BANKWEST LANDSCOPE PROJECT FINAL REPORT

ASSESSING THE TAXONOMIC STATUS OF *BANKSIA BROWNII* AND PATTERNS OF GENETIC DIVERSITY IN EXTINCT AND EXTANT POPULATIONS

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Introduction

Banksia brownii was declared as Rare Flora under the Western Australian Wildlife Conservation Act 1950 in November 1980 and is currently ranked as Critically Endangered (CR) under World Conservation Union Red List criterion A3ce (IUCN, 2001). Although it was previously ranked as Endangered the threat category of *B. brownii* was upgraded because of a projected decline in population size of ≥ 80% within the next three generations due to *Phytophthora* dieback. It is also listed as Endangered under the Commonwealth Environment Protection and Biodiversity Conservation Act 1999 (EPBC Act).

Seventeen populations, together containing approximately 10,000 mature plants, are currently known to be extant and ten populations have been recorded as extinct since 1996 due to *Phytophthora* dieback.

B. brownii grows in a range of habitats from mountain tops and slopes, in thicket and mallee-heath on rocky sand clay loam soils in the Stirling Range; and in mallee heath and low woodland, on sandy clay soils on lateritic ridges or granite in southern populations. The associated shrubland is rich in proteaceous and myrtaceous species. It occurs within the Montane 01 TEC (Montane Thicket and Heath of the South West Botanical Province, above approximately 900m above sea level) which is listed as Endangered under the Commonwealth Environmental Protection and Biodiversity Conservation Act 1999 and the Montane Mallee Thicket of the Stirling Range TEC (Mallee-heath and mallee-thicket community on mid to upper slopes of Stirling Range mountains and hills)

The type collection of *B. brownii* (Baxter ex R. Br) was made from King George Sound in 1829 by William Baxter who described it in 1830. Collections by various botanists since 1829 include those from Vancouver Peninsula in 1902, Millbrook in 1917, and the summits of Bluff Knoll and Coynanarup (Stirling Range National Park) in 1923.

Two forms of *Banksia brownii* are recognised (Keighery 1988); a northern form confined to the Stirling Range with short, thin, hard leaves and a southern form with long, wide, soft leaves occurring in the Albany-Cheyne Beach area. Two forms that breed true to form from seed have been identified also by Kevin Collins (Banksia Farm, Mt Barker) who considers there may be three forms.

The primary aims of this project were to develop suitable DNA based population genetic markers, in this case microsatellites, and to undertake genotyping to assess population genetic structure and levels of genetic diversity. In particular we sought to assess whether the Stirling Range populations and the Milbrook to Cheyne Beach (southern) populations are significantly genetically differentiated. This would provide supporting evidence for observations by Keighery (1988) that there are two forms and that these two forms may warrant consideration as separate taxa. Since we had seed from five extinct populations another key objective was to determine the amount of genetic diversity lost following the extinction of these populations and the value such *ex situ* seed collections have in the reintroduction and conservation of this species.

Materials and Methods

Detailed materials and methods are available on request and are currently being prepared for publication. Seed previously collected from 6 extinct populations in the late 1980s and early 1990s was successfully germinated and seedlings sent to the Kings Park nursery to be grown on for DNA harvesting and eventual translocation. DNA was extracted from 103 seedlings and a total of 116 plants from the extinct populations were available for translocation to two sites (Fig 1).

Leaf samples were collected from 11 extant *Banksia brownii* populations covering the geographical extent of the species as shown in Fig 1. DNA was extracted from a further 283 plants providing a total sample size of 386 plants from 16 populations for this investigation.

Microsatellite markers were developed and trialed on *B. brownii* DNA from a number of populations. Nineteen primer pairs that exhibited polymorphism were selected for screening and optimisation and of these we identified twelve polymorphic primer pairs that produced clear and interpretable PCR products.

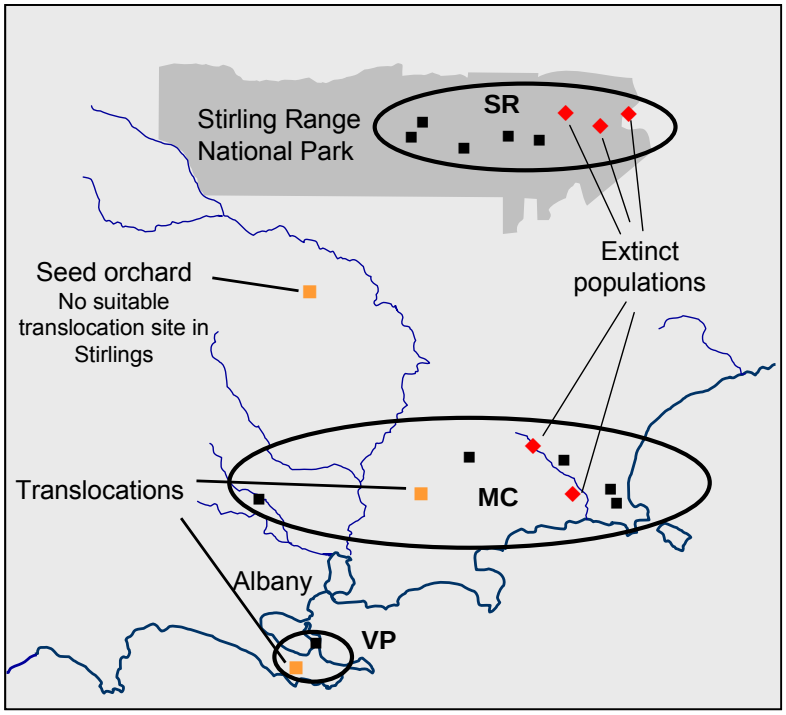

Figure 1. Location of sampled populations indicating three geographically disjunct population groups Stirling Ranges (SR), Milbrook to Cheyne Beach (MC) and Vancouver Peninsula (VP). Extinct populations that were genetically assayed using previously collected and stored seed are shown as are sites where translocations and a seed orchard were set up with seed from this study.

To investigate population genetic structure two different approaches were used. A multivariate principal component analysis (PCA) was conducted using a covariance genetic distance matrix constructed by analysis of molecular variance (AMOVA) in GenAlEx 6.1. An Unweighted Pair Group Method with Arithmetic Mean Analysis

(UPGMA) was also carried out based on Nei's unbiased genetic distance by generating a pairwise distance matrix from gene frequency data using the program GENDIST and then constructing a UPGMA using the program NEIGHBOR. both of which are available in the PHYLIP computer package (PHYLIP 3.4c; Felsenstein, 1995)

Rarefaction analysis using HP-RARE 1.0 (Kalanowski, 2005) was performed on the number of alleles per sample (allelic richness, A) and the number of unique alleles per sample (private allele richness, P) to take into account sample size differences between populations. These data were then used to investigate genetic diversity lost following the extinction of the five populations and to compare genetic diversity levels between the three geographic regions

Results and Discussion

An investigation of patterns of genetic variation based on the microsatellite markers revealed findings in two key areas. The first concerned the geographical pattern and genetic structure within *B. brownii* and clearly showed that this species is divided into three genetically discrete population groups that are geographically disjunct(Figure 2). The second involved the amount of genetic diversity lost from natural populations following extinction and how *ex situ* storage of the seed from those populations has ensured that significant levels of genetic variation are available for use in translocations and a seed production area.

Genetic Structure and Geographical patterns of genetic variation

The AMOVA (Fig 2) indicates that 39% of the total genetic variation detected in *B. brownii* in this study is distributed between populations and regions. This is indicative of relatively high levels of genetic structure, reduced gene flow between populations within regions and an extended period of isolation of each region. These findings are further supported by PCA and UPGMA studies.

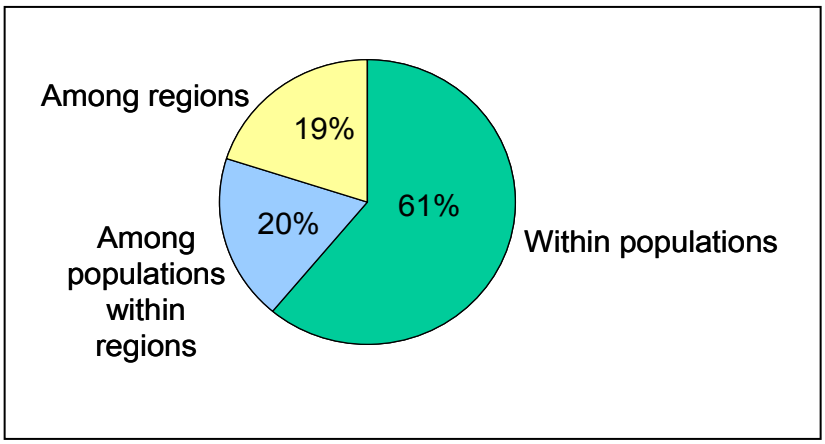

Figure 2. Analysis of molecular variance showing the percentage distribution of genetic variation within and among populations and among regions.

Both the PCA and UPGMA (Fig 3) clearly show that *B. brownii* is genetically subdivided into three discrete population groups that correspond to the three geographic regions shown in Fig 1. Two of these groups, the Stirling Ranges and the Milbrook – Cheyne

Beach groups, have been recognized previously on morphological grounds (Keighery 1988; Collins pers comm.) and given the level of the genetic differences observed in this study we suggest that further investigation is warranted into the taxonomic status of these two forms. The Vancouver Peninsula population falls out as the third distinct entity although there is no suggestion, at present, that it is morphologically different from either of the other two entities, but this also requires further taxonomic investigation.

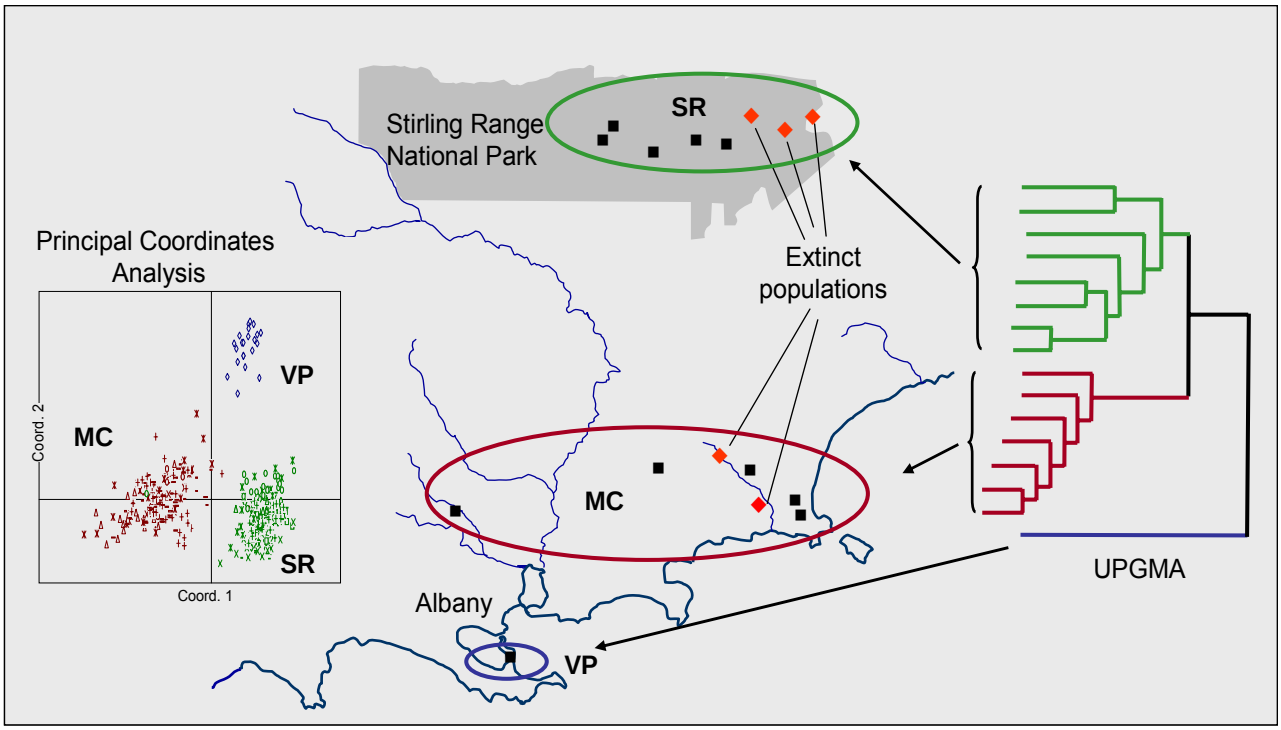

Figure 2. Both the PCA and UPGMA methods reveal significant differentiation between the three geographically discrete population groups of *Banksia brownii*: Stirling Range (SR), Milbrook-Cheyne Beach (MC) and Vancouver Peninsula (VP)

Regional Patterns of within population genetic diversity

Estimates of genetic diversity within populations indicate that two key measures allelic richness (A) and the proportion of private alleles (P) range from lowest in the Vancouver Peninsula population to highest in the Stirling Range populations (Fig 3). Although more detailed phylogeographic analysis is needed these preliminary findings suggest that this increased genetic diversity in the Stirling Range populations is likely to be due to the increased age and stability of the populations in this region. It is also possible that the Stirling Ranges have acted as climate change refugia for *B. brownii* and that the southern populations are the result of the expansion of this species following a period of climate amelioration. The significantly lower level of genetic diversity in the Vancouver Peninsula population also indicates that it has probably been founded only relatively recently compared to the other southern populations.

An alternative interpretation of reduced genetic diversity due to recent bottlenecks in the Milbrook-Cheyne Beach southern and Vancouver Peninsula populations appears not to be the case as a Bottleneck analysis indicated that none of the populations had been through recent bottlenecks. These data therefore provide some support for the notion that the original source populations for *B. brownii* came from the Stirling Ranges or, at

the very least, that the Stirling Ranges have provided critical refugial habitats for this species to persist during periods of increased climatic instability.

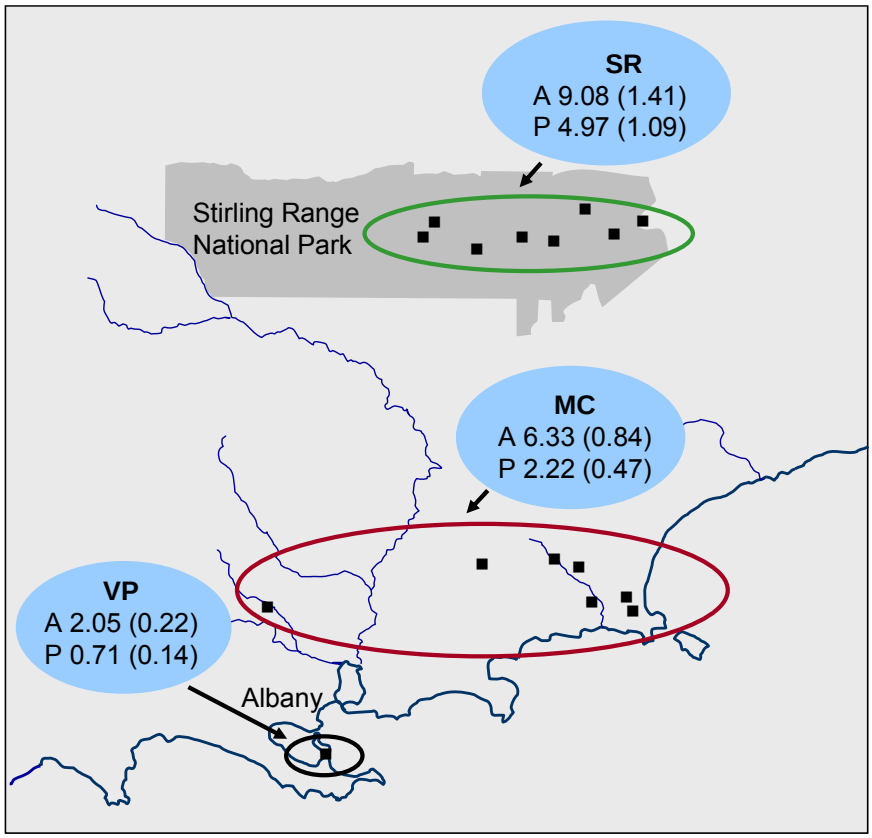

Figure 3. Genetic diversity based on allelic richness (A) and private allele richness for the three population groups of *Banksia brownii*: Stirling Range (SR), Milbrook-Cheyne Beach (MC) and Vancouver Peninsula (VP)

Genetic diversity and population extinction

The comparison of levels of genetic diversity based on differences in allelic richness (A) and the proportion of private alleles (P) between extinct and extant populations, showed that despite fewer populations there was an increased overall loss of genetic diversity in the extinct populations (Figure 4) compared with all extant populations including the Vancouver Peninsula population. When this is further investigated at the regional level it is evident that this increased loss is due to the higher levels of genetic diversity in the three extinct Stirling Range populations on the eastern side of the range (Bluff Knoll to Ellen's Peak) with A = 7.34 (1.06), P = 2.23 (0.56) compared to A = 7.11 (0.97), P = 2.00 (0.51) in the extant populations. In contrast there is slightly less genetic diversity in the two extinct southern populations [A = 4.19 (0.39), P = 1.11 (0.33)] populations compared to the extant populations [A = 4.27 (0.35), P = 1.19(0.26)].

It is unclear why there is more genetic diversity in the extinct eastern Stirling Range populations. It does not appear to be associated with seed storage conditions as the extinct southern populations have slightly less genetic diversity than the extant

populations. It is possible that the increased genetic diversity in these populations may indicate that the eastern Stirling Range populations have somewhat different evolutionary histories to the more western populations. However, this requires further investigation with different molecular markers such as the maternally inherited chloroplast DNA genes.

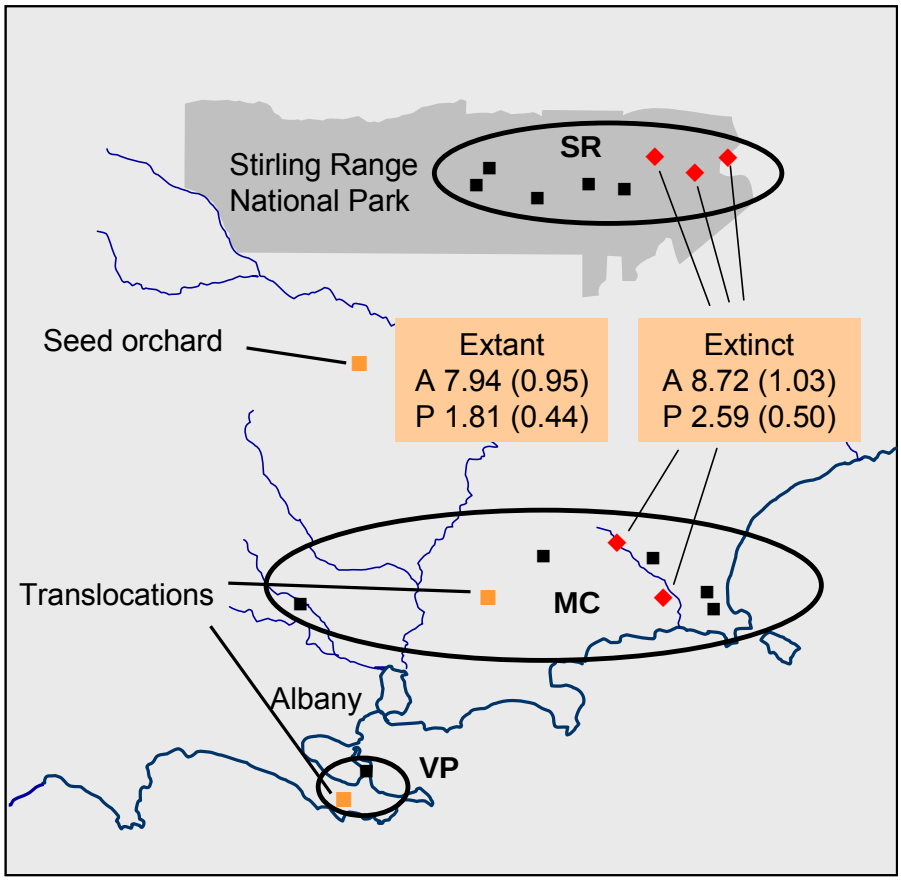

Figure 4. Genetic diversity based on allelic richness (A) and private allele richness (P) in the five extinct and the 10 extant populations of *B. brownii* sampled in this study.

While extinction of the five populations investigated in this study has resulted in a significant loss of genetic resources from natural populations of *B. browni* it is also clear that *ex situ* conservation measures through seed collections and storage have made an important contribution to reducing this loss. Seed material on which this study has been based is still available in the Threatened Flora Seed Centre and the seed used in this study has been grown on and planted out in the seed orchard and translocation sites. This highlights the importance of these seed collections, which were in some cases carried out over 20 years ago, in a situation where *Phytophthora* dieback has lead to the extinction of these populations and where all known populations of *B brownii* are currently infected with the pathogen.