victorian populations of *O. pannosa* subsp. *cardiophylla* are restricted to shallow, rocky soils in woodland and open forest areas usually dominated by *Eucalyptus macrorhyncha* F.Muell. ex Benth., *Acacia pycnantha* Benth. and *Xanthorrhoea australis* R. Br., where mean annual rainfall ranges from 600 to 650 mm. It occurs across a range of aspects, except
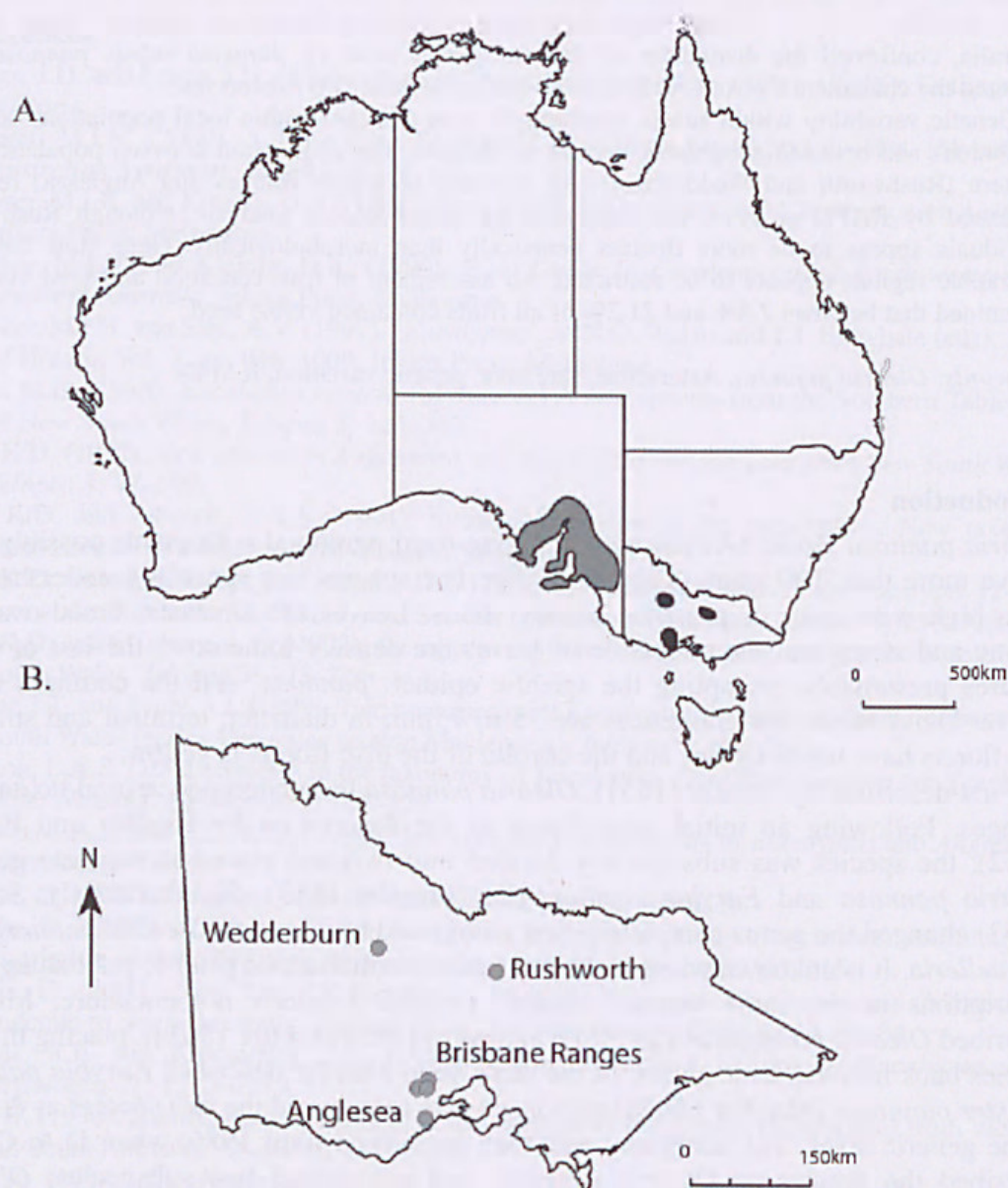

Figure 1. (A.) Distribution of *Olearia pannosa* in Australia. Grey area: Distribution of subsp. *cardiophylla* and subsp *pannosa* in South Australia. Black area: Distribution of subsp. *cardiophylla* in Victoria. (B.) Distribution of *Olearia pannosa* subsp. *cardiophylla* populations in Victoria.

on exposed north-western slopes (Galbraith 1967; Wisniewski *et al.* 1987; Cropper 1993).

A number of small populations occurs in the Brisbane Ranges, and also near Wedderburn, Rushworth and Anglesea (Fig. 1) with some morphological variation noted across this range. Few known large plants remain, and populations occur in unprotected areas where they are subject to threats such as browsing by mammals, roadworks, erosion and rubbish dumping (Wisniewski *et al.* 1987; Bartley 1990).

Flowering occurs between August and October, and seed is ripe from early December until May (Wisniewski *et al.* 1987; Cropper 1993). Seed set appears to be low, and Bartley (1990) reports that unexpanded fruits may be damaged by mould or a fungus-eating beetle (*Corticaria* sp.). Seedling recruitment is negligible (Wisniewski *et al.* 1987) and, with the added pressure of habitat loss, poses a threat to long-term survival of the subspecies. Although Cooke (1986) describes the species as 'root suckering', Wisniewski *et al.* (1987) confirmed by sectioning that shoots arise from decumbent stems. Cloning by shoot suckering has been found in at least one population in the Brisbane Ranges National Park, Victoria (N. Walsh pers. comm.). The small shoot suckers, although resembling seedlings, can be distinguished from the latter by the absence of small teeth on the leaf margins (Cropper 1993).

The aims of this study were to clarify patterns of morphological and genetic variation and provide data on seed set to improve conservation strategies. *Olearia pannosa* was compared morphologically across its range to evaluate the current taxonomic status of the two subspecies. The genetic variation of all known populations of the rarer subspecies *cardiophylla* in Victoria was assessed using the molecular random amplified polymorphic DNA (RAPD) technique. Seed set, viability and predations were also assessed.

Methods

Morphology

Within Victoria, *Olearia pannosa* subsp. *cardiophylla* populations were sampled from four locations, including four populations in and around the Brisbane Ranges (Demott's Rd, Anakie Gorge, Maude–Sheoaks Rd and Steiglitz), two at Anglesea (Point Addis and Ironbark Basin) and single populations at Wedderburn and Rushworth. At least five plants were sampled from each population except where small population size and rarity restricted sample size to three.

In total, collections for morphological analysis included 59 samples from nine sites within the four locations. One stem was taken from each plant for morphological measurements, preferably with a terminal, solitary inflorescence. Three to five specimens from each site were lodged as voucher herbarium specimens at The University of Melbourne Herbarium (MELU) and the National Herbarium of Victoria (MEL). For this study, herbarium specimens of both subspecies of *O. pannosa* at MEL and MELU supplemented these collections and included specimens from South Australia. Eighty-one specimens were initially compared for 15 morphological leaf characters, including binary, multi-state and continuous measurements (Table 1). However, six characters were uninformative and removed from the analyses (leaf width, leaf width 1 cm from tip, leaf length: leaf base at widest point, presence of hairs on upper lamina, colour of lower lamina, leaf apex angle). In an attempt to eliminate any ontogenetic variation, only mature leaves were measured. Although five leaves were considered a reasonable minimum sample size, only three mature leaves were available for many specimens. Hence, for consistency, the three largest leaves on each specimen were measured and mean values recorded.

Ratios were derived from some of the continuous character measurements in order to express leaf shape. The use of ratios allowed the inclusion of more informative characters in the analysis where the constituents were not largely variable. The use of ratios has been criticised by some authors (Atchley and Bryant 1976; Phillips 1983; Frampton and Ward

Table 1. Characters scored for morphometric analysis

Continuous characters
1. Leaf length (mm)
2. Leaf base to widest point (mm)
3. Petiole length (mm)

Ratios
4. Leaf length: leaf width at widest point (mm)
5. Leaf width at widest point: leaf width 1 cm from tip
6. Leaf apex angle

Binary and multistate characters
7. Leaf lamina elliptic to ovate/ broad-ovate (0/1)
8. Fine hairs on lower surface of leaf appressed strongly/ partially (0/1)
9. Leaf base obtuse/ acute/ truncate- shallowly cordate (0/1/2)

1990) because ratios may have undesirable statistical properties and often result in a high correlation between the ratio and its constituent size variables (Phillips 1983). Problems with ratios have been overcome in this study by the removal of the least informative variable, which is in effect redundant, adding nothing new to the analysis (Wright and Ladiges 1997).

Morphological data were analysed phenetically using the PATN pattern analysis package (Belbin 1995). A matrix of dissimilarity was produced using the Gower metric, and a hierarchical clustering was produced using the Unweighted Pair Group Method of Averaging (UPGMA) fusion strategy. The cophenetic correlation coefficient was used to measure how well the dendrogram represented the information in the dissimilarity matrix. An ordination of the data was produced in two and three dimensions using the non-metric multidimensional scaling (NMDS) technique. A two dimensional ordination was produced using the PATN package, while a three dimensional ordination was performed using the Ntsys numerical taxonomy and multivariate analysis system (Rohlf 1998) in order to compare these data with the genetic analysis.

A comparison of appressed leaf hairs in *O. pannosa* subsp. *pannosa* and subsp. *cardiophylla* was made using 50 mm^2 sections of leaf material. Sections were dehydrated in an ethanol series (70, 80, 90 and 100%), mounted on carbon-coated stubs and coated with silver. Specimens were then sputter coated with gold, viewed under a Phillips FEG Scanning Electron Microscope (2.00 kV) and photographed at x30 and x90 magnifications.

Genetic analysis

RAPD analysis is one of the most extensively used DNA fingerprinting techniques, based on the Polymerase Chain Reaction, for measurements of diversity (Karp *et al.* 1996; Hoelzel 1998). RAPD technique has proved a useful method for detecting polymorphisms where *a priori* knowledge of the genome is not available and can enable assessment of genetic relationships and diversity estimations among individuals (Maxted *et al.* 1997; Ayres and Ryan 1999). The dominant RAPD markers reveal polymorphism as band presence/absence, with each band assumed to represent a single locus (Krauss and Peakall 1998).

For DNA isolation and RAPD analysis, four or five young leaves were collected from 10–20 plants within each population (with the exception of Wedderburn, where only two samples were taken from the small patch at this site). Care was taken to avoid sampling obvious clones, or suckers, and where possible samples were taken at least 10 metres apart. The 47 samples were wrapped in moist paper towel and kept on ice for

transportation, before storage at –86°C within 24 hours of collection.

DNA was isolated from approximately 50 mg of frozen leaf material from a number of samples from each population (Wedderburn, 2; Rushworth, 10; Anglesea, 18; Brisbane Ranges, 17) using the Nucleospin Plant DNA extraction kit. Twenty-nine RAPD primers were screened, and five were chosen for the analysis. Seventeen did not amplify the DNA at all, while remaining primers only amplified a small number of fragments. PCR reaction mixtures (20 μl final volume) contained 10 μl HotStartaq mastermix reaction buffer (Promega) (containing *Taq* polymerase buffer, *Taq* polymerase, dNTPs, MgCl$_2$ and BSA (bovine serum albumin), 8.2 μl H$_2$O and 1.0 μl of template DNA (approx. 20 ng). Amplification was carried out in an Eppendorf Mastercycler ® gradient thermocycler programmed as follows: initial 15 m at 95°C, then 35 cycles of 30 s at 95°C denaturation, 30 s 38°C annealing, 30 s 72°C extension and an additional 4 m 30 s period for extension followed the last cycle. Reproducibility of amplification product profiles between reactions was tested by performing duplicate PCR runs for three samples with each primer, as in Cambecedes *et al.* (1999), and a dilution series of four Rushworth samples was used to ensure that the RAPD patterns were consistent over a range of DNA concentrations. A negative control with DNA omitted was included for every PCR run to check for contamination.

The amplification products were separated by electrophoresis in 1.5% agarose using x 1 TBE buffer at 80 V for 1 to 1.5 hours, depending on the primer. Ethidium bromide was added to the agarose to stain the DNA. Resultant gels were photographed on an ultraviolet transilluminator using Polaroid film, or scanned using Kodak DCS 120 imaging software and saved as a digital image.

Amplified fragments were scored as present or absent, with ambiguous and monomorphic bands removed from the data set. Typically, 5–20 bands were produced per primer. A similarity matrix was generated using the Jaccard coefficient, which excludes negative matches, because the mutual absence of bands among individuals may not be the result of a common cause. An agglomerative hierarchical classification was generated using the UPGMA method of clustering. Three-dimensional NMDS ordinations were also generated from the similarity matrix. All analyses were performed using the Ntsys numerical taxonomy and multivariate analysis system (Rohlf 1998).

Seed production and viability

In January 2002, 20 capitula were collected, one per plant, at random from each of three locations (Rushworth, Anglesea and Brisbane Ranges) for the assessment of fruit condition and seed viability. The mean number of fruits per capitulum was calculated for each population under each of the three categories specified by Wisniewski *et al.* (1987) and Bartley (1990): (i) fruits unexpanded or empty, (ii) partially or completely damaged by predation, and (iii) filled fruits with developed and apparently viable seed.

The viability of approximately 100 seeds, pooled from all capitula collected, was tested using tetrazolium. Seeds were cut in half longitudinally and seed coats removed. Half of each seed was soaked in a filtered TTC (triphenyl tetrazolium chloride) solution (1g in 100 ml phosphate buffer, pH 6.5–7.0) for 40 minutes in darkness at 30°C and rinsed in sterile distilled water. Embryos were considered viable if completely coloured pink or red and not viable if embryos were partially coloured or white, yellow or brown (Rasmussen 1995).

A test of self-compatibility was done during this study on the Brisbane Ranges populations. Cotton interfacing bags were tied over capitula in bud for six plants. After a few weeks the bags were removed to determine whether any flowers had set seed.
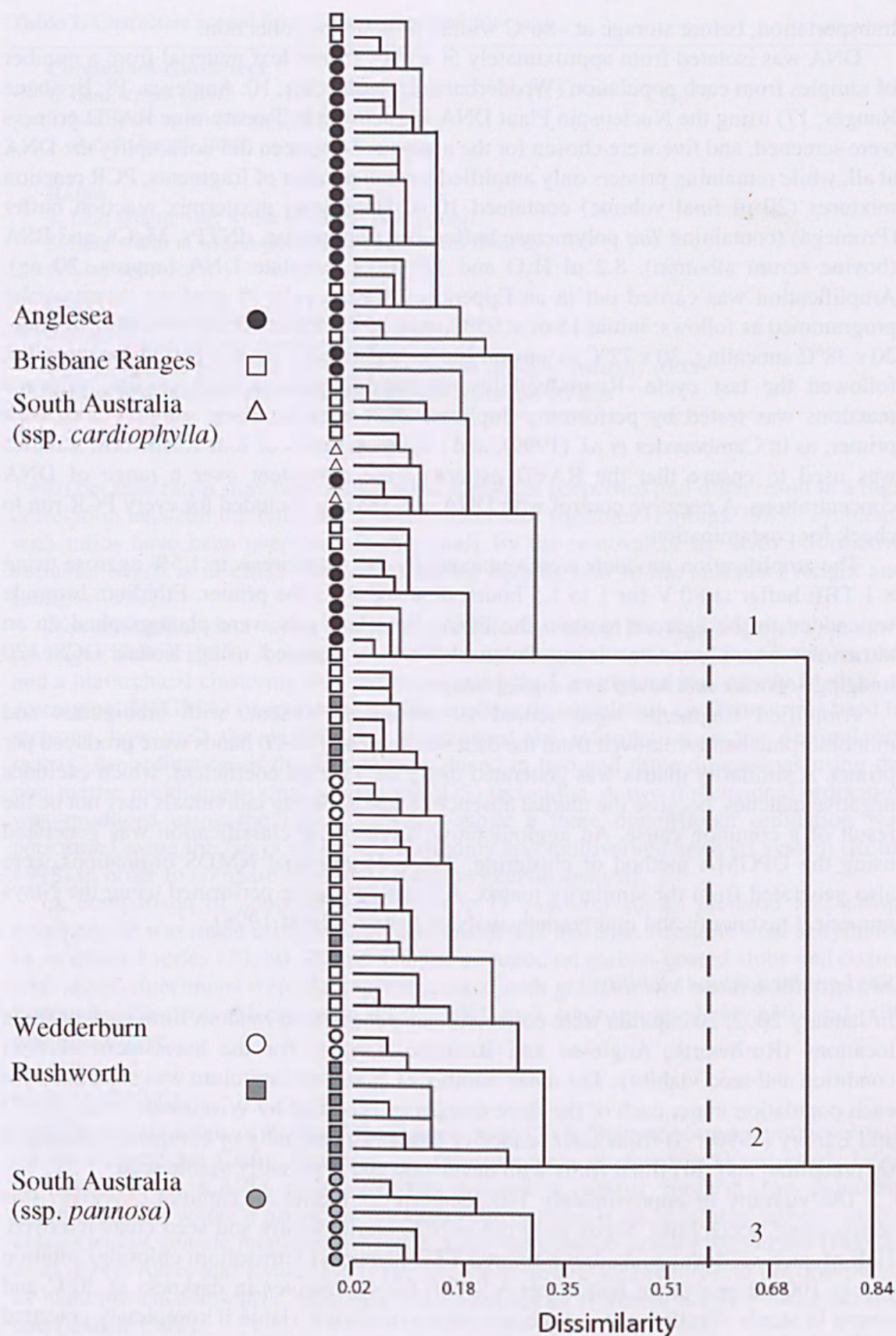
Figure 2. Agglomerative hierarchical classification of 81 specimens based on morphology using UPGMA clustering technique. Co-phenetic correlation coefficient = 0.81. Groups referred to in the results and discussion are numbered above the branches on the dendrogram.

Results

Morphology

The hierarchical classification of the 81 specimens of subsp. *cardiophylla* and subsp. *pannosa* (Fig. 2) identified three main groups with a high correlation coefficient (0.81). Group one contains all individuals of subsp. *cardiophylla* from Anglesea (Point Addis and Ironbark Basin), whilst group two contains all individuals from Wedderburn and Rushworth. Individuals from populations within the Brisbane Ranges region were distributed in groups one and two, with Demotts Rd and Steiglitz populations mostly clustering within group one and Maude–Sheoaks Rd and Anakie Gorge populations clustering entirely within group two. Herbarium specimens of subsp. *cardiophylla* from South Australia cluster in group one with the field-collected specimens from Anglesea and the Brisbane Ranges in Victoria. Group three consists solely of herbarium specimens of subsp. *pannosa* from South Australia.

Three binary characters, appression of hairs on the underside of the leaf (character 8), leaf lamina shape (character 7) and leaf base shape (character 9) were highly informative in separating the groups (Kruskal-Wallis values 66.16, 40.31 and 75.70 respectively). Leaf hairs of both subspecies are shown in Figure 3, illustrating that the hairs of group 3 are markedly more appressed than those of groups 1 and 2. Of the continuous characters, the most informative were the ratio of leaf length to width (character 4, Kruskal-Wallis value 42.65) and leaf apex angle (character 6, 20.14). The least informative character in separating the groups was distance between leaf base and widest point (character 2, 8.81). An example of the variation observed in leaf lamina shape is shown in Figure 4.

The NMDS ordination (Fig. 5) demonstrated two discrete groups corresponding to the two subspecies; subsp. *pannosa* (group 3) and subsp. *cardiophylla* (groups 1 and 2). Individuals from the Anglesea region, and those from Wedderburn and Rushworth, tended to cluster separately, based on leaf lamina shape and angle of the leaf base (Table 2). However, discrete groups were not evident in the ordination. Populations within the Brisbane Ranges region were the most variable. Half of those individuals grouped closely
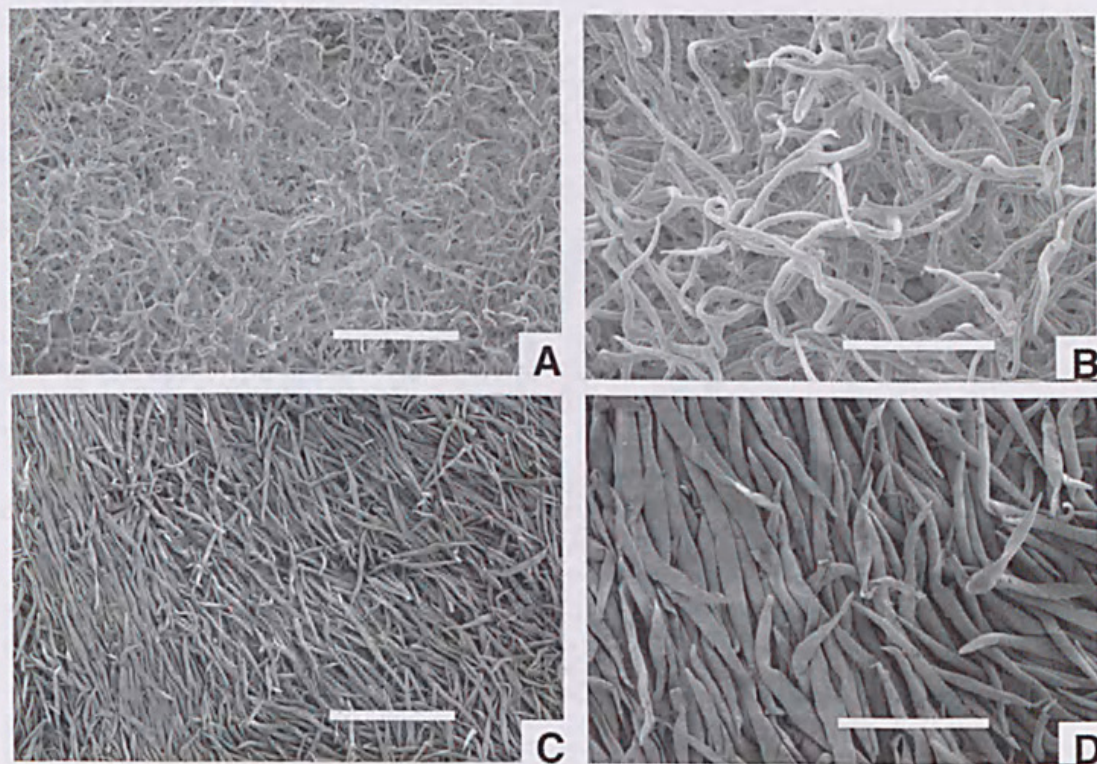

Figure 3. Comparison of leaf hairs on the underside of leaves in *O. pannosa* subsp. *cardiophylla* (A and B) and *O. pannosa* subsp. *pannosa* (C and D). Scale bar = 200 µm (A and C), 500 µm (B and D).
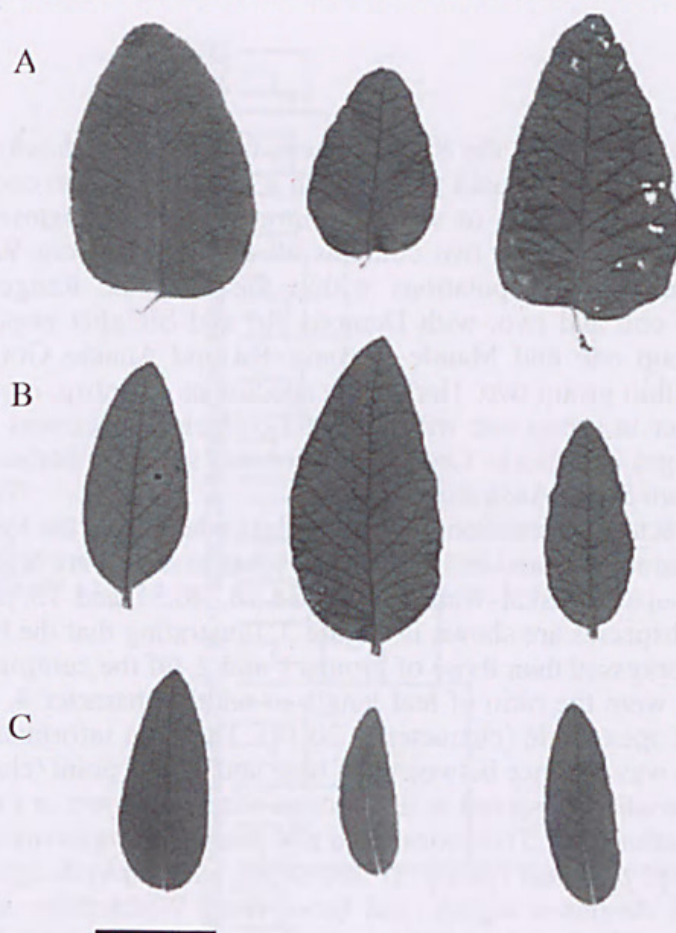
Figure 4.   Variation in leaf shape of *O. pannosa* subsp. *cardiophylla* in Victoria. Three leaves are
shown from each geographic location: (A) Anglesea, (B) Rushworth and (C)
Wedderburn. Brisbane Ranges populations exhibit a range of forms, corresponding to
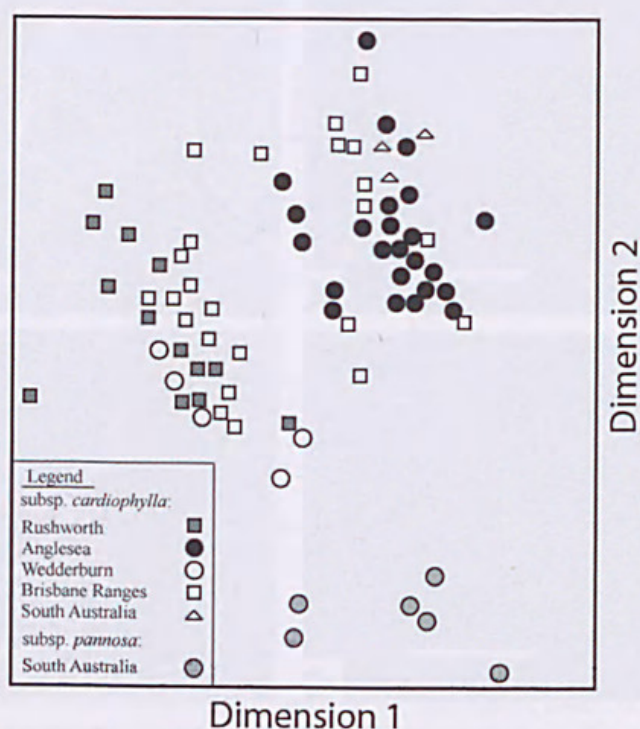A, B or C. Scale bar = 2.5 cm.

Figure 5.   NMDS ordination in two dimensions of individual plants based on morphometric data.
Stress = 0.14.

Table 2. Kruskal-Wallis statistics and mean values (and range) for nine morphological characters, based on groups defined in Figure 2.

Character	Kruskal-Wallis Statistic	Group 1	Group 2	Group 3
Leaf lamina elliptic to ovate/broad ovate%	40.31	80.5	0	66.7
Fine hairs on lower surface of leaf appressed strongly/ partially %	66.16	100	96.9	0
Leaf base obtuse/ acute/ truncate-shallowly cordate %	75.70	100	9.4	0
Leaf length (mm)	10.63	68.2 (45–86)	66.6 (47–92)	49.8 (40–64)
Leaf base to widest point (mm)	9.01	19.3 (14–26)	22.0 (16–31)	20.6 (15–28)
Petiole length (mm)	13.23	7.3 (4–12)	6.9 (3–8)	5.5 (4–6)
Leaf length: leaf width at widest point	42.99	0.7 (0.5–1.7)	0.5 (0.4–0.8)	0.4 (0.3–0.5)
Leaf width at widest point: leaf width 1 cm from tip	12.51	2.0 (1.6–2.6)	1.9 (1.4–2.7)	1.6 (1.3–1.8)
Leaf apex angle	21.27	64 (50–77)	58 (42–80)	39 (30–50)

with those from Anglesea, and the other half with the Wedderburn and Rushworth individuals. One Rushworth specimen appears as an outlier in the ordination due to the occurrence of appressed hairs on the underside of the leaves, as was seen in specimens of subsp. *pannosa*.

Genetic Analysis

Genetic analysis was conducted on specimens sampled from Victoria only.

Five primers (OPA-2, OPA-3, OPA-13, OPB-10 and OPF-4) generated a total of 42 RAPD bands, from which 29 polymorphic fragments were scored. No band was exclusive to an individual population apart from a single band found in individuals from Rushworth. Ironbark Basin and Wedderburn individuals shared a single band with three Rushworth individuals but with no other individuals from the Anglesea or Brisbane Ranges populations.

Cluster analysis of the 47 specimens shows two main groups (Fig. 6). Group one includes all individuals except those from Rushworth, which form group two. Two subgroups are formed within group one. Sub-group A includes half of the Anglesea individuals and a single Brisbane Ranges individual, while the other half of the Anglesea individuals group with remaining Brisbane Ranges individuals and the two samples from Wedderburn in Sub-group B.

The three dimensional NMDS ordination (Fig. 7) identified similar clustering to the dendrogram. Rushworth individuals cluster together, as do individuals from the two Anglesea populations. Populations within the Brisbane Ranges region also tended to cluster together. Although the dendrogram showed the two Wedderburn samples to be identical, the ordination indicates that they have a small amount of genetic variation, with two band differences and therefore, two individuals are present.

The results indicate that there is genetic variation both within and among local populations as well as between geographic regions. A pattern of divergence between northern (Wedderburn and Rushworth) and southern (Anglesea and Brisbane Ranges) populations is evident. The four Brisbane Ranges populations, the closest geographically to one another, were the most similar. A minimum spanning tree, depicted as a dendrogram, effectively displays these results (Fig. 8).
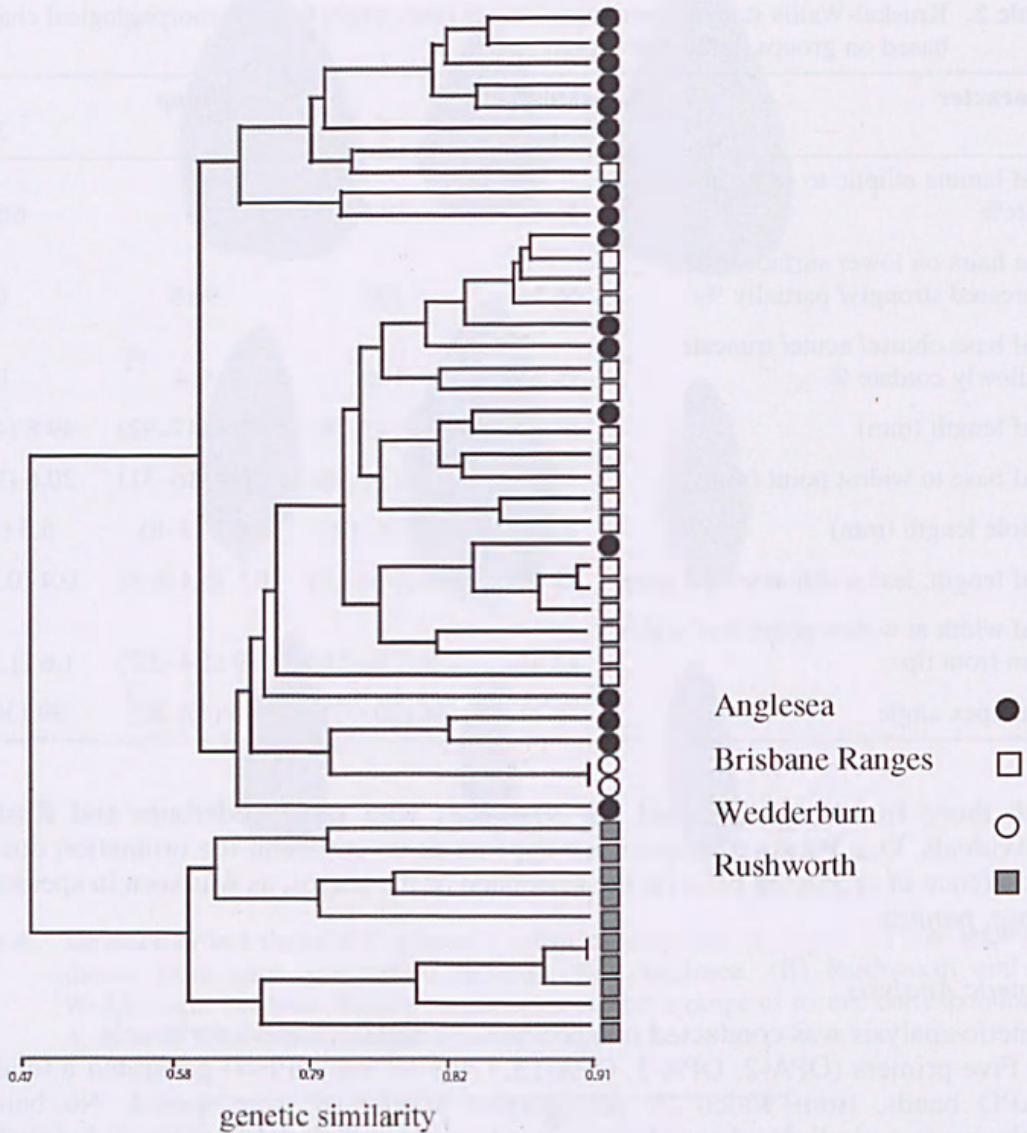
Figure 6. UPGMA classification of individual plants based on RAPD data. Co-phenetic correlation coefficient = 0.83. Groups discussed in the text are indicated on the dendrogram.

Fruit condition and seed viability

A total of 1131 fruits from Rushworth, 1097 from the Brisbane Ranges region and 1198 from the Anglesea region was extracted from 20 fruiting capitula from each location. Fruits were categorised as: unexpanded or empty; partly or completely damaged by predation; or filled fruits with developed and apparently viable seed (Table 3). Point Addis had the highest percentage of filled fruit, at 29.2%. Anakie Gorge and Rushworth populations were almost as high, with 27.0% and 17.2% of filled fruit respectively, while remaining populations had less than 6% of filled fruit. Capitula collected from Demotts Rd contained no filled fruit. Anakie Gorge had the highest level of predated fruit, with 29.5% damaged. Point Addis was the only other population with a significant level of predation (23.7%). The majority of fruits in all populations were unexpanded or empty; 99.7% of fruits collected from Steiglitz fell into this category.

Staining with TTC revealed that on average 91% of filled seeds were viable: 93.4% of Rushworth seeds, 93.1% of Brisbane Ranges seeds and 87.2% of Anglesea seeds. Seed was set in every bagged inflorescence and hence it was concluded that subsp. *cardiophylla* is either self-fertile or that apomixis can occur.
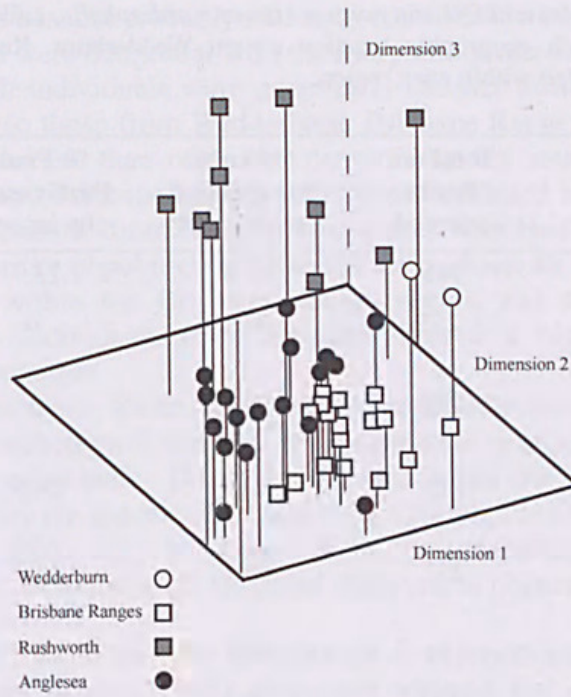
Figure 7. NMDS ordination in three dimensions of individual plants based on RAPD data. Stress = 0.29.
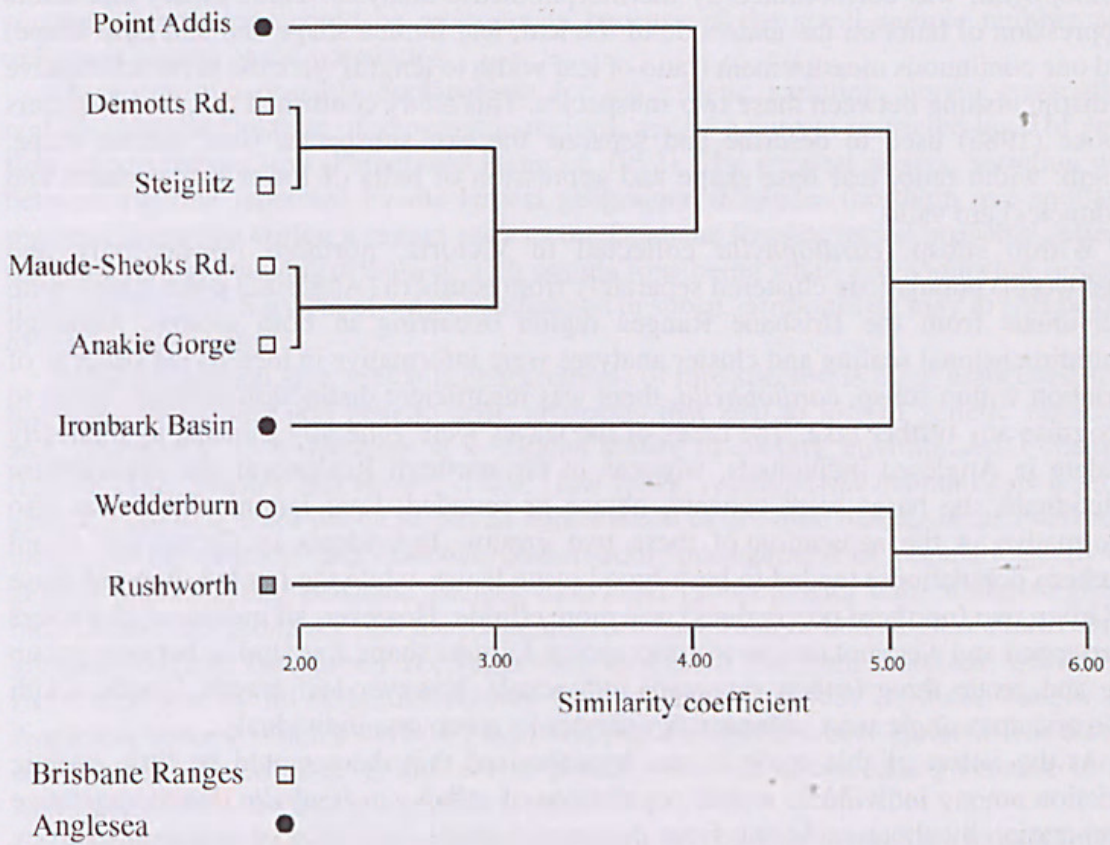

Figure 8. Minimum spanning tree (presented as a dendrogram) showing phenetic relationships of populations based on RAPD data.

Table 3. Characteristics of fruit of *Olearia pannosa* subsp. *cardiophylla,* collected from 20 mature capitula from each geographic location except Wedderburn. Results are shown for populations sampled within each region.

Population	Total no. fruits counted	%Fruits unexpanded and/or empty	% Fruits Partly eaten by insects	% Fruits filled
Point Addis (Anglesea)	945	47.1	23.7	29.2
Ironbark Basin (Anglesea)	253	94.1	0	5.9
Maude-Sheoaks Rd (Brisbane Ranges)	297	96.0	0	4.0
Anakie Gorge (Brisbane Ranges)	278	43.5	29.5	27.0
DeMotts Rd (Brisbane Ranges)	231	86.1	13.9	0
Steiglitz (Brisbane Ranges)	291	99.7	0	0.3
Rushworth	1131	79.1	3.6	17.2

Discussion

The distinction between the two subspecies, *Olearia pannosa* subsp. *pannosa* and subsp. *cardiophylla*, was corroborated by the morphometric analysis. Three binary characters (appression of hairs on the underside of the leaf, leaf lamina shape and leaf base shape) and one continuous measurement (ratio of leaf width to length) were the most informative in distinguishing between these two subspecies. This study confirmed that the characters Cooke (1986) used to describe and separate the two subspecies (leaf lamina shape, length: width ratio, leaf base shape and appression of hairs of lower leaf surfaces and peduncles) are valid.

Within subsp. *cardiophylla* collected in Victoria, northern (Wedderburn and Rushworth) populations clustered separately from southern (Anglesea) populations, with individuals from the Brisbane Ranges region occurring in both groups. Although multidimensional scaling and cluster analyses were informative in identifying patterns of variation within subsp. *cardiophylla*, there was insufficient distinction between forms to recognise any further taxa. The bases of the leaves were generally truncate to shallowly cordate in Anglesea individuals, whereas in the northern Rushworth and Wedderburn individuals, the bases were cuneate, obtuse or rounded. Leaf lamina shape was also informative in the separation of these two groups. Individuals in Group one (from southern populations) tended to have broad ovate leaves while the lamina shape of those in Group two (northern populations) was more elliptic. However, all measured characters overlapped and were not unique to either group. Lamina shape was similar between group one and group three (subsp. *pannosa*) individuals, however leaf length, length: width ratio and apex angle were substantially greater in group one individuals.

At the outset of this study it was hypothesised that there would be little genetic variation among individuals within populations of subsp. *cardiophylla* due to vegetative regeneration by shoot suckering from decumbent stems, limited seed dispersal (Bartley 1990) and the small, isolated nature of the populations. The RAPD analysis indicated, however, that within subsp. *cardiophylla* genetic variation among and within populations was evident in Victoria. No single population was found to be a clone, indicating that

sexual reproduction has and probably still does contribute to recruitment in populations.

The RAPD data were congruent with the morphological analysis in showing that the northern Rushworth individuals were genetically distinct from individuals in southern populations, and also those from Wedderburn. Brisbane Ranges populations were more variable in both datasets than other populations. Among southern populations, Point Addis (Anglesea) and Brisbane Ranges individuals exhibited some genetic differences. Individuals from Ironbark Basin (Anglesea) clustered with Brisbane Ranges individuals, even though the former population is geographically closer to Point Addis. Variation in local populations within the Brisbane Ranges region was evident from the cluster analysis, although individuals from Steiglitz showed a high degree of similarity, clustering closely together.

In the genetic analysis, Rushworth clustered separately from remaining populations, whereas in the morphological analysis this population grouped with Wedderburn and Brisbane Ranges individuals. This indicates that while the two populations overlap morphologically they are genetically distinct. Morphological analysis included only leaf characters, hence there may be other informative characters that distinguish these populations, or the morphological variation observed is phenotypic and not reflected in the genotype.

Two subgroups formed between Wedderburn, Rushworth and Anglesea individuals in the genetic analysis, where RAPD characters allowed for greater resolution of the variation. The few specimens available from the Wedderburn population were less than the ideal number of individuals sampled per population. The results were, nevertheless, included to indicate their genetic variability. The two samples from the small Wedderburn population appeared to be morphologically identical, but the two band differences noted from the RAPD profiles indicated that the small multi-stemmed bush, originally assumed to be one plant, is more than one individual. The placement of Wedderburn samples with southern populations could be an anomaly because of the small sample number and unknown history of the population.

There are two possible explanations for the genetic variation among geographic regions: historic isolation of geographic regions, and/or a current restricted level of gene flow due to habitat loss (Parker and Hamrick 1992). The greatest genetic variation was between regions separated by the largest geographic distances (northern and southern regions). Variation within a region such as the Brisbane Ranges region probably reflects the plant's fragmented distribution, with populations being small and somewhat isolated from one another. Survival of small populations in subsp. *cardiophylla* may in part be due to self-compatibility.

Small population size, which is characteristic of rare species, is often associated with increased inbreeding and genetic drift, processes that lead to loss of genetic variation, and, potentially, to a decrease in a species ability to survive environmental changes (Lande 1988; Tansley and Brown 2000). How subsp. *cardiophylla* maintains its level of genetic variation is not yet clear, but an appreciation of possible mechanisms involved is important for efficient and effective conservation management of populations. In the genetic analysis, one Brisbane Ranges individual grouped with half of the Anglesea individuals (sub-group A), and the remaining Brisbane Ranges and Anglesea individuals grouped together (sub-group B), suggesting that there has been historical gene flow. However, there are no herbarium records for populations between Brisbane Ranges and Anglesea, hence it is unknown how long these populations have been isolated. The cluster of Rushworth individuals (group two) is possibly a result of inbreeding because of the small population size and geographic isolation.

A comparison of previous records (>ten years old) of area covered by populations of subsp. *cardiophylla* indicates some decline, but also persistence in areas; some small populations have survived in areas for over 50 years (M. Bartley pers. comm.). There are several features of small, rare populations that might play a role in preventing genetic

deterioration. The ability of *Olearia pannosa* subsp. *cardiophylla* plants to sucker may provide a mechanism to survive fire and disturbance, and infrequent but significant seedling establishment could act to maintain genetic variation. Bartley (1990) notes that subsp. *cardiophylla* has slow initial shoot growth, invests early in underground storage structures and contains axillary vegetative buds and dormant buds in older stems, which could sprout after plant damage by fire, browsing or other physical injury. The species is also slow-growing and long-lived, possibly more than 100 years (Cropper 1993). One Brisbane Ranges individual was noted by H.A.Boardman to be at least 38 years old (Wisniewski *et al.* 1987). Slow growth was observed during this study in seedlings planted at Wedderburn by the Department of Sustainability and Environment.

Although insect predation has previously been noted to have an impact on the number of viable seeds in subsp. *cardiophylla* (Bartley 1990), it was found in this study that predation had a minimal impact in most populations, with levels of unexpanded or empty fruits more significant. The low level of filled fruits and rarely observed seedlings is an indication of the species' limited regeneration by seed, so vegetative reproduction is an important mechanism for retaining genotypic variation. Previous studies of fruit condition in populations from Brisbane Ranges and Anglesea (Wisniewski *et al.* 1987) revealed that only 3% of fruits contained developed seed (filled fruits), and up to 68% were damaged by predation. In this study, 29.2% of fruits in the Point Addis population contained developed seed, while the highest level of predation was comparably lower, at 29.5% at Anakie Gorge. This is an indication that predation is currently having a relatively small effect on the development of seed.

The majority of filled fruits from populations at Rushworth, Brisbane Ranges and Anglesea were determined to contain viable seed (>87%), so even though the number of filled fruits was relatively low, viable seed had been set and regeneration from seed is potentially possible. Previous germination trials found that up to 90% of filled fruits produce seed that germinates (Bartley 1990), which corresponds to the level of viable seed (91%) found in this study. Taking into account the level of viability and the percentage of filled fruits (Table 3) 5–26% of fruits per population contain potentially germinable seed.

It was concluded from this study that plants are self-compatible, hence inbreeding may be a problem. James (2000) showed that in three genera, *Laxmannia, Drosera* and *Stylidium,* species that exhibit high levels of genetic diversity have limited evolutionary potential because of impediments to recombination at meiosis, whereas species with little genetic diversity within populations and few or no lethal polymorphisms exhibit few restrictions to recombination. He considered that if populations with historically high levels of genetic diversity are forced to inbreed, due to reduced population size and increased geographic isolation, then polymorphic recessive lethals could cause low percentages of filled fruits (James 2000). Conversely, recessive lethals may provide genetic barriers to inbreeding, and hence enable the small populations to maintain levels of genetic diversity. Further study of female and male function including pollen viability is required to identify limits to sexual reproduction. A comparison of seed set following controlled selfing and cross pollination would be useful in determining whether lethal polymorphisms are present in populations of *Olearia pannosa* subsp. *cardiophylla.*

An important management consideration is that small populations are vulnerable to events that might have a relatively small impact on larger populations. Such an event occurred when a colony of 30–40 mature plants at Meredith, near the Brisbane Ranges, was severely damaged by roadworks in about 1973. In 2001, one of the study populations at Point Addis was damaged by trail bike users.

In order to improve long-term persistence, establishment of new populations in surrounding areas and augmentation of small populations is important, particularly for Wedderburn, which currently consists of only one small clump. The Department of Sustainability and Environment has replanted 29 seedlings and cuttings (originating from

the small clump) in a nearby road reserve, which will increase the effective population size. Long term monitoring will be required to see if any seedling recruitment occurs.

Protection of the habitat is the first priority for *in situ* conservation. Considering its genetic uniqueness, the conservation of the Rushworth population, which currently resides on a recently sold block of land, is essential. Either seedlings or cuttings could be used for population reinforcement or reintroduction provided that consideration is given to the maintenance of genetic diversity. The small populations at Rushworth and Wedderburn provide a dilemma for conservation because there is a risk that translocation procedures that combine divergent genomes from differentiated populations (ie. northern and southern populations) may result in reduced fitness (James 2000). However, their small population size currently makes them highly vulnerable to genetic erosion and extinction resulting from habitat loss.

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