An integrated approach to evaluating species vulnerability and conservation strategies in a changing climate

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Declaration

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Cover: *Callistemon teretifolius* growing along a ridgeline in Dutchman's Stern Conservation Park, Southern Flinders Ranges, South Australia.
(Photo taken by author)

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Abstract:

Climate change is predicted to put many species at risk of extinction, especially range restricted species and those occurring at high altitudes. *Callistemon teretifolius*, which has a restricted, high altitude, disjunct distribution, was chosen as a model species for evaluating climate change impacts and developing effective management and conservation strategies. An integrated approach was used combining on-ground assessment, population genetics, species distribution modelling and spatial analysis. Sixteen geographic populations were located and sampled across the species range, along the Adelaide Geosyncline in South Australia. Population size ranged from one to 400 individuals, but the majority of populations contained 20 or fewer individuals. Genetic analysis of these populations with chloroplast microsatellites (or cpSSRs) and Amplified Fragment Length Polymorphisms (AFLPs) indicated low species-level genetic diversity (He = 0.079), which is consistent with the small population size observed in contemporary populations. Two historical genetic refugia were identified in the centre of the species range. Expansion out of these areas in northern and southern directions was indicated from these population genetic data (most likely due to post glacial warming), allowing the species to occupy its current range. The sixteen geographic populations were assigned to three genetic clusters, which aligned with the Mt. Lofty, Southern Flinders and Northern Flinders Ranges. High levels of population genetic structure, and therefore low gene flow between populations, was evident for both marker types (Gst = 0.573; PhiPT = 0.242). Two major genetic disjunctions (between the Mt. Lofty and Southern Flinders Ranges and in Mt. Remarkable National Park) were apparent and coincided with the major genetic cluster assignments. Species distribution modelling using MAXENT, BIOCLIM and GARP supported the scenario of a historically restricted range, with a number of climatically suitable areas predicted along the Adelaide Geosyncline. Modelling of predicted future climates suggest *C. teretifolius* will experience a southerly range shift and further reduction and fragmentation of current range as a result of increased temperature and rainfall redistribution.

Based on this evidence, *C. teretifolius* is at high risk of climate change due to its low genetic diversity and low levels of gene flow, which will limit its potential for adaptation and migration, and may make it prone to extinction. The Southern Flinders Ranges was identified as a priority area for conservation as a result of highest genetic

diversity and predicted range restriction. The current reserve system in South Australia may not adequately protect the species in the future as a result of predicted range reductions and southerly shifts. The Cape Borda to Barrossa and Flinders-Olary NatureLinks corridors have the potential to increase the species capacity to adapt or migrate, by linking up intact sections of habitat through revegetation; but for species like *Callistemon teretifolius*, with a naturally disjunct distribution and low gene flow capacity, additional management may be necessary, such as assisted dispersal and habitat restoration. Management strategies recommended in this report are focussed on increasing adaptive capacity, maintaining genetic diversity, overcoming barriers to gene flow and increasing population size and connectivity. Ongoing monitoring of the future status and distribution of *C. teretifolius* and implementation of adaptive management strategies to respond to observed changes will be essential to track the effectiveness of conservation works.

Introduction:

Increased temperature and shifting rainfall redistribution will have, and are already having, significant impacts on many vegetation types (Walther *et al.* 2002; Hughes 2003). The current bioclimates of some species are predicted to disappear with relatively small climatic change, putting these species at high risk of extinction (Hughes 2003; Thomas *et al.* 2004). Extinction risk is determined by a species' ability to respond to climate change through migration or adaptation and these responses are generally idiosyncratic due to variable physiological tolerances, adaptive capacities and life histories (Brubaker 1986; Travis 2003; Aitken *et al.* 2008). Some species may be pre-disposed to greater risk than others, specifically range restricted species and those occurring at high altitude (Willi *et al.* 2006; Parmesan 2006).

Endemics and other range restricted species also often possess traits that make them vulnerable to climate change, including narrow geographic range, limited dispersal capacity and high habitat specialization (Gibson *et al.* 2010). In combination with these, endemics generally exhibit lower genetic diversity than widespread species, as a result of smaller and more ecologically limited populations (Ellstrand and Elam 1993), which can limit their adaptive capacity (Parmesan 2006). Climate change may further reduce population size and genetic diversity by reducing climatically suitable

area (Wilson *et al.* 2005; Hoffman and Willi 2008). Consequently, species may enter an 'extinction vortex' as positive feedback between genetic and demographic factors gradually reduces genetic variability and population size (Giplin and Soule 1986; Shrestha *et al.* 2002; Fagan and Holmes 2006). Climate change risk can therefore be assessed by analysing genetic structure and diversity, climate tolerances and population demographics; an integrated approach such as this is often more effective than focussing solely on one discipline (Williams *et al.* 2008; Andreou *et al.* 2011; Loss *et al.* 2011).

As climate change is not a new phenomenon, vegetation responses to past fluctuations can give an insight into species' responses to predicted future change. During past climatic fluctuations, migration was often the primary response, as plants dispersed seed and established in new regions more easily than evolving new climate tolerances (Davis and Shaw 2001; Pearson 2006; Petit *et al.* 2008). However, migrations were often accompanied by genetic changes as species adapted to different environmental conditions and community compositions (Hughes 2003; Davis and Shaw 2001). The ability of plants to respond to future climate change may be impeded by habitat fragmentation and as such concern surrounds their ability to respond to predicted changes (Pearson and Dawson 2002; Travis *et al.* 2003). If migration is limited, species will be under increased pressure to adapt *in situ* and consequently at greater risk of extinction (Jump and Penuelas 2005; Loss *et al.* 2011).

Genetic diversity is critical for adaptation as it is the raw material for evolution (Shresta *et al.* 2002; Andreou *et al.* 2011). Current patterns of genetic diversity are often shaped by previous range contractions and expansions and these events can leave a genetic signature in modern populations (Williams *et al.* 2003; Petit *et al.* 2008; Larcombe 2011). Past events influence a species' ability to respond to further climate change, so an understanding of past population fluctuations is important when developing management strategies (Davis and Shaw 2001; McKinnon *et al.* 2004). Past population structure can be investigated using chloroplast DNA (cpDNA) as it tends to evolve slower than non-coding nuclear DNA. Chloroplast DNA is usually variable within species and geographically structured enabling identification of genetic diversity patterns that may reflect the position of past genetic refugia and possible expansion routes (Byrne and Hines 2004; Byrne 2008; Lemes *et al.* 2010;

Kato *et al.* 2011). Analysis of cpDNA has been used previously to investigate the phylogeography of montane Australian tree species, to examine the signatures of refugia and subsequent expansion routes and to examine patterns of haplotype diversity. Conservation and management strategies were then developed, with high priority areas assigned to populations that were genetically distinctive or had high diversity (Nevill *et al.* 2010).

Gene flow is also a major determinant of population genetic structure and as such, knowledge of gene flow is often important when developing management plans (Ellstrand 1992; Escudero *et al.* 2003; Tero *et al.* 2003). Estimates of gene flow can give an indication of migration capacity and possible routes (King and Ferris 1998; Davis and Shaw 2001; Garant *et al.* 2007). Since plants cannot physically move, migrations are achieved through passive seed dispersal and seedling establishment in new areas or through pollen mediated gene flow (Brubaker 1986; Huntley 1991). Gene flow through pollen occurs relatively independently from seed dispersal and is often substantial (Ellstrand 1992). Pollen dispersal may play a large role in the organisation of genetic diversity within and among populations and is thought to be more important in maintaining the genetic structure of populations than gene flow through seed (Ellstrand 1992; Petit *et al.* 2005). Past levels of gene flow can be inferred from population genetic structure through determination of population differentiation, based on allelic frequencies (Escudero *et al.* 2003; Tero *et al.* 2003). An estimate of '0' indicates no differentiation and high gene flow, while an estimate of '1' indicates complete differentiation (no shared alleles) and therefore no gene flow (Bensch and Åkesson 2005; Bonin *et al.* 2007).

The Amplified Fragment Length Polymorphism (AFLP) method is useful for assessing genetic diversity and differentiation between individuals and populations, as well as evaluating gene flow, dispersal and out-crossing (Vos *et al.* 1995; Mueller and Wolfenbarger 1999). AFLP is a DNA fingerprinting technique (Vos *et al.* 1995; Coppi *et al.* 2008). The method is based on the separation of DNA fragments based on length differences (Vos *et al.* 1995). DNA fragments are scored as follows: if a fragment is present at a certain length it represents an allele and is scored "1". If no fragment is present then it is scored "0". Although, the per loci type of information obtained is relatively poor, advantages of this method are that it is relatively fast,

cheap and reliable and numerous loci can be studied at once (Krauss 2000; Tero *et al.* 2003). As such, AFLP's are becoming an increasingly popular technique in ecological studies and have been used previously in the development of conservation and management strategies (Gaudel *et al.* 2000; Lucchini 2003; Agrimonti *et al.* 2007).

If population genetics indicates restricted gene flow, then ability to track changing climates may be limited and species may become restricted to the parts of their current range that remain climatically suitable (Pearson and Dawson 2002; Loss *et al.* 2011). Therefore, combining population genetics with knowledge of future climatic suitability can help develop more comprehensive, climate change accommodated management strategies. Species distribution models can be used to gain an understanding of potential climatically suitable area as they correlate a species' current distribution with associated climatic variables in those areas. Likely distribution under different climate scenarios can then be predicted, with projected area corresponding to the region(s) assumed to be climatically similar to those now occupied (Midgley *et al.* 2002; Thuiller 2004; Hijmans and Graham 2006). Consequently, these models can be used to evaluate species' range changes, redistributions and climatic tolerances, and to assess changes in distribution, identify priority areas for conservation and examine the effectiveness of reserves (Beaumont *et al.* 2007; Yates *et al.* 2010).

Species distribution models provide a useful preliminary assessment of climate change impacts, but uncertainty is an inherent feature of all models as uncertainty surrounds both the magnitude and direction of future climate change (Beaumont *et al.* 2007; Wiens *et al.* 2009). Uncertainty is also inherent in models that simplify the complex interactions determining species' distributions (Pearson and Dawson 2002; Buisson *et al.* 2010). Species distribution models assume climate is the major factor limiting distribution and as such do not account for all other variables which determine species distributions (Pearson and Dawson 2002; Barry and Elith 2006; Heikkinen *et al.* 2006). Modelled projections can be further validated by incorporating spatial data and/or population genetics, since factors such as habitat fragmentation and gene flow also play significant roles in determining species' distributions (Pearson 2006; Huntley *et al.* 2010). If habitats are highly fragmented, species may not be able migrate through or survive in areas that are otherwise deemed climatically suitable

(Walther *et al.* 2002; Trakhtenbrot *et al.* 2005). Therefore, an accurate prediction of plant re-distribution requires combined knowledge of suitable climatic area and the land condition of that area (Hannah *et al.* 2002b).

Developing conservation strategies for climate change management pose a number of challenges, as a result of the complex nature of factors involved and the uncertainty surrounding predicted climate change (Hannah *et al.* 2002a; Pearson 2006). The need for an integrated approach to overcome some of these issues has been raised (Hannah *et al.* 2002b), encompassing species distribution modelling, on-ground assessment and population genetics (Scoble and Lowe 2010; Loss *et al.* 2011). To assess the merits of an integrated approach and to examine components of ecosystem change, species distribution and population genetics, this project has focussed on the mainland portion of the Adelaide Geosyncline (Fig. 1). The Adelaide Geosyncline is a 600km region of raised elevation extending across southern Australia and encompassing the Mt. Lofty and Flinders Ranges. The raised elevation results in the region being cooler and wetter than the surrounding arid land and this wetter region has greater biodiversity in part due to range overlap between the northern arid and moister southern species (Bardsley 2006; DEH 2006a; DEH 2006b Byrne 2008). This type of environment may be particularly susceptible to climate change, potentially putting many species adapted to cool, moist, montane environments at risk (Theurillat and Guisan 2001; Bardsley 2006; Fitzpatrick *et al.* 2008). As the effects of climate change may become apparent relatively early in this system, the Adelaide Geosyncline is an important area to monitor the impacts of climate change in southern Australia, especially as climate change may exacerbate a system already stressed by habitat fragmentation (Bardsley 2006).

One of the major conservation strategies currently being developed in South Australia are NatureLinks corridors, which tie in with South Australia's *no species loss* strategy (DEH 2007). Two of the five proposed NatureLinks (NL) encompass areas of the Adelaide Geosyncline and these are the Cape Borda to Barossa NL and the Flinders-Olary NL. One of the major aims of these corridors is to increase the resilience of the region to climate change by managing and restoring large areas of habitat (DEH 2011a; 2011b). These corridors will build on existing reserves (National and Conservation Parks), but a broader and more connected approach will be used (DEH

2007). However, research has questioned the effectiveness of other NatureLinks as a conservation tool (Costion *et al.* Unpublished data). Concern also surrounds the relevance of fixed reserve systems as viable conservation tools in a changing climate, as species ranges may become more dynamic (Araujo *et al.* 2004; Hannah *et al.* 2007).

To date, the Adelaide Geosyncline and its biota have been poorly studied, so a great deal of uncertainty surrounds the impacts of climate change on this area. *Callistemon teretifolius* (Muell, 1853) is endemic to the region and will be used here as a model species to examine genetic and distributional changes under a range of future climate scenarios to assess likely impacts and responses. *C. teretifolius* is not currently listed as threatened, but it may be particularly vulnerable to climate change due to its restricted distribution (Trakhtenbrot *et al.* 2005). With populations generally constrained to high elevation ridgelines or rocky environments (DENR 2011), the potential of this species to cope with climate change through migration or adaptation may be limited. Therefore the aims of this research are:

- To use an integrated approach to evaluate the impacts of climate change on *Callistemon teretifolius* and to then develop management strategies
- To prioritise populations and regions for conservation based on on-ground assessment, population genetics, species distribution modelling and spatial analysis.
- To identify overall genetic structure, potential refugium and likely post glacial migration routes.
- To assess potential range restrictions, fragmentation and shifts with predicted climate change.
- To assess the impacts of habitat fragmentation and the effectiveness of the current reserve system and proposed NatureLinks on the survival of *C. teretifolius*.

Methods:

Data sets and study area

Biological data for *Callistemon teretifolius* were sourced from the Biological DataBase of South Australia (BDBSA) through the Department of Environment and Natural Resources (DENR). Data included information on population location, date recorded, reserve name and spatial accuracy of recording. These data were used to determine the distribution of the species and then to establish sampling locations (Fig. 1). Field based sampling sites were chosen based on coverage of species distribution, abundance of locality records, position of parks (Conservation (CP), National (NP) and privately owned) and accessibility. Sites ranged from Warren CP in the south to Arkaroola Wilderness Sanctuary in the north, covering a distance of approximately 600km and encompassing the Mt. Lofty and Flinders Ranges. This region has a Mediterranean type climate (Bardsley 2006) with average annual temperature ranging from 12^{o}C to 17^{o}C and average annual rainfall ranging from 350mm to 1100mm (Hijmans *et al.* 2005a).

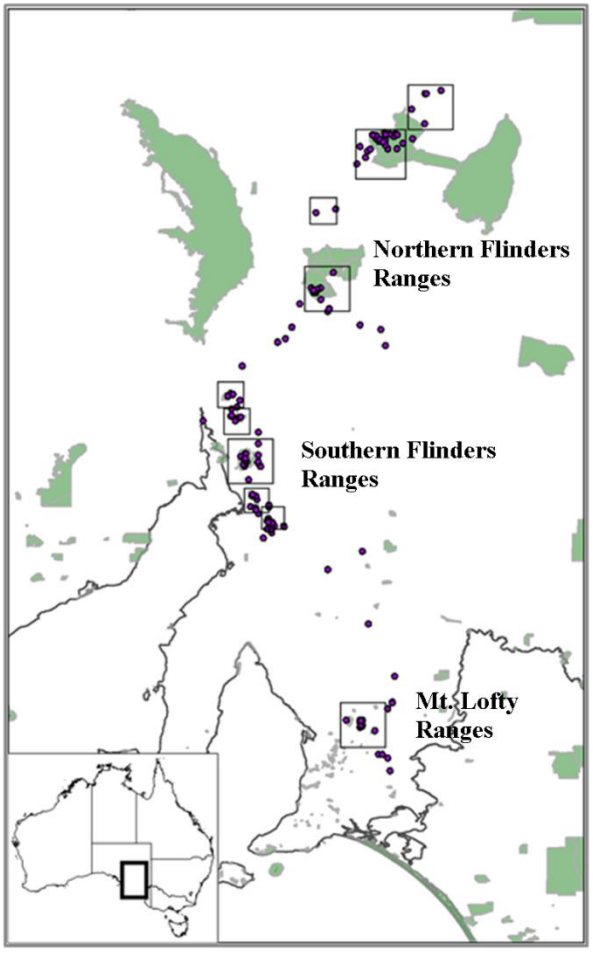


Figure 1: Distribution of *Callistemon teretifolius* along the Adelaide Geosyncline region of South Australia. The purple circles represent locality records (BDBSA records). Populations occur in three major areas, the Mt. Lofty Ranges and the Southern and Northern Flinders Ranges. The black squares represent general field sampling locations and green polygons show the position of Conservation and National Parks. *Refer to Appendix A and B for reserve positions.*

Field work

Field sampling was completed in April 2011, with sites ranging from Warren CP in the south to Arkaroola Wilderness Sanctuary in the north, representative of the range of *Callistemon teretifolius* (Fig. 1). Exploration for populations in the following areas was undertaken (listed from south to north): Mt. Lofty Ranges (Warren CP, Hale CP), Southern Flinders Ranges (Telowie Gorge CP, Mt. Remarkable NP - Mambray Creek Block and Mt. Remarkable, Mt. Brown CP, Dutchman's Stern CP) and Northern Flinders/Gammon Ranges (Flinders Ranges NP – Wilpena Pound, Vulkathunha-Gammon Ranges NP and Arkaroola Wilderness Sanctuary). Populations were found in all locations except Mt. Brown CP, Vulkathunha-Gammon Ranges NP and Arkaroola Wilderness Sanctuary. Multiple populations were located in Hale CP, Mt. Remarkable NP - Mambray Creek Block, Dutchman's Stern CP and on the rim of Wilpena Pound.

A small number of healthy leaves were removed from selected plants within each population and placed into individual material bags, labelled and dried using silica. The GPS position of each individual sampled, along with details of population size, plant size and maturity, soil type, habitat, community composition (predominant plants) and access were recorded. Two voucher specimens were collected per population for herbarium records, with seed clusters attached if possible. Small populations (<15 individuals) were exhaustively sampled, while larger populations were randomly sampled across the entire population. The number of samples collected per population was dependent on overall population size, with collections ranging from 1 to 32 samples. Photographs were taken at each site to document plant size, growth position and community composition.

Herbarium Collection

Leaf samples were collected from 12 voucher specimens stored at the State Herbarium of South Australia (Appendix C). Samples were sourced from specimens collected in the Northern Flinders Ranges from the 1960's onwards to supplement field sampling. One to two leaves were collected from each voucher specimen depending on leaf size and condition.

Genetic analysis

DNA was extracted from dried leaf tissue of 183 samples, collected from field (*n* = 171) and herbarium samples (*n* = 12). Leaf tissue was extracted using the Machery-Nagel Nucleospin Plant II Kit at the Australian Genomics Research Facility (AGRF, Adelaide, Australia).

Chloroplast microsatellites – Sequencing and genotyping

PCR amplifications were performed in 25µL reaction volumes containing 2.5µL 10x *Taq Gold* buffer, 2µL $MgCl_2$, 2µL dNTP, 0.2µL *Taq Gold* polymerase, 1.5µL each of 10mM forward and reverse primer, 1.5µL template DNA and 13.8µL PCR grade water. PCR reactions were run on a Palm Cycler (Corbett Research) for 2 hrs and 43mins with the following conditions: initial denaturation step of 94^{o}C (9mins), followed by 34 repeats of 94^{o}C (45sec), 50^{o}C (45sec) and 72^{o}C (1min), followed by 72^{o}C (6mins) and 25^{o}C (30sec). PCR amplification was performed for all 10 universal chloroplast microsatellite (ccmp) primer pairs (Weising and Gardner 1999), for ITS-1 ribosomal spacer region (Brown *et al.* 2001) and for 9 other primer pairs developed previously for various regions of the chloroplast DNA (Olmstead and Sweere 1994; Demesure 1995; Shaw *et al.* 2005). Refer to appendix for primer details (Appendix D).

Amplification products were run on 1.5% agarose gel at 104 volts for 20mins in 1 x Tris-borate ethylenediaminetetraacetate (TBE) buffer, to determine reactions which amplified successfully. Gels were stained in ethidium bromide solution or GelRed for 30 mins and then visualised by UV transillumination. Successful reactions were cleaned for sequencing using a 0.3mM TRIS wash. Clean PCR products were resuspended with 25µL of water. Sequencing reactions were carried out directly on PCR product and were performed in 20µL of solution containing 1µL Big Dye, 5x Big Dye buffer, 1 µL of 10mM forward or reverse primer, 1 or 2 µL of DNA template and 14 µL PCR grade water. The sequencing reaction was run for 2 hrs and 46 mins with 24 repeats of 96^{o}C (30sec), 50^{o}C (15sec), 60^{o}C (4min) and then one repeat of 25^{o}C (1min). Sequence products were cleaned using a 0.3 mM EDTA and PCR grade water wash. Clean product was rehydrated in 25µL of PCR grade water and samples were sent to AGRF for sequencing using capillary separation. Electropherograms were analysed using RIDOM TRACEEDIT version 1.1.0 and BIOEDIT version 7.0.9

software (Freely available from http://www.ridom.de/traceedit/ and http://www.mbio.ncsu.edu/bioedit/page2.html). Sequence traces were viewed using RIDOM TRACEEDIT and 'noisy' data were removed. Edited sequences were imported into BIOEDIT where they were manually aligned to check for base-pair differences between sequences.

Genotyping was carried out on universal chloroplast microsatellite (ccmp) pairs 1,2,3,4,5,6,7 and 10 (Weising and Gardner 1999; *Appendix D*) using fluorescent-labelled forward primers and standard reverse primers. Forward primers were labelled with the following: FAM (2, 6), VIC (1, 4, 5) and NED (3,7,10). The same PCR and cleaning methods as specified above were used. Fragments were separated on an AB3730 genetic analyser with a 36cm capillary array (Applied Biosystems, Foster City, MA, USA). Results were viewed using GENE MAPPER® version 4.0 software (Applied Biosystems) and peaks were double-checked manually. Genotyping was continued with primer pairs 1, 2, 3 and 5. All 12 herbarium samples and 83 field samples from the 16 populations were analysed. PCR products for each sample were pooled for fragment separation by adding 30µl of H_2O, 1µl VIC (primer 1), 3µl NED (primer 2) and 2.5µl of FAM (primer 3).

Subsequent scoring found only primer pairs ccmp2 and 3 to be variable so further analysis was restricted to these data. Many of the alleles differed by 1bp, so to minimise ambiguity with scoring, results containing stutter peaks were not used and, if necessary, samples were re-amplified to confirm ambiguous genotypes. Number of haplotypes was determined from the combination of alleles present within individuals and distribution was mapped. A haplotype is a unique combination of alleles that occur at a set of loci and are inherited as a block (Lowe *et al.* 2004; Nevil *et al.* 2010). PERMUT version 1.0 (Pons and Petit 1996) was used to determine diversity within and between populations and differentiation between populations using both ordered and unordered alleles. Allele and haplotype frequency and AMOVA testing was completed with GENALEX version 6.41 (Peakall and Smouse 2006). For statistical testing, the two Mambray Creek populations with one individual (MC2 and MC3) and all herbarium samples from the Gammon Ranges were combined, as testing was not possible on populations with only one individual. NETWORK version 4.6.0.0 (available at http://www.fluxus-engineering.com/sharepub.htm#a10) was used to develop a

haplotype tree, which allowed haplotype frequency and base pair differences between haplotypes to be visualised. A median-joining network was developed using equal weighting of all characters after data had been manually entered.

AFLPs

Amplified fragment length polymorphism (AFLP) analysis was carried out on all field samples (n = 171). Three primer pairs were selected from the 12 trialled using a combination of three labelled forward primers (Eco RI) and four standard reverse primers (Msel I). *Refer to Appendix E and F for primers*. Primers were selected based on peak quality, number of peaks and range/spacing of peaks. All 171 field samples were amplified along with two negative controls, six positive controls and 18 samples of re-extracted DNA (approx. 10%).

Double-stranded adapter (Eco RI and Mse I) pairs were prepared. The Eco RI adapter pair (adapter 1) consisted of 25µl of 100µM forward adapter (G773), 25µL of 100µM reverse adapter (G774) and 450µL TE buffer, producing a 500µL solution with a concentration of 5µM. Mse I adapter pair consisted of 25µL of 1000µM forward adapter (G775) and 25µL of 1000µM reverse adapter (G776) and 450µL TE buffer, producing a 500µL solution at 50µM. Solutions were heated for 5mins at 95^{o}C and then left to cool for 120mins.

Restriction digest of DNA was carried out in 20µL reaction volumes containing 15µL DNA, 6.3µL PCR grade water, 2µL 10xNEBbuffer4 (New England Biolabs), 1µL MseI (10U/ µL), 0.5µL EcoRI (20U/µL) and 0.2µL 100x BSA. Samples were incubated at 37^{o}C for 3hrs. Temperature was raised to 65^{o}C for 20mins to prevent further enzyme activity and then lowered to 4^{o}C for 10mins. Samples were cooled on ice for 1min before the ligation reaction was commenced. Ligation occurred in 40µL solution containing 20µL digested DNA (from restriction digest step), 11.8µL PCR grade water, 4µL10x T4 ligase buffer, 2µL of Adapter 1 (Eco RI), 2µL of Adapter 2 (Msel I) and 0.2µL of T4 DNA ligase (2000U/µL). Samples were incubated at 16^{o}C in a Palm Cycler (Corbett Research) for 16.5hrs. Samples were then diluted 1/10 in PCR grade water.

Pre-selective amplification was run in 25µL reaction volumes containing 13.25µL of PCR grade water, 2.5µL of 10X Finnzyme PCR buffer with 1.5mM Mg, 2.0µL dNTP, 2.5 µL of 5µM EcoRI +A primer G783 and MseI +C primer 790, 0.25µL of Finnzyme Taq polymerase (1U/µl) and 2µL of dilute (1/10) ligated DNA (from ligation step). Reaction was completed on an Eppendorf thermalcycler with the following cycle: 72°C (2min), 94°C (30sec), 56°C (30sec), 74°C (2min), return to step 2 and repeat 25 more times, then 60°C (10min) and 30°C (10sec). The success of the amplification was tested by running 5µL of product on 1.5% agarose gel for 20mins. The remaining pre-amplified PCR products were diluted 1/30 in PCR grade water.

Selective amplification occurred in 15µL volumes containing 7.85µL PCR grade water, 1.5µL 10X *Taq Gold* PCR buffer, 1.2µL $MgCl_2$, 1.5µL dNTP, 0.15 *Taq Gold* polymerase, 1µL of dilute (1/30) pre-selective amplified PCR product and 0.9µL each of EcoRI +3 labelled primer and MseI +3 standard primer at 5µM. Solutions were incubated on an Eppendorf thermalcycler with the following cycle: 95°C (9min), 94°C (30sec), 65°C (30sec) (reduce by 1°C per cycle), 72°C (2min), return to step 2 and repeat 8 more times, 94°C (30sec), 56°C (30sec), 72°C (2min), return to step 6 and repeat 20 times, 72°C (5min) and 25°C (30sec). Fragments were separated on an AB3730 genetic analyser with a 36cm capillary array (Applied Biosystems, Foster City, MA, USA).

Reaction success was verified with GENE MAPPER version 4.0 (Applied Biosystems) to check sample and peak quality. All electropherograms were imported into PEAK SCANNER version 1.0 (Applied Biosystems) to detect and size peaks; this information was imported into R-cran library RAWGENO version 2.0-0 (Arrigo *et al.* 2009). Samples were filtered to remove low quality samples, identified by an abnormally low number of peaks. All remaining samples were then scored with reproducibility cut-off set at 90%. Bins assigned by RAWGENO were manually verified and altered if necessary and any bins that occurred in the negative controls were removed. Quality of scored data was determined using the Ibin value and mismatch error rate (E_b) provided by RAWGENO (Arrigo *et al.* 2009). Only samples that amplified successfully across all primer pairs were used in final analysis. Controls were removed from the dataset before analysis. Number of polymorphic loci, percent of polymorphic loci, Nei's expected heterozygosity, Shannon's Index, allele frequencies, Principle

Coordinates Analysis (PCA), spatial autocorrelation and AMOVA testing was undertaken with GENALEX version 6.41 (Peakall and Smouse 2006), using 9999 permutations where appropriate. PCA was undertaken with the genetic distance between individuals as determined by GENALEX. Spatial autocorrelation using populations as distance classes was completed and a correlogram was constructed depicting the correlation coefficient (r) as a function of each population. The correlation coefficient provided a measure of genetic similarity between pairs of individuals occurring within a specified distance class (Smouse and Peakall 1999).

To assess the number of genetic groups (k) within the species and the distribution of individuals within these groups STRUCTURE version 2.2 (Pritchard *et al.* 2000) was used with a burn-in period of 500 000 and 1 000 000 iterations (Markov Chain Monte Carlo). These values were selected as it was long enough to stablilize alpha and Ln Likelihood and to obtain consistent end results (Pritchard *et al.* 2000; Breinholt *et al.* 2009). An admixture model with correlated allele frequencies was used to estimate the number of clusters with k ranging from 1-6, with 6 repetitions. Phylogeographic analysis was completed with PAUP® version 4.0b10 software (Swofford, 2003). Parsimony analysis included those 132 of the original 158 characters that were parsimony-informative, with equal-weighted, unordered, characters and 5000 random addition sequence replicates. Support for nodes was tested with 1000 bootstrap replicates each with 50 random additional sequence replicates. The monophyly of individual populations was constrained.

Species Distribution Modelling and Spatial Analysis

Data obtained from the BDBSA was used as the presence data for species distribution modelling and subsequent spatial analysis. Climate layers were sourced from WorldClim (www.worldclim.org) for tile 410, which covered southern Australia and had a resolution of 1 km^2. The WorldClim data for Australia was sourced from the Bureau of Meteorology (BOM) up to 2003 (Hijmans *et al.* 2005a). Modelling was completed with MAXENT version 3.3.3 (Phillips *et al.* 2006), DIVA-GIS version 7.3.0 (Hijmans *et al.* 2005b) and OPENMODELLER DESKTOP version 1.0 (de Souza Muñoz *et al.* 2011) software, along with ArcGIS® 9.3.1 programs ArcMap and ArcCatalogue (ESRI).

Modelling

BIOCLIM and MAXENT were used for presence-only modelling with modelled outputs restricted to an area of: -30°S to -35.7°S, 137.8°E to 139.65°E (Fig. 2). The historical model was based on cooler and drier conditions during the Pleistocene, with a 6°C reduction in temperature and a 10% decrease in rainfall (McKinnon *et al.* 2004; Larcombe 2011). Future climate scenarios were based on CSIRO predictions for 2030 and 2070 (Suppiah *et al.* 2006). As predictions were given as a range, the least and most severe changes were modelled for each period. A 0.5°C and 1.5°C increase in temperature was projected for 2030 in combination with a 1% increase and 9% decrease in rainfall respectively. The projected changes for 2070 included a 1.2°C and 4.7°C increase in temperature and a 4% increase and 25% decrease in rainfall (Appendix G).

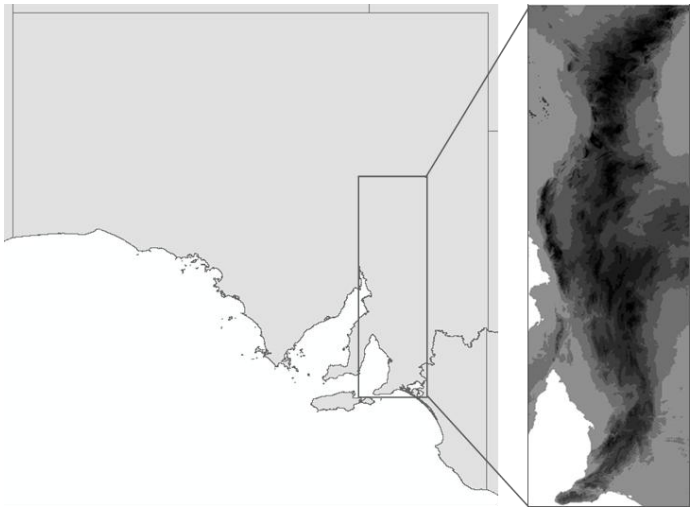


Figure 2: Map of South Australia showing the modelled extent (-30°S to -35.7°S, 137.8°E to 139.65°E) and inset featuring the altitude grid of the area, with darker shades representing higher elevations.

The BIOCLIM function was used in DIVA-GIS to model current, historical and future climate scenarios. A climate database was established using monthly minimum and maximum temperature and precipitation as well altitude to generate 19 bioclimatic variables (Appendix H). Train datasets were developed using 75% of the data and were then used to run the models. The remaining 25% of data and pseudo-absence data was used to test the model. The climate change function in BIOCLIM was used to alter the 19 bioclimatic layers to project potential past and future changes in rainfall and temperature.

Bioclim variables sourced from WorldClim (Appendix H) were adjusted in DIVA-GIS, to be used with MAXENT. Layers were stacked so adjustments to temperature and rainfall could be made. Layers 2, 3, 4 and 7 were excluded from the temperature stack (layers 1-11), while layer 15 was excluded from the precipitation stack (layers 12-19). The scalar function was used on each stack to change the temperature or rainfall accordingly before layers were exported into MAXENT. Current climate data was used as the input for the environmental variables. Presence points were compared with 10 000 background points. Models were trained using 75% of the presence data and the remaining 25% was used to test the model. A logistic output was used, so each cell was given a score between zero and one based on its climatic suitability. The maximum test sensitivity and specificity threshold rule was applied to each model, which converted the logistic output to a binary presence/absence output. The contribution of each of the 19 bioclimatic variables to the modelled output was calculated (Table 1); from this the 10 bioclimatic variables which contributed 2% or greater to the model were selected and models (including BIOCLIM) were then re-run using only these variables.

Table 1: Contribution of the 19 bioclimatic variables to the MAXENT model.

Species distribution modelling was restricted to the bioclimatic variables that contributed 2% or more to the MAXENT model, indicated by grey shading.

Variable	Percent contribution	Permutation importance
bio11	18.1	0
bio3	17.5	1.1
bio13	17.5	1.9
bio4	13.8	60.6
bio6	8.3	0.2
bio2	5.9	2.8
bio15	4.1	1.2
bio8	3.1	0.1
bio10	2.1	0
bio9	2	4.2
bio14	1.7	0.4
bio12	1.5	1.8
bio7	1.2	0.4
bio5	1.1	13.8
bio18	0.8	2.8
bio16	0.7	0.2
bio17	0.5	6.5
bio19	0.2	1.9
bio1	0	0

The GARP (Stockwell and Peters 1999) function in OPENMODELLER DESKTOP was used with presence/absence data sourced from Biological Survey of South Australia data (DENR 2011). Modelling was completed using the four GARP algorithms available (Single run Desktop GARP, Single run with new implementation software, Best Subsets Desktop GARP and Best Subsets new implementation software). Modelling was restricted to the 10 bioclimatic values which contributed 2% or more to the MAXENT model (Table 1). The Best Subsets new implementation software algorithm was selected for further analysis based on its AUC, kappa values and visual evaluation, as it produced the best fit for the data.

Model accuracy was assessed with AUC and kappa values. AUC is the area under the Receiver Operated Curve (ROC) and can be used to evaluate how well the model fits the data. AUC ranges from 0-1; with a value of >0.9 indicating an excellent fit for the data and <0.5 indicating that the model provides a fit that is no better than random. Comparing the AUC values of different algorithms is an effective way to determine how well each fits the data, as long as species and study area are held constant (Wisz *et al.* 2008). Kappa was used to determine the most appropriate threshold to apply to the model, allowing predictions to be converted to presence/absence outputs (Elith *et al.* 2006).

To analyse changes in distribution, the reclass function in DIVA-GIS was applied to each modelled output layer, with suitable area classed '1' and unsuitable area classed '0'. The area function was applied with a threshold of '1' and the identify tool was used to determine total suitable area. This procedure was repeated, with only climatically excellent area classed '1'. The percent change in suitable area from current was calculated for each climate scenario and algorithm.

Spatial analysis

ArcMap and DIVA-GIS were used to analyse the impact of land clearance and habitat fragmentation on potential species redistribution as well as effectiveness of National and Conservation Parks and the proposed Cape Borda to Barossa and Flinders-Olary NatureLinks. Analysis was restricted to MAXENT models as these produced the best fit for the data. Raster output files were assigned to the Geographic coordinate system GCS_GDA_1994 and converted to polygon layers in ArcMap. Areas deemed

climatically suitable were selected and clipped from the original layer before total suitable area was calculated. This procedure was repeated for excellent climatic area.

A polygon feature class of the proposed Cape Borda to Barossa and Flinders-Olary NatureLinks was developed by geo-referencing an image sourced from NatureLinks documents (DEH 2011a; 2011b) using the Australia outline and reserve layers as controls (Appendix I). The effectiveness of georeferenced points was assessed using the residual and Total RMS values. Values of less than 1 were attained indicating a good fit to the data.

The intersect tool was used to analyse reserve and NatureLinks placement by selecting area of overlap with modelled output. The modelled area which fell within these features was calculated before total area was determined. Percent of suitable area outside the NatureLinks was calculated. Reserve specific analysis was completed with the Vulkathuna-Gammon Ranges NP, Flinders Ranges NP, Mt. Remarkable NP, Dutchman's Stern CP and Hale CP as modelling, on-ground assessment and population genetics suggested these reserves may be of particular interest. The area of suitable climate which remained in each of the reserves was determined for each future climate scenario.

The native vegetation layer was more complex than the other layers, due to high levels of habitat fragmentation in some parts (Appendix J). As a result the layer was clipped to the modelled extent and then dissolved for simplification. The total amount of intact vegetation left in the modelled area was calculated. The union tool was used to create a new feature class, with a combination of native vegetation and modelled area. The regions of the modelled output which fell on cleared land were then selected and clipped, creating a new layer containing only this data, from which area was calculated and the percent of area occurring on cleared land determined.

Results

Field sampling

In total 171 samples were collected from 16 populations, spanning approximately 400km from Warren CP in the south to Wilpena Pound, Flinders Ranges NP in the north. Samples were collected in the regions shown in Fig. 3 below. Population size ranged from 400 individuals at Dutchman's Stern CP in the north of the Southern Flinders Ranges, to a population size of one in Mt. Remarkable NP also in the Southern Flinders. Overall, mean population size was approximately 38 and median was 10, with the majority of populations having 20 or less individuals (Table 2).

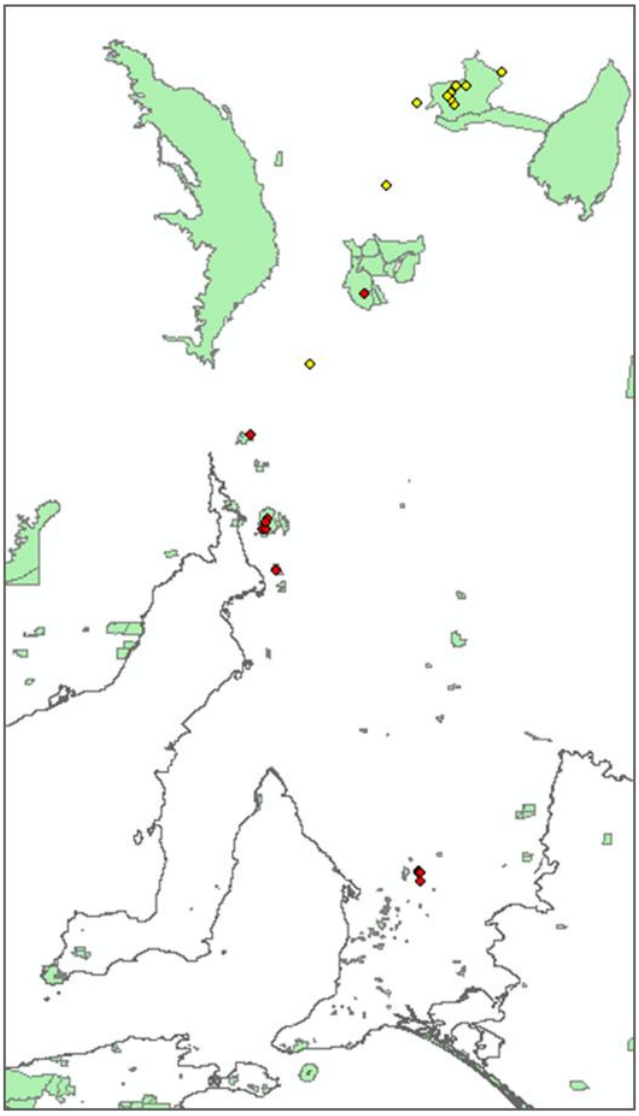


Figure 3: Location of sampled populations along the Adelaide Geosyncline region of southern Australia (Red circles). The most southerly population sampled was at Warren CP in the Mt. Lofty Ranges, the most northern is on the rim of Wilpena Pound in the Flinders Ranges NP. In total 16 populations were sampled with leaves collected from 171 individuals. The yellow circles show the position of the 12 herbarium specimens sampled.

In general, there was a trend for the elevation of populations to increase from south to north, with the exception of many of the Southern Flinders Ranges populations. The two populations found on the rim of Wilpena Pound (Flinders Ranges NP) were both at elevations greater than 800m, while many of the populations in the Southern Flinders, especially those in the Mambray Creek section of Mt. Remarkable NP were found at low elevations often below 300m (Table 2). A large proportion of populations were geographically restricted, with many populations constrained to an area of $10m^2$ or less and this was most evident in Mt. Remarkable NP and Warren CP.

Table 2: **Name, position, population size and elevation (m) of sampled populations**. Standard error in parentheses.

Population	Latitude	Longitude	Estimated Population Size		Mean elevation (m)
Warren (W)	-34.73159	138.91272		10	393 (3.38)
Hale 1 (H1)	-34.68153	138.90741		20	385 (3.79)
Hale 2 (H2)	-34.68526	138.90463		10	338 (2.13)
Hale 3 (H3)	-34.6878	138.90773		30	388 (1.88)
Hale 4 (H4)	-34.68721	138.90939		20	408 (9.02)
Telowie Gorge (T)	-33.04369	138.11889		40	359 (4.29)
Mambray Creek 1 (MC1)	-32.82552	138.04846		2	307 (1.08)
Mambray Creek 2 (MC2)	-32.82355	138.0614		1	182 (-)
Mambray Creek 3 (MC3)	-32.81944	138.0616		1	274 (-)
Alligator Gorge 1 (AG1)	-32.76522	138.07183		4	342 (1.60)
Alligator Gorge 2 (AG2)	-32.78186	138.06642		15	296 (2.46)
Dutchman's 1 (D1)	-32.30831	137.98074		10	665 (2.19)
Dutchman's 2 (D2)	-32.30781	137.97905		400	668 (3.59)
Dutchman's 3 (D3)	-32.30776	137.97689		10	691 (4.17)
Wilpena 1 (WP1)	-31.54078	138.60263		20	825 (15.78)
Wilpena 2 (WP2)	-31.54419	138.60543		10	857 (2.07)
			Mean	38 (11)	513 (192)
			Median	10	391

A number of the populations occurred in close proximity, most commonly in Dutchman's Stern CP and on the rim of Wilpena Pound, where populations were separated by a distance of 60-100m. The three populations in Dutchman's Stern CP were found along a 400km distance of ridgeline, with a large central population of 400 individuals occurring in association with two small peripheral populations. A large proportion of plants in Telowie Gorge CP were juveniles, especially those in the region bordering the 4WD track, which suffered from disturbance (Appendix K). In

Telowie Gorge CP and Mt. Remarkable NP there were few other medium sized species growing in close association with *C. teretifolius*. In general, this species was found in lightly vegetated areas and if other species were present they were usually *Eucalyptus sp., Allocasurina sp.*or *Exocarpus sp. Refer to Appendix K for field images.*

Population Genetics

Sequencing and genotyping

Eight other primer pairs B, D, E, F, G, H, I and J were sent away for sequencing on four samples from across *Callistemon teretifolius's* range encompassing four populations (*Refer to Appendix D for primers trialled*). Clean sequence results were obtained for six of the primers trialled. These sequences contained A or T mononucleotide repeats which were 8 to 12bp in length. No nucleotide differences were evident across four widely spaced populations. Sequencing of the ITS-1 spacer region also showed no variation between individuals.

In total six alleles were found across the two variable loci amplified with universal primers pairs ccmp2 (5' to *trnS*) and ccmp3 (*trnG* intron) across 24 populations (field and herbarium), forming five different haplotypes (Table 3). The 5' to *trnS* region had four alleles which differed by 1bp, with peaks occurring at 241 (A1), 242 (A2), 243 (A3) and 244 (A4), with variation found across the range of *Callistemon teretifolius*. The *trn*G intron had two alleles separated by 5bp, with peaks at 133 (A5) and 138 (A6). Only two individuals with allele A6 were amplified and were sampled from the Telowie Gorge CP in the Southern Flinders Ranges. All other samples were fixed for A5 (Table 3). Results were obtained from eight of the twelve herbarium samples, but two samples (H8, H10) only amplified successfully for the *trn*G intron and were excluded from subsequent haplotype analysis.

Table 3: Population allele and haplotype number and composition

Alleles 1-4 (A1-A4) are from the 5' to *trn*S region amplified by ccmp2 and alleles A5 and A6 were from the *trnG* intron region amplified by ccmp3. Haplotypes are determined by the combination of alleles each individual has. In total the results from 83 samples are shown, including the 8 herbarium (HB) samples and 75 field samples. A unique allele is one that occurs only in that population. The shading represents the three major regions in which populations occur (Mt. Lofty, Southern and Northern Flinders Ranges).

	Population	Number of alleles	Number of unique alleles	Alleles	Haplotypes
1	W	2	0	A2, A5	H2
2	H1	2	0	A2, A5	H2
3	H2	2	0	A3, A5	H4
4	H3	2	0	A3, A5	H4
5	H4	2	0	A3, A5	H4
6	T	3	1	A2, A5, A6	H2, H3
7	MC1	2	0	A4, A5	H5
8	MC2	2	0	A3, A5	H4
9	MC3	2	0	A2, A5	H2
10	AG1	3	0	A2, A3, A5	H2, H4
11	AG2	3	0	A3, A4, A5	H4, H5
12	D1	2	0	A2, A5	H2
13	D2	3	0	A2, A3, A5	H2, H4
14	D3	3	0	A2, A3, A5	H2, H4
15	W1	3	0	A2, A3, A5	H2, H4
16	W2	2	1	A1, A5	H1
17	H1	2	0	A3, A5	H4
18	H2	2	0	A3, A5	H4
19	H4	2	0	A3, A5	H4
20	H5	2	0	A3, A5	H4
21	H6	2	0	A3, A5	H4
22	H8 *	1	0	A5	-
23	H10 *	1	0	A5	-
24	H12	2	0	A2, A5	H2

* Only one loci amplified successfully

A5 occurred most frequently and was fixed for 23 of the 24 populations, occurring in 98% of samples. The least frequent allele (A6) was only found in two samples, both from Telowie Gorge CP in the Southern Flinders. The most frequent allele amplified for the 5' to *trnS* region was A2, occurring in 51% of samples, followed by A3 with 36% and then A1 and A4 which occurred in five samples each. Two private alleles were found, one at each loci. Private alleles occurred in the Telowie Gorge CP and on the rim of Wilpena Pound (WP2). In total eight individuals contained a private allele (Table 3). The private allele found in the Wilpena Pound population (WP2) was fixed for all individuals within that population. A4 only occurred in two populations in the Mambray Creek Block of the Mt. Remarkable NP and was therefore private to that region (Fig. 4).

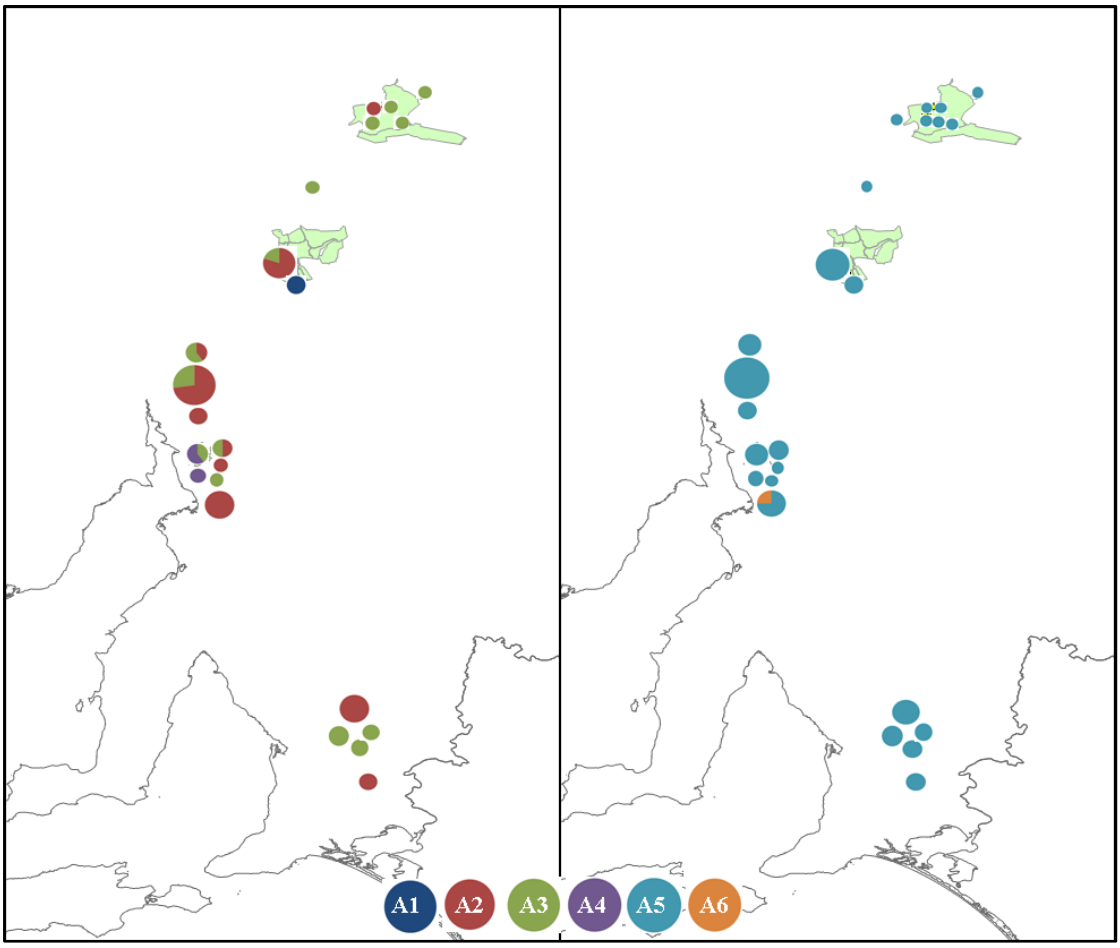


Figure 4: Arrangement of alleles across the range of *Callistemon teretifolius* for the 5' to *trn*S region (left) and *trnG* intron region (right). A1-A4 occurred in the 5' to *trn*S region and A5-A6 occurred in the *trnG* intron region. Circle size represents size of populations as indicated below.

The maximum number of alleles found per population was three and occurred in six of the populations, five of which occurred in the Southern Flinders Ranges and the other at Wilpena Pound (Table 3). The Southern Flinders Ranges, with populations in Dutchman's Stern CP, Mt. Remarkable NP and Telowie Gorge CP had the greatest allelic diversity with five alleles, of which three occurred in the 5'*trn*S region and two in the *trnG* intron (Table 3). The allelic composition of Dutchman's Stern and Alligator Gorge populations were the same at that recorded for the Mt. Lofty and Gammon Ranges (herbarium samples).

Wilpena Pound was the most diverse area in the Northern Flinders Ranges, with four alleles occurring across two populations (Fig. 4). Of the six herbarium samples that amplified successfully for both loci, only one sample differed in allelic composition, bringing the number of alleles in the region to three (Table 3). There was a more even spread of alleles in the Mt. Lofty Ranges, with two of the five populations containing A2, compared to the herbarium samples where only one of six populations contained A2. There were no polymorphic loci in the Mt. Lofty or Gammon Ranges.

Haplotypes

The six alleles produced a combination of five haplotypes and of the 22 populations in which haplotypes could be determined, sixteen contained only one haplotype (Fig. 5). All other populations contained two haplotypes. H2 was the most common haplotype, found in 41 individuals across ten populations, followed by H4 which was found in 30 individuals across fourteen populations. Of the variation present, 27% occurred within populations and 73% among populations. No variation occurred between regions.

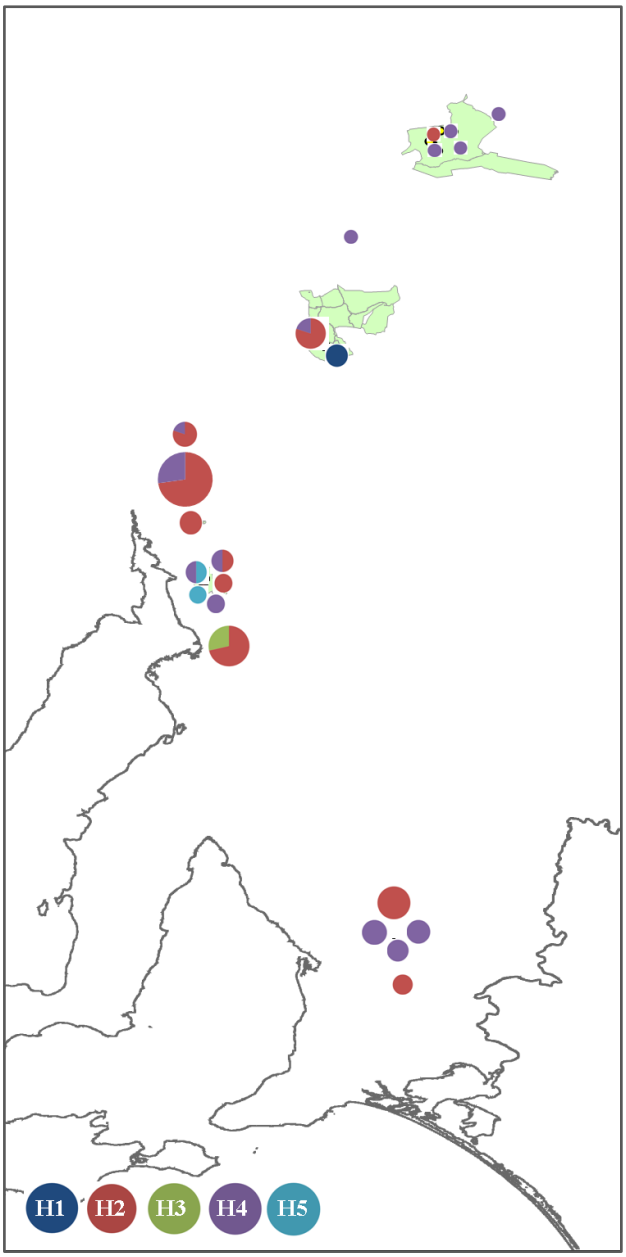
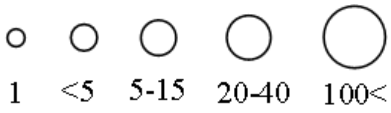


Figure 5: Haplotype diversity map, showing distribution of the 5 haplotypes (H1, H2, H3, H4 and H5) across 22 populations along the Adelaide Geosyncline. Size of chart indicates relative population size (not to scale), as shown below.



The Southern Flinders Ranges contained the most haplotypes with four recorded for this region, comprising H2, H3, H4 and H5, of which H3 and H5 were unique to the region (Fig. 6). Three haplotypes were recorded on Wilpena Pound, with H1 unique to the area. The Mt. Lofty and Gammon Ranges contained H2 and H4, but the ratio of these varied between the regions. The two haplotypes were more evenly distributed in the Mt. Lofty Ranges, so genetic diversity was higher in this region (Fig. 6).

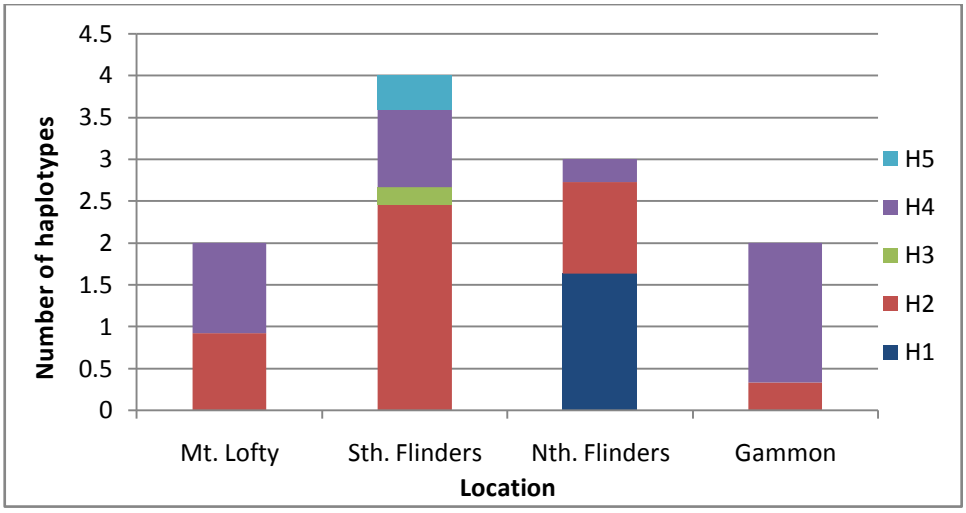


Figure 6: Haplotype number and composition for each of the four regions: Mt. Lofty Ranges, Southern Flinders Ranges, Northern Flinders Ranges (Wilpena Pound) and Gammon Ranges (herbarium samples). Height of each bar represents total number of haplotypes per region and colouring indicates proportion of haplotypes comprising that region. Haplotype specific colours are consistent with Fig. 5.

The statistical parsimony tree indicated that H2 or H4 were probably the basal condition as these were most frequent and other haplotypes diverged from these. H2 was more common in terms of number of individuals, but H4 was found in a greater number of populations and was more dominant in the Gammon and Mt. Lofty Ranges. H1 and H3 probably diverged from H2 and H5 from H4 (Fig. 7). The divergences from H4 to H5 and H2 to H3 probably occurred in the Southern Flinders Ranges, while divergence from H2 to H1 probably occurred at Wilpena Pound in the Northern Flinders Ranges (Fig. 7).

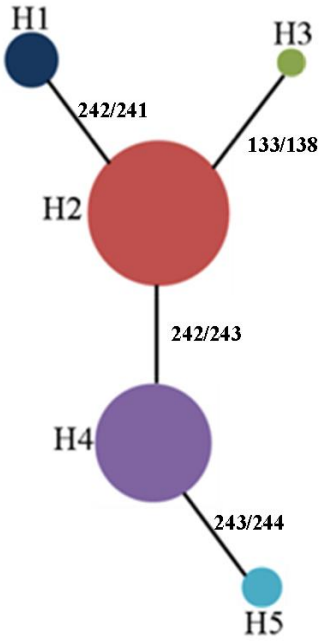


Figure 7: Statistical parsimony tree for haplotypes of *Callistemon teretifolius*. Branch length represents number of base pairs different between haplotypes and circle size represents haplotype frequency. Haplotypes are a combination of alleles from two variable cpDNA regions. The shift between haplotypes is shown along each branch.

Callistemon teretifolius had lower genetic diversity within populations (h_S=0.28, v_S=0.36) than among populations (h_T=0.66, v_T=0.65). G_{ST} was greater than $N_{ST,}$ indicating that geographic position may not be related to genetic relatedness between haplotypes (p>0.05; Table 4). Consequently, there was no significant geographic structure to haplotype distribution. Populations in the Mt. Lofty Ranges had the lowest within population diversity with h_s and V_s values of zero. This was followed by the Northern Flinders Ranges which had low diversity (hs=Vs=0.244) and the Southern Flinders Ranges which had moderate diversity (hs = 0.497, Vs = 0.472). The Northern Flinders Ranges has the highest among population diversity (hT=VT=0.900), followed by the Southern Flinders and then Mt. Lofty Ranges (Table 12). In the Mt. Lofty Ranges and Northern Flinders Ranges, G_{ST} was equal to N_{ST} which indicated all haplotypes were phylogenetically equivalent. In the Southern Flinders N_{ST} was greater than G_{ST} indicating some geographic structure to the haplotypes, but this was not significant (p=0.1).

Table 4: Chloroplast haplotype diversity and population differentiation

Chloroplast haplotype diversity (within population diversity h_S and v_S; among population diversity h_T and v_T) and population differentiation (G_{ST} and N_{ST}) (s.e.). for *Callistemon teretifolius* populations along the Adelaide Geosyncline region of southern Australia. NC = not calculated.

	Number of haplotypes	**h_S**	**h_T**	**G_{ST}**	**V_S**	**V_T**	**N_{ST}**
Entire Range	5	0.281 (0.0771)	0.657 (0.0582)	0.573 (0.1214)	0.355 (0.2501)	0.652 (0.2949)	0.455 (0.1463)
Mt Lofty Ranges	2	0.000 (0.000)	0.600 (0.0980)	1.000 (NC)	0.000 (0.000)	0.600 (0.0980)	1.000 (NC)
Southern Flinders	4	0.497 (0.1013)	0.625 (0.0998)	0.205 (0.0837)	0.472 (0.0962)	0.629 (0.1067)	0.250 (0.0778)
Northern Flinders	3	0.244 (0.1237)	0.900 (0.0081)	0.728 (0.1879)	0.244 (0.1237)	0.900 (0.0081)	0.728 (0.1879)

AFLPs

In total, 158 bands were produced from three primer pairs and were amplified successfully for 153 individuals. Of the original 171 samples used, five of these were removed from final analysis due to poor amplification for one or more of the primer pairs and a further 14 samples were removed as they produced ambiguous results that could not be overcome with re-amplification. The number of individuals per population that were successfully amplified and scored ranged from one to 32. Unique bands were found in five populations and the number of bands per population ranged from 52 to 116 (Table 5). Total number of bands and bands occurring in at least 5% of individuals were the same for every population except for Telowie Gorge CP and Dutchman's Stern 2 (D2), indicating rare bands in these two populations (23 in each). Private bands occurred in Dutchman's Stern CP (D2), Alligator Gorge (AG2), Telowie Gorge CP and Hale CP (H1 and H3) and ranged from one to nine per population. The majority of unique bands occurred in the Southern Flinders Ranges (Table 5).

Table 5: Band pattern across populations.

The total number of different bands (No. Bands), the number of bands that occurred in at least 5% of individuals (No. Bands>=5%) and the number of bands only found in that population (No. Private Bands).

Population	No. Bands	No. Bands >=5%	No. Private Bands
W	52	52	0
H1	90	90	1
H2	61	61	0
H3	77	77	1
H4	65	65	0
T	95	72	2
MC1	55	55	0
MC2	55	55	0
AG1	58	58	0
AG2	74	74	2
D1	69	69	0
D2	116	93	9
D3	66	66	0
WP1	56	56	0
WP2	63	63	0

On average 29.09% of bands per population were polymorphic (Table 6). The geographic population with the greatest percent of polymorphic loci was the population of 400 individuals in Dutchman's Stern CP (D2), with 70.70%; this was followed by the Telowie Gorge CP population with 52.87% and then Hale CP (H1) population with 48.41% (Table 6). All other populations had 35% or fewer polymorphic loci. The lowest percent of polymorphic loci occurred in Warren CP with only 10.80% of bands polymorphic, indicating that nearly 90% of loci were fixed. The Warren CP population also had the lowest Shannon index and Nei's expected heterozygosity, with the highest occurring in Dutchman's Stern CP (Table 6). A similar trend was found for the three genetic diversity indices across all populations. There was a positive correlation between sample size and percent of polymorphic loci, with $y=11.642 + 1.721x$ ($r^2 = 0.900$). Sample size was related to geographic population size.

Table 6: Genetic diversity indices for *Callistemon teretifolius* populations

Population	**Estimated population size**	**Percent of polymorphic loci (%)**	**Shannon Index**	**Nei's expected heterozygosity**
W	10	10.8	0.051	0.033
H1	20	48.41	0.181	0.113
H2	10	19.75	0.105	0.069
H3	30	34.39	0.128	0.079
H4	20	22.29	0.103	0.066
T	40	52.87	0.173	0.107
MC1	2	12.74	0.077	0.053
MC2	2	12.74	0.077	0.053
AG1	4	21.02	0.113	0.075
AG2	15	31.21	0.123	0.077
D1	10	29.94	0.126	0.081
D2	400	70.70	0.227	0.140
D3	10	29.30	0.139	0.090
WP1	20	17.83	0.097	0.065
WP2	10	22.29	0.119	0.079
Mean (SE)	38 (11)	29.09 (4.33)	0.123 (0.005)	0.079 (0.003)

Overall, within population genetic diversity was low with Shannon Index of 0.123 and heterozygosity 0.079 (Table 6). The genetic variance was partitioned with 76% occurring within populations, 16% among regions and the remaining 8% among populations. The total differentiation between populations was 0.242 (PhiPT), of which 0.158 (PhiRT) was due to the among region component and 0.099 (PhiPR) due to the among-population within region component ($p<0.001$).

PCA based on pairwise genetic distance showed three major groupings between populations (Fig. 8), with the first three axes explaining 83.58% of the variation. All Mt. Lofty populations were clustered together. A second cluster consisted of the Mambray Creek, Alligator Gorge and Telowie Gorge CP populations, while the Dutchman's Stern populations clustered more closely with the Wilpena Pound population WP1, rather than the other Southern Flinders Ranges populations. The other Wilpena Pound population WP2 was quite distinct from all other populations. PCA suggests that genetically, little separates W and H1.

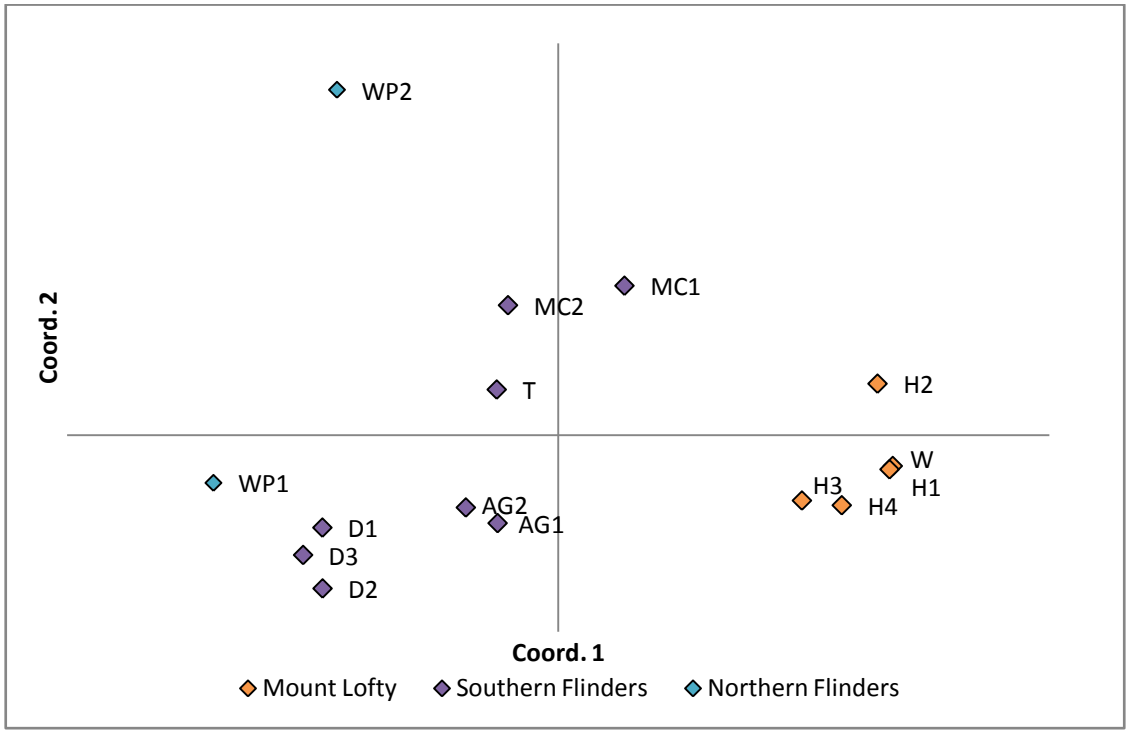


Figure 8: Principle Coordinate Analysis (PCA) showing genetic distance between populations. W = Warren CP, H = Hale CP, MC = Mambray Creek, AG = Alligator Gorge, T = Telowie Gorge CP, D = Dutchman's Stern CP and WP = Wilpena Pound.

Three major genetic clusters were indicated by STRUCTURE (k=4) and these corresponded to some extent with geographical regions, while the fourth group was shared between regions (Fig. 9). All Mt. Lofty populations grouped together, a second cluster consisted of the Telowie Gorge CP and Mambray Creek populations and a final group of the Alligator Gorge, Dutchman's Stern and the WP1 population. The other Wilpena Pound population (WP2) appeared to share more genetic characteristics with the Telowie Gorge cluster (Fig. 9). Clusters were not highly distinct, with each cluster having some individuals sharing genetic material with the other clusters. The Mt. Lofty Cluster was the most differentiated, but some gene flow may have occurred throughout the Southern Flinders Ranges as far north as the Mambray Creek Block in Mt. Remarkable NP.

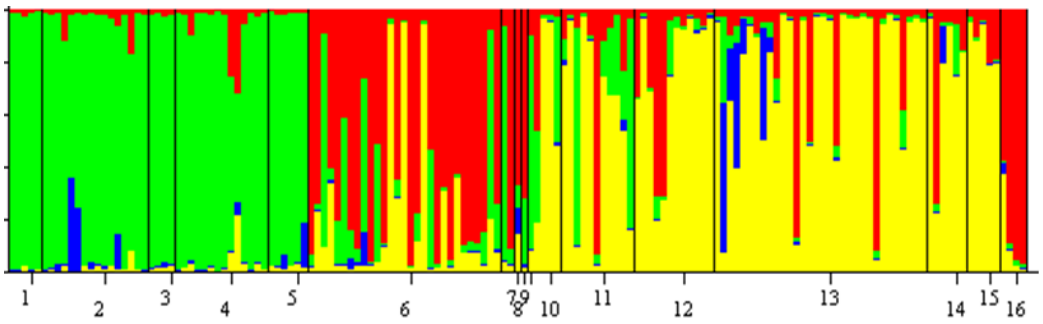


Figure 9: STRUCTURE cluster analysis showing natural genetic clusters across the 16 geographic populations sampled. Each colour represents genetic material from one cluster. Cluster 1 (Red), Cluster 2 (Green), Cluster 3 (Blue) and Cluster 4 (Yellow). Each vertical line represents one individual. The black vertical lines represent the division between geographic populations. Populations are ordered from south (left) to north (right).

Results from PCA and STRUCTURE indicated a likely barrier to gene flow between the Mt. Lofty and Southern Flinders Ranges and within the Mt. Remarkable NP. There was evidence of differentiation as a result of geographic distance, supported by a positive Mantel Test. A significant positive correlation between genetic and geographic distance was found ($r^2 = 0.0983$; $p<0.001$).

The three major genetic groupings were generally supported by the phylogeographic results (Appendix L). However, there were some inconsistencies when compared to Figures 8 and 9, possibly due to high homoplasy as a result of constrained population monophyly. Phylogeographic analysis indicated that both Wilpena Pound populations were nested within the Southern Flinders Ranges populations. The Mt. Lofty populations did not form a clade as H2 was aligned with the Southern Flinders Ranges. Results had high homoplasy index (0.8941), when population monophyly was constrained, indicative of high levels of homoplasy and a weak phylogenetic signal. Therefore, derived characters were shared between individuals in unrelated clades.

Pairwise population PhiPT values suggest high levels of differentiation between many of the populations. The highest levels of differentiation were recorded between the Warren Population and populations from Wilpena Pound (PhiPT = 0.539, 0.507; $p<0.005$). The Warren population was highly differentiated from all populations, except those in Hale CP, where moderate to moderate-high differentiation occurred (Table 7). PhiPT values tended to increase with geographic distance, consistent with isolation by distance. Three major clusters of low differentiation were evident, occurring in Hale CP around Telowie Gorge and Mambray Creek and in Dutchman's Stern CP, which align with the three genetic clusters suggested by PCA and STRUCTURE. The two populations that were most differentiated were W and WP1 (Table 7), which corresponded to the most southerly and northerly regions sampled.

Table 7: Pairwise population PhiPT matrix

AFLP pairwise population comparisons. PhiPT values are shown below the diagonal. P-values based on 9999 permutations are shown above the diagonal. Orange shading represents low differentiation estimates. W=Warren, H = Hale, T=Telowie, MC=Mambray Creek, AG= Alligator Gorge, D=Dutchman's Stern and WP = Wilpena Pound.

	W	H1	H2	H3	H4	T	MC1	MC2	AG1	AG2	D1	D2	D3	WP1	WP2	
W	0.000	0.012	0.002	0.000	0.028	0.000	0.048	0.048	0.000	0.000	0.000	0.000	0.000	0.008	0.000	**W**
H1	0.132	0.000	0.166	0.000	0.008	0.000	0.119	0.019	0.002	0.000	0.000	0.000	0.000	0.000	0.000	**H1**
H2	0.178	0.036	0.000	0.024	0.082	0.001	0.329	0.061	0.004	0.000	0.001	0.001	0.003	0.020	0.030	**H2**
H3	0.163	0.099	0.091	0.000	0.035	0.000	0.033	0.009	0.000	0.000	0.000	0.000	0.000	0.000	0.001	**H3**
H4	0.174	0.117	0.069	0.060	0.000	0.000	0.142	0.035	0.002	0.000	0.000	0.000	0.002	0.005	0.006	**H4**
T	0.271	0.181	0.186	0.182	0.214	0.000	0.169	0.078	0.024	0.000	0.000	0.000	0.001	0.000	0.000	**T**
MC1	0.418	0.074	0.071	0.184	0.107	0.063	0.000	0.341	0.391	0.036	0.011	0.149	0.104	0.043	0.066	**MC1**
MC2	0.504	0.238	0.168	0.314	0.275	0.112	0.000	0.000	0.196	0.030	0.011	0.123	0.114	0.046	0.264	**MC2**
AG1	0.396	0.178	0.196	0.200	0.184	0.064	0.038	0.129	0.000	0.055	0.010	0.027	0.218	0.050	0.008	**AG1**
AG2	0.377	0.238	0.241	0.250	0.233	0.132	0.156	0.173	0.067	0.000	0.000	0.000	0.000	0.002	0.002	**AG2**
D1	0.397	0.297	0.329	0.297	0.295	0.114	0.196	0.189	0.092	0.096	0.000	0.032	0.460	0.060	0.001	**D1**
D2	0.305	0.258	0.250	0.261	0.233	0.116	0.118	0.135	0.082	0.102	0.031	0.000	0.415	0.116	0.012	**D2**
D3	0.397	0.263	0.280	0.269	0.249	0.097	0.114	0.101	0.028	0.094	0.000	0.000	0.000	0.367	0.004	**D3**
WP1	0.539	0.357	0.385	0.372	0.382	0.151	0.230	0.230	0.152	0.112	0.059	0.036	0.009	0.000	0.030	**WP1**
WP2	0.507	0.351	0.348	0.394	0.384	0.189	0.235	0.068	0.204	0.212	0.177	0.159	0.141	0.172	0.000	**WP2**
	W	**H1**	**H2**	**H3**	**H4**	**T**	**MC1**	**MC2**	**AG1**	**AG2**	**D1**	**D2**	**D3**	**WP1**	**WP2**	

A significant spatial autocorrelation (r) was found for all populations, indicating spatial genetic structure within populations (Fig. 10). The highest level of spatial autocorrelation was found for the Warren CP population (r=0.590). All other populations in the Mt. Lofty Ranges had r values greater than 0.200. Telowie Gorge had the smallest positive correlation (r=0.082), followed by AG2 and all three Dutchman's Stern populations (r<0.180). High positive correlations were found for AG1 and both Wilpena Pound populations. In general there was a trend for r to increase at the range margins, indicating higher genetic structure in those areas. All correlations were significant at the 0.05 level.

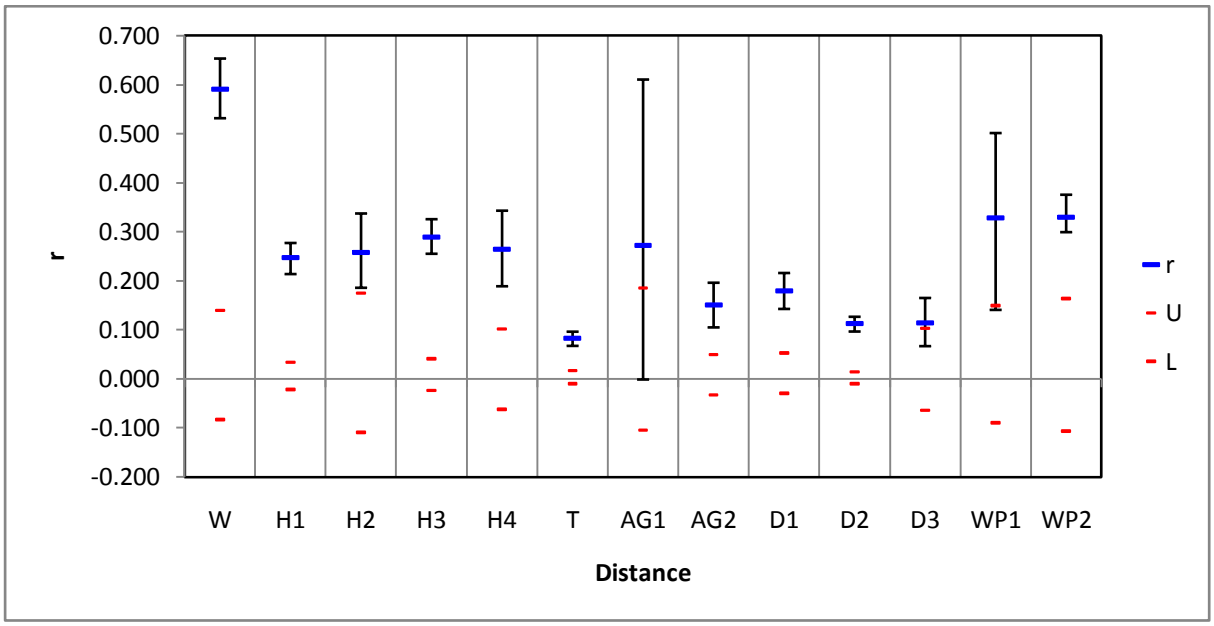


Figure 10: Spatial autocorrelation (r) between members of 13 populations (MC populations were excluded due to small population size). Population r is determined by the pairwise correlation between all individuals within the population. The blue line = r, upper and lower error bars bound the 95% confidence interval as determined by bootstrap re-sampling. Red lines represent upper (U) and lower (L) 95% confidence limits about the null hypothesis as determined by 9999 permutations. All results are significant at the 0.05 level.

Modelling and Spatial Analysis

Species Distribution Models

Eighteen final models were produced using historical (Pleistocene), current and four future climate scenarios and three algorithms (BIOCLIM, MAXENT and GARP best subsets new implementation software). Approximately 91% of data fell within the climate envelope developed by BIOCLIM. The MAXENT model had an AUC > 0.9 indicating an excellent fit of the model to the data, GARP and BIOCLIM had an AUC >0.80 indicating a good fit to the data (Table 8). The MAXENT algorithm produced the best fit for the data, demonstrated by the highest AUC value (Table 8). GARP and BIOCLIM both appeared to over-estimate range, especially to the east of the Southern Flinders and Mt. Lofty Ranges.

Table 8: AUC, Kappa and threshold values for all three species distribution algorithms

	AUC	**Kappa**	**Logistic threshold/ threshold at max Kappa**
BIOCLIM	0.840	0.610	16.10
MAXENT	0.954	0.186	15.53
GARP (Best subsets new implementation)	0.870	0.050	-

Declines in suitable area were predicted by all algorithms for each of the four future climate scenarios modelled (Table 9). GARP predicted the largest areas of excellent climate under current, all 2030 and 2070 least severe climate scenarios. MAXENT predicted increases in excellent area in 2030 and 2070 with the least severe climate changes with increases of 48.4% and 68.1% respectively (Table 9). Changes in total suitable and excellent area did not occur proportionately and large differences in the percent change occurred for some scenarios. This was evident for MAXENT predictions, where reductions in total suitable area were accompanied by increases in excellent area and for BIOCLIM and GARP outputs, where excellent area declined by approximately 50% more than total suitable area under some scenarios (Table 9).

Table 9: Percent change from current conditions for each algorithm and climate scenario. Suitable is the total of the areas deemed low-excellent. Excellent is the subset of the total area which is most climatically suitable for survival (indicated by red on modelled outputs). Negative values represent a decrease in area compared to current and positive represent an increase.

	BIOCLIM		MAXENT		GARP	
	Suitable	**Excellent**	**Suitable**	**Excellent**	**Suitable**	**Excellent**
2030 Low	-12.1	-66.7	-2.8	+48.4	-85.3	-14.7
2030 High	-49.6	-96.3	-49.2	-18.3	-36.0	-64.0
2070 Low	-29.7	-91.9	-5.3	+68.1	-54.2	-45.8
2070 High	-99.9	-100.0	-80.4	-88.8	-90.2	-99.8
Pleistocene	-77.5	-100.0	-78.5	-89.9	-62.4	-75.5

Apart from over-estimation by BIOCLIM and GARP the most notable difference between algorithm projections occurred for the historical (Pleistocene) scenario. BIOCLIM predicted suitable climate in the Northern Flinders Ranges, MAXENT predicted a number of suitable areas, with the largest in the Mt. Lofty Ranges and GARP predicted restriction to the northern limits of current range, but on either side of the Adelaide Geosyncline (Figs. 11-13).

BIOCLIM

In general BIOCLIM tended to over predict range, especially for the following scenarios: current, all 2030 and 2070 least change, with the majority of over-prediction occurring to the east of the Southern Flinders and Mt. Lofty Ranges. Projection of current conditions suggests the Southern Flinders Ranges is the most climatically suitable region for the species, but much of the predicted area occurred along the coast or on cleared, low elevation areas further inland (Fig. 11). An increase of 75% total suitable area was predicted from the Pleistocene to current scenario (Table 9). The 2030 climate scenarios resulted in a 12.1-49.6% reduction in total suitable area and a 66.7-96.3% reduction in excellent area (Table 8), with restriction most pronounced along the coast and in the northern areas of the Adelaide Geosyncline (Fig. 11). The 2070 low scenario resulted in a 30% reduction in total suitable area and 91% reduction in excellent area from current (Table 9). With the most severe changes (2070 high), severe range restrictions were predicted, with a 99.9% decline in total suitable area. For all scenarios modelled, suitable area became more restricted across the entire range and more fragmented.

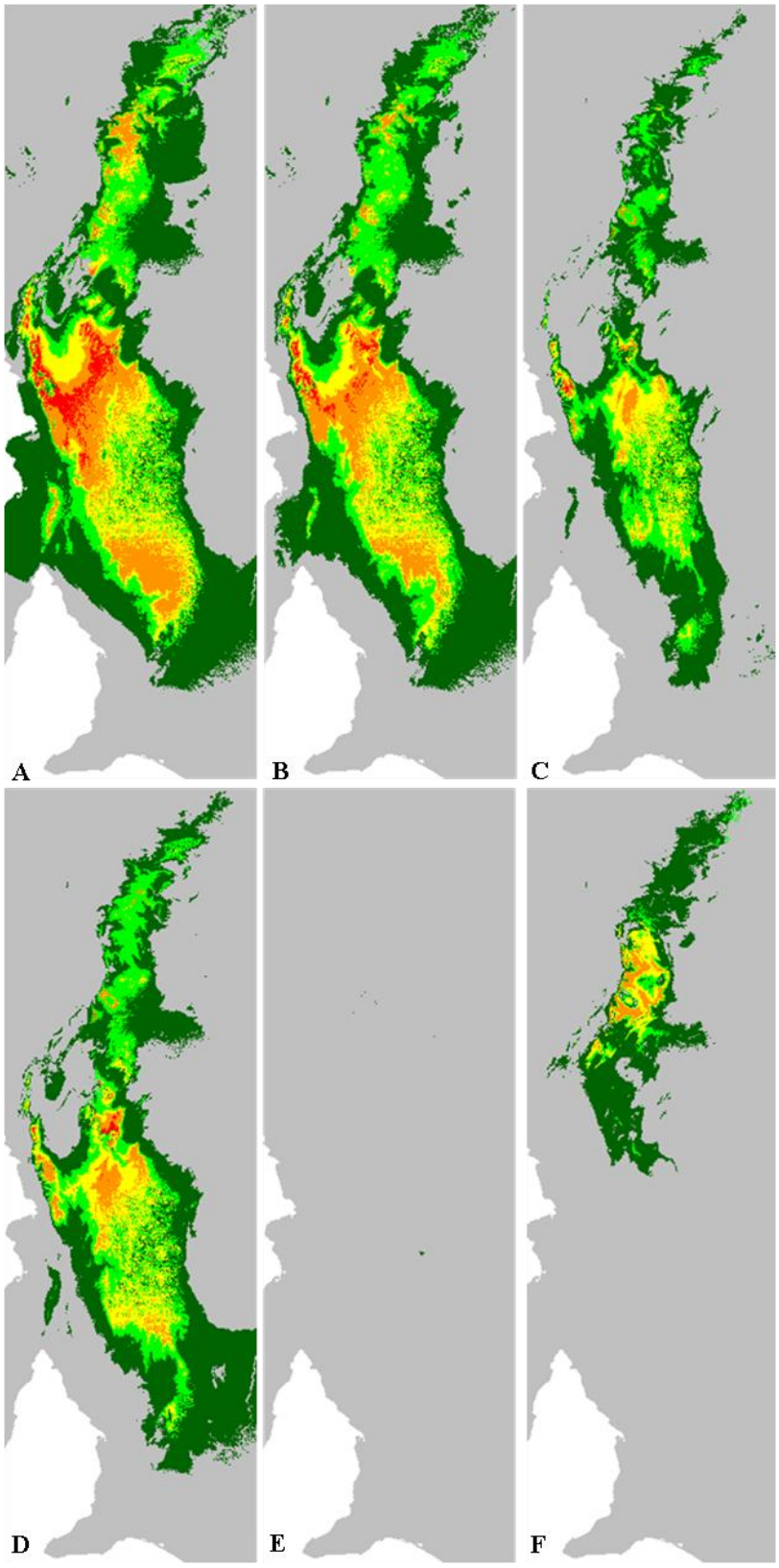


Figure 11: From left to right (BIOCLIM projections): A. Current conditions, B. 2030 least change (0.5°C temperature increase; 1% increase in rainfall), C. 2030 greatest change (1.5°C temperature increase; 9% rainfall reduction), D. 2070 least change (1.2°C temperature increase; 4% rainfall increase), E. 2070 greatest change (4.7°C temperature increase; 25% rainfall reduction) and F. Pleistocene (6°C temperature decrease; 10% reduction in rainfall). Grey represents the areas which are unsuitable for survival, dark green is low, light green medium, yellow high, orange very high and red is excellent suitability.

MAXENT

A number of climatically excellent regions were predicted by MAXENT under the current climate scenario. The largest patches of excellent climate were projected along the Southern Flinders Ranges and in the Vulkathunha-Gammon Ranges NP. There were also smaller areas around the rim of Wilpena Pound, in Arkaroola Wilderness Sanctuary and in the Mt. Lofty Ranges. There was some evidence of range fragmentation, which was most evident between the Mt. Lofty and Southern Flinders Ranges and between the Southern Flinders and Northern Flinders Ranges (Fig. 12). A distance of approximately 175km separated excellent area in the Southern Flinders and Mt. Lofty Ranges, with the majority of intervening area either climatically unsuitable or low suitability. A 78.5% increase in suitable area was predicted to have occurred from the Pleistocene to present (Table 9).

Excellent climate was predicted to increase in the Southern Flinders for the least severe climate changes predicted for 2030 and 2070 (Table 9). These increases occurred in association with a decline in total suitable area across the species range. The more severe changes for 2030 resulted in a 50% reduction in suitable area and an 18% reduction in excellent area (Table 9), leaving only 1km^2 of excellent climate in the Northern Flinders Ranges. The majority of the Southern Flinders Ranges was deemed climatically excellent, but overall there was pronounced range restriction and fragmentation, especially in the Northern Flinders Ranges (Fig. 12).

The greatest increase in climatically excellent area was predicted for the 2070 low scenario, with the majority of this increase occurring in the Southern Flinders Ranges (Fig. 12). Range restrictions were predicted for the northern regions; under these conditions Arkaroola Wilderness Sanctuary no longer contained any excellent area. In the Northern Flinders Ranges excellent area became restricted to a small pocket within the Vulkathunha-Gammon NP and around the rim of Wilpena Pound (Fig. 12). Severe range loss and restrictions were projected for the 2070 high scenario, with 80% reductions in suitable area and 89% reductions in excellent area (Table 9). This scenario resulted in almost total decline of suitable area in the Flinders Ranges, as only small sections of low climatically suitable area remained. A southerly shift from the Mt. Lofty Ranges towards the coast was predicted (Fig. 12).

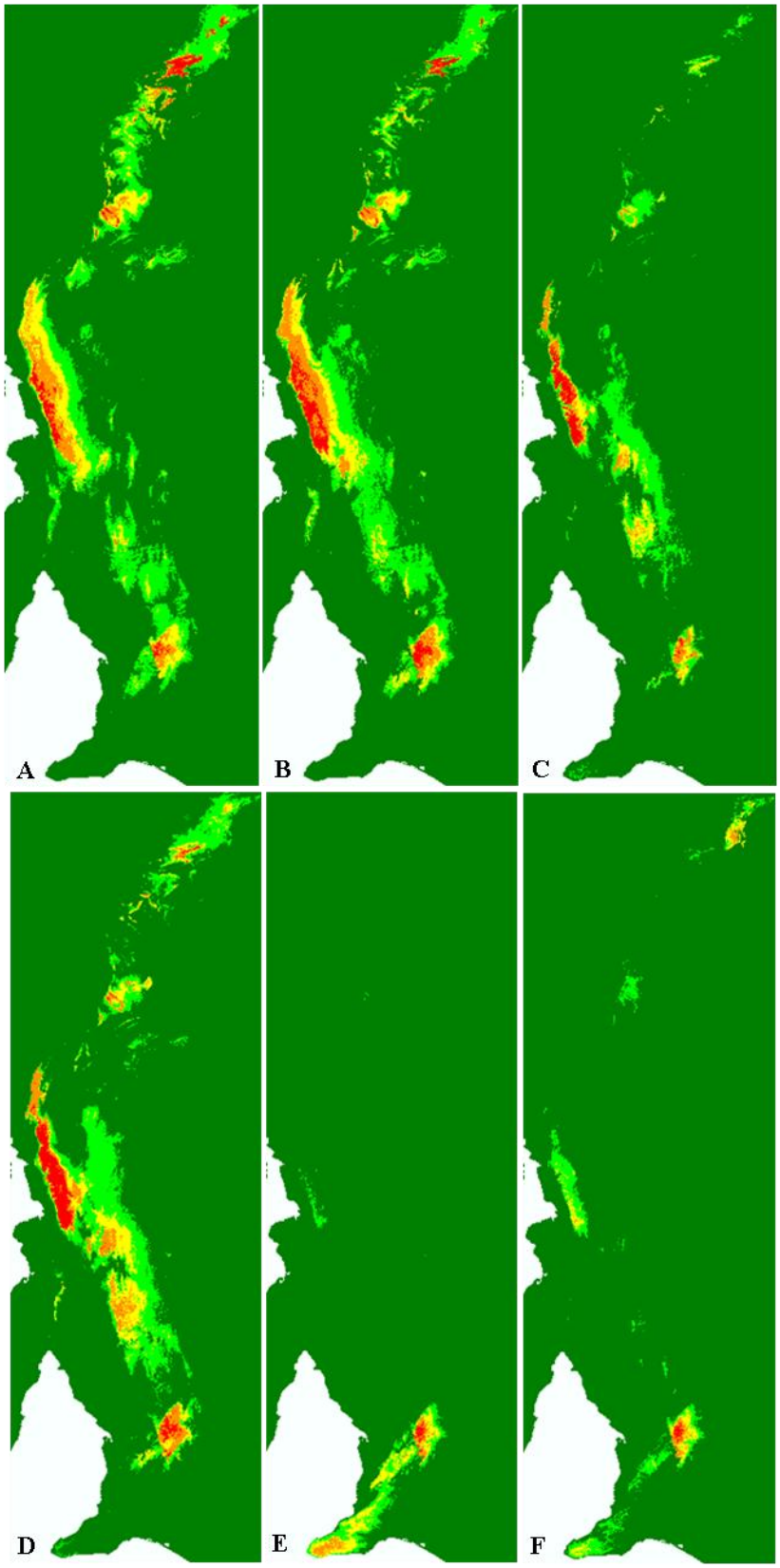


Figure 12: From left to right (MAXENT projections): A. Current conditions, B. 2030 least change (0.5°C temperature increase; 1% increase in rainfall), C. 2030 greatest change (1.5°C temperature increase; 9% rainfall reduction), D. 2070 least change (1.2°C temperature increase; 4% rainfall increase), E. 2070 greatest change (4.7°C temperature increase; 25% rainfall reduction) and F. Pleistocene (6°C temperature decrease; 10% reduction in rainfall). Dark green represents unsuitable, light green is low, yellow is medium, orange is high and red is excellent.

GARP Best subsets new implementation software

GARP did not fit the data as well as MAXENT in terms of AUC, visual comparison to presence points and field knowledge. The outputs for current, all 2030 scenarios and the 2070 least change scenario appear to overestimate range in the Flinders Ranges and under-estimate range in the Mt. Lofty Ranges. The Southern Flinders Ranges was the region predicted to have the highest climate suitability for the species. Range restrictions were predicted for all future climate scenarios, with restrictions occurring along the species entire range, but restrictions were most severe in the Northern Flinders Ranges (Fig. 13). The 2070 high scenario resulted in a severe reduction in predicted range (99%), with most suitable area restricted to a small pocket in the Mid-North of the state.

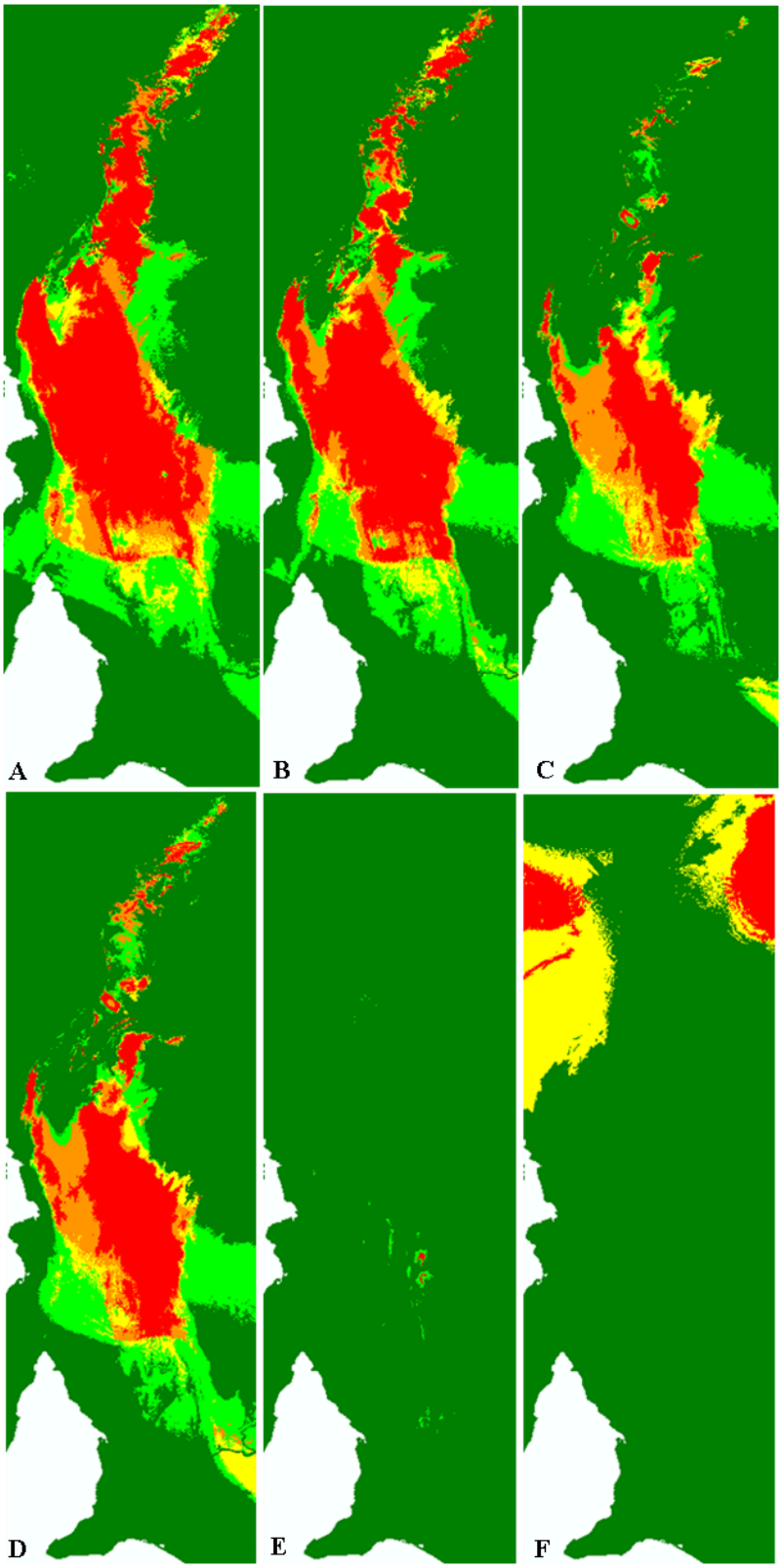


Figure 13: From left to right (GARP projections): A. Current conditions, B. 2030 least change (0.5°C temperature increase; 1% increase in rainfall), C. 2030 greatest change (1.5°C temperature increase; 9% rainfall reduction), D. 2070 least change (1.2°C temperature increase; 4% rainfall increase), E. 2070 greatest change (4.7°C temperature increase; 25% rainfall reduction) and F. Pleistocene (6°C temperature decrease; 10% reduction in rainfall). Dark green represents unsuitable, light green is low, yellow is medium, orange is high and red is excellent.

Spatial Analysis

Spatial analysis was limited to MAXENT outputs as these produced the best fit for the data (Table 8). Under the current climate scenario there was approximately 26 000 km^2 of area deemed climatically suitable and 544km^2 of this was classed as excellent (Table 10). Total suitable area declined under all future climate scenarios, with only 2 500km^2 of suitable area predicted under the most severe climate scenario (2070 High). Only 60km^2 of this area was excellent, of which only 4km^2 was protected by reserves (Table 10). Under current conditions 51% of excellent area was protected by reserves, but less than 8% of total suitable area occurred within the reserve system. In both 2030 scenarios and 2070 least change scenario there was an increase in the total suitable area protected by reserves, but a large drop in the amount of excellent area protected. With the most severe changes predicted, less than 6% of total suitable and excellent area was expected to occur within the current reserve system (Table 10).

Table 10: Total modelled area and area within reserves.
Area of modelled outputs for each climate scenario for the MAXENT algorithm, with total suitable area (suitable) and the subset of that, which is climatically excellent for *Callistemon teretifolius*. The proportion of this area which falls within the current reserve systems (National and Conservation Parks) and the percent. 2030 Low represents a 0.5°C and 1% increase in rainfall, 2030 High 1.5°C temperature increase and 9% rainfall decrease, 2070 Low 1.2°C temperature increase and 4% rainfall increase and 2070 High 4.7°C temperature increase and 25% rainfall reduction. Pleistocene represents predicted historical climate of a 6°C temperature and 10% rainfall reduction.

		Total area (km^2)	**Area within reserves (km^2)**	**Percent (%) area within reserves**
Current	Suitable	26 027	2 029	7.8
	Excellent	544	278	51
2030 Low	Suitable	13 630	1 361	10.8
	Excellent	820	289	27.9
2030 High	Suitable	6 553	823	12.6
	Excellent	456	126	27.7
2070 Low	Suitable	12 271	1 307	10.7
	Excellent	940	200	21.3
2070 High	Suitable	2 533	144	5.7
	Excellent	60	4	5.9
Pleistocene	Suitable	2 762		
	Excellent	55		

The total modelled area contained 61 900km^2 of intact native vegetation, the majority of which occurred in the northern parts of the state, where large expanses of land remain relatively uncleared. With the current climate scenario, 14 496km^2 (55%) of the area predicted to be climatically suitable fell on cleared land (Table 11). The amount of suitable area on fragmented land decreased with all future climate scenarios due to overall reductions in total suitable area. However, percent of suitable area on cleared land stayed relatively constant or increased. The largest percent of suitable area on cleared land occurred for 2070 high with 1 951km^2 of the predicted area falling on cleared land, accounting for over 75% of climatically suitable area (Table 11).

Table 11: Modelled area on cleared land and in between the NatureLinks.
For current and all future scenarios as predicted by MAXENT. 2030 Low represents a 0.5^{o}C and 1% increase in rainfall, 2030 High 1.5^{o}C temperature increase and 9% rainfall decrease, 2070 Low 1.2^{o}C temperature increase and 4% rainfall increase and 2070 High 4.7^{o}C temperature increase and 25% rainfall reduction. Pleistocene represents predicted historical climate of a 6^{o}C temperature and 10% rainfall reduction.

	Modelled suitable area on cleared land (km^2)	**Percent (%) of modelled output on cleared land**	**Area outside NatureLinks (km^2)**	**Percent (%) of modelled area outside of NatureLinks**
Current	14 469	55.6	9 107	35
2030 Low	4 142	63.2	5 418	40
2030 High	7 024	55.6	3 292	50
2070 Low	7 339	59.8	5 534	45
2070 High	1 951	77.0	0	0

A large proportion of suitable area fell between the two NatureLinks corridors for all climate scenarios modelled, except 2070 high. The current output had the greatest net area outside the NLs, with 9 107km^2 falling between the two corridors (Table 11). Similar levels of climatically suitable area fell in between the two corridors for 2030 and 2070 low climate scenarios, with approximately 5 500km^2 outside the corridors. Under the 2070 high scenario, all predicted area fell within the Cape Borda to Barossa NL.

Reserve specific analysis was undertaken on the Vulkathunha-Gammon, Flinders Ranges and Mt. Remarkable NPs, along with Dutchman's Stern and Hale CPs. The Mt. Remarkable NP consisted of three separate blocks and as such, the area for this section is the total area of these regions combined. The amount of suitable area in Hale CP did not change across the climate scenarios, with the entire 1.93km^2 of the park remaining suitable under all possible future climates modelled. Dutchman's Stern CP and the Vulkathunha-Gammon NP are predicted to lose all suitable climate by 2070 with most severe climate changes, while suitable area remaining in the Flinders Ranges NP was restricted to 2.3km^2 (Table 12). In the Mt. Remarkable NP reserve system 16.9km^2 of suitable area remained in reserves, but this was highly fragmented and the majority occurred within the Napperby Block. Only 5.1km^2 of suitable area remained in the Mambray Creek Block and on Mt. Remarkable, but area was fragmented into six sections.

Severe range restrictions within the reserves were predicted for the 2070 high scenario, with range reductions between 90 and 100% (excluding Hale CP). Different climate scenarios affected reserves to different extents. The Flinders Ranges NP and Dutchman's Stern CP were predicted to lose a higher percent of suitable area than Vulkathuna-Gammon Ranges NP in 2030 low (Table 12). In 2030 high, the Vulkathunha-Gammon Ranges NP suffered a 70% decline in total suitable area, compared to declines of approximately 40 and 30% for the Flinders Ranges NP and Dutchman's Stern CP respectively (Table 12). There was less than a 2% difference in decline predicted for the Vulkathunha-Gammon Ranges NP under the 2030 and 2070 low scenarios. The Mt. Remarkable NP lost very little range under all 2030 scenarios and the 2070 low scenario, with all reductions less than 5.5% (Table 12). There was a general trend for the percent decline to be greater in more northern areas and this was especially evident for 2030 low. No increases in protected suitable area occurred.

Table 12: Reserve specific analysis.

Total suitable area (km^2) and percent change from current in the Vulkathunha-Gammon Ranges NP, Flinders Ranges NP, Dutchman's Stern CP, Mt. Remarkable NP and Hale CP, under current and all future climate scenarios. 2030 Low (0.5^oC and 1% increase in rainfall), 2030 High (1.5^oC temperature increase and 9% rainfall decrease), 2070 Low (1.2^oC temperature increase and 4% rainfall increase) and 2070 High (4.7^oC temperature increase and 25% rainfall reduction).

	Current	**2030 Low**		**2030 High**		**2070 Low**		**2070 High**	
	Area (km^2)	**Area (km^2)**	**% Change**	**Area (km^2)**	**% Change**	**Area (km^2)**	**% Change**	**Area (km^2)**	**% Change**
Vulkathunha-Gammon NP	591.25	561.87	-5	176.87	-70.1	552.04	-6.6	0	-100
Flinders Ranges NP	655.31	507.19	-22.6	384.79	-41.3	469.74	-28.3	2.31	-99.6
Dutchman's Stern CP	35.89	32.45	-9.6	25	-30.2	28.98	-19.2	0	-100
Mt. Remarkable NP	181.73	179.17	-1.6	172.03	-5.4	179.51	-1.2	16.90	-90.7
Hale CP	1.93	1.93	0	1.93	0	1.93	0	1.93	0

Discussion and Conclusions

The primary aim of this research was to evaluate the risk of climate change on *Callistemon teretifolius* with field assessment, population genetics, species distribution modelling and spatial analysis to inform the development of management strategies. Sixteen geographic populations were located, with population size ranging from one to 400 individuals. The majority of geographic populations located and sampled contained 20 or fewer individuals. Genetic analysis of these populations with chloroplast microsatellites (cpSSRs) and Amplified Fragment Length Polymorphisms (AFLP) indicated past refugium in the Southern Flinders Ranges and Wilpena Pound and subsequent expansions into the Mt. Lofty and Northern Flinders Ranges. The sixteen geographic populations were clustered into three major genetic groups, which aligned largely with geographic location. Two major barriers to gene flow were evident, which were consistent with these genetic clusters. Overall, genetic diversity was low (He = 0.079) and population differentiation was high, indicating low gene flow between populations (PhiPT = 0.242). Species distribution modelling supported historical range restriction and predicted future range reductions and fragmentation under all four climate scenarios modelled. Actual area suitable for growth may be more restricted than predicted by the models, as large proportions of modelled area often corresponded with fragmented habitats. Habitat fragmentation may impede range shifts and limit survival in areas otherwise deemed climatically suitable. Climate change may also reduce the effectiveness of the current reserve system as a result of range shifts and range contractions.

Historical population structure

Refugial populations have a longer demographic history and as such tend to contain higher levels of genetic diversity and private alleles/haplotypes (Provan and Bennet 2008). Results from the two variable chloroplast loci suggest that the Southern Flinders Ranges, especially the Mambray Creek Block of Mt. Remarkable NP and Telowie Gorge CP may have been refugium for this species during the past. Another refuge may have occurred on the rim of Wilpena Pound as a population in this area was fixed for a unique divergent haplotype (Fig. 5). The Gammon Ranges, Mt. Lofty Ranges, Dutchman's Stern CP and the second Wilpena Pound population (WP1) have lower haplotypic diversity, indicative of expansions (potentially post-glacial) into these regions (Cozzolino *et al.* 2003; Johansen and Latta 2003). However, care needs to be taken when analysing the Gammon Ranges results as herbarium

material may suggest lower genetic diversity than what is actually present due to allelic drop out as a result of DNA degradation (Wandeler *et al.* 2007).

The AFLP results support the pattern of diversity found in the chloroplast DNA. Genetic structure was indicative of two expansion events from the Southern Flinders Ranges into the Mt. Lofty and Northern Flinders Ranges. Expansion from the refugial Wilpena Pound population was unlikely, due to the genetic isolation of this population (Fig. 8). The recolonised Wilpena Pound population (WP1) was genetically more similar to populations from Alligator Gorge and Dutchman's Stern CP (Fig. 9), which indicates colonisation of this area from the Southern Flinders Ranges. Populations were clustered into three major genetic groups (Figs 8 and 9), supporting two expansion events away from the central refugial area. Peripheral populations were more structured than central populations (Fig. 10), which supports two expansion events, with more restricted gene flow in peripheral areas resulting in fine scale genetic structure (Loveless and Hamrick 1984; Escudero *et al.* 2003). However, AFLP results need to be analysed with caution due to small sample sizes available. A minimum of 30 individuals per population is often required to yield accurate results with AFLP analysis (Bonin *et al.* 2007), but samples of this size were not attainable in most instances due to highly restricted population size.

Modelling of expected historical climate conditions during the Pleistocene supported range restriction during the past and subsequent expansion during post-glacial warming (Fig. 12G). The range of *Callistemon teretifolius* may have been at least 75% less than the range currently occupied, indicating likely survival during past climatic fluctuations even with severe range restrictions (> 3 000 km^2). MAXENT indicated a number of possible refugium across the Adelaide Geosyncline during past climatic fluctuations, which compared to cpDNA structure where a number of refugium were apparent (Fig. 5). The MAXENT algorithm supported the cpDNA to a greater extent that the other two algorithms. However, there were some inconsistencies between the modelled results and haplotype distribution. MAXENT indicated the most suitable area for survival probably occurred in the Mt. Lofty Ranges, but genetic analysis indicated low haplotype diversity in this area and hence a refugium was unlikely. Inconsistencies may be a result of uncertainty surrounding the magnitude of past climate changes (Pearson and Dawson 2002; Beaumont *et al.* 2007), but may also indicate *C. teretifolius* survived in climates that differed to some extent from those currently occupied.

Current range, demographics and genetic structure

Population demographics

Of the sixteen populations located during field sampling, fifteen of those were estimated to contain less than 40 individuals, with overall median population size of ten individuals (Table 2). Populations were generally highly geographically restricted, often at high altitudes, and as such, there may be limited opportunity for future population expansion or range shifts (Grabbher *et al.* 1994; Wilson *et al.* 2005; Loss *et al.* 2011). As effective population size tends to be much smaller than census size, very few individuals may contribute genetic material to the next generation, increasing risk of genetic drift and inbreeding (Ellstrand and Elam 1993; Young *et al.* 2006; Frankham 2005). This was especially evident in Telowie Gorge CP, where a high proportion of the population were juvenile. Genetic drift and inbreeding may cause genetic erosion of small populations and reduce population fitness, increasing extinction risk (Garant *et al.* 2006; Aguilar *et al.* 2008). However, these geographic populations may not be completely isolated as gene flow can occur over large distances, effectively increasing population size, potentially mitigating against small population size issues (Tero *et al.* 2003; Garant *et al.* 2006).

Population genetics

The sixteen geographic populations were grouped into three major genetic clusters and some gene flow between populations was likely within these clusters (Table 7). Actual population size may be larger than geographic population size in these areas. However, across the range of the species populations were highly differentiated (PhiPT = 0.242) and pairwise differentiation between populations was generally moderate to high (Table 7), indicating low levels of past gene flow (Ellstrand and Elam 1993; Petit *et al.* 2005) and likely population isolation. Five population's also maintained unique alleles (Table 5) and there was evidence of isolation by distance, which supports restricted gene flow. Therefore, gene flow may not play a major role in mitigating issues of small population size in parts of the species range. Gene flow through seed dispersal may also be limited as the genetic signature of past population fluctuations was still evident in contemporary genetic structure (Fig. 5). The genetic signature of past refugia and subsequent colonisations was not eroded over time by gene flow (cpDNA); indicating seed dispersal may be rare (Petit *et al.* 1993; King and Ferris 1998). Therefore *Callistemon teretifolius* may have limited ability to track changing climates

through seed dispersal and establishment in new areas (Davis and Shaw 2001; Petit *et al.* 2003).

Barriers to gene flow may also limit the species ability to track changing climates. The most pronounced division between genetic clusters occurred between the Mt. Lofty and Southern Flinders Ranges (Figs. 8 and 9) indicating a large barrier to gene flow in this area (Ellstrand 1992; Lienert 2004; Aguilar *et al.* 2008). This barrier is supported by MAXENT results as the two regions were separated by a matrix of low suitability or unsuitable climate (Fig. 12). This region is also highly fragmented, which may further limit gene flow (Appendix J). A second division occurred between populations in the Mambray Creek section of the Mt. Remarkable NP (Fig. 9). A barrier in this area was unexpected as populations were separated by a relatively small geographic distance (<5km). A gorge which separates these populations (DEH 2006a) may act as a barrier to gene flow. However, as the species is probably, predominantly bird pollinated, a barrier to gene flow between geographically close populations is unusual (Sampson *et al.* 1989; Campbell 1991). AFLP results need to be analysed with caution due very small sample sizes in this region and as such results may be affected by chance sampling bias (Bonin *et al.* 2007). Some evidence suggests the second division may actually occur between Mt. Remarkable NP and Dutchman's Stern CP (Fig. 8).

If barriers to gene flow and low dispersal ability limit the species ability to track changing climates, genetic diversity will be important to facilitate *in situ* adaptation. However, *Callistemon teretifolius's* ability to respond to climate change though adaptation may be limited as a result of low genetic variation. Only two cpDNA regions were found to be variable and mean number of polymorphic loci per population was approximately 29%, indicating the majority of loci were fixed within populations (Table 6). Low within population and species-level diversity (He = 0.079) indicate the species may not have the necessary variation to overcome climate change and associated changes in community and environmental conditions (Hewitt 1999; Walther *et al.* 2002). The populations/areas that probably have the greatest capacity to respond to climate change are Hale, Telowie Gorge and Dutchman's Stern CPs, due to high genetic diversity (Table 6). These higher diversity populations aligned to some extent with population size, indicating a correlation between contemporary diversity and number of individuals. Consequently, maintaining or increasing population size will be important for maintaining the species genetic diversity (Ellstrand and Elam 1993; Gaudel *et al.* 2000; Frankham 2005).

Distribution and landscape features

Modelling of current conditions suggest that a number of areas along the Adelaide Geosyncline should be climatically excellent for the species (Fig. 12A). The largest of these occurred in the Southern Flinders Ranges, but the Mt. Lofty Ranges, Wilpena Pound and Gammon Ranges were also predicted to be important areas for growth. These modelled results support on-ground assessment and population genetics as the Southern Flinders Ranges was the most important area for survival, supporting the highest genetic diversity and greatest number of individuals. The Mt. Lofty Ranges and Wilpena Pound were also important based on field assessment, due to population size and contemporary genetic diversity in those areas (Table 6). Modelled results also suggest that current reserve placement may be important for maintaining *Callistemon teretifolius* as over 50% of predicted excellent climatic area is currently protected (Table 10).

Habitat fragmentation may reduce the viability of areas deemed suitable as 55.6% of projected area occurred on cleared land and therefore, the actual area that could support the species may be more restricted than suggested by the models (Table 11). Habitat fragmentation may impede gene flow between populations, which may contribute to the high levels of population differentiation evident (Jump and Penuelas 2006; Aguilar *et al.* 2008). Populations were generally moderate to highly differentiated when the intervening matrix consisted of cleared land (Table 7). Gene flow was also more limited around the Mt. Lofty and Southern Flinders Ranges, where habitats are more fragmented than those in the Northern Flinders Ranges (Appendix J).

Future changes

In general, all algorithms predicted reductions in total suitable area under all future climate scenarios (Table 9). Area occupied may be less than predicted by species distribution modelling, as the climate envelopes of individual populations are generally much smaller than the climate envelope of the entire species as a whole (Aitken *et al.* 2008). Therefore, suitable area for survival may be more restricted than indicated by modelled outputs. Restrictions in suitable area were predicted for all four future climate scenarios modelled and these restrictions were most pronounced in the Northern Flinders Ranges (Figs. 11-13). However, MAXENT also predicted an increase in excellent climatic area with a 0.5-1.2^{o}C temperature rise and 1-4% increase in rainfall. Range increases occurred predominantly in the

Southern Flinders Ranges, indicating this area may actually become more favourable for the species with a small degree of climate change (Fig. 12).

Increases in climatically excellent area may facilitate population expansions, mitigating some issues currently facing small populations (Frankham 2005; Willi *et al.* 2006). Increases in population size should help maintain the genetic diversity of the area, potentially preserving the unique alleles occurring in this region (Fig. 5 and Table 5). Increases in the Southern Flinders Ranges were accompanied by range restrictions in the Northern Flinders and fluctuations in the Mt. Lofty Ranges (11-13). Populations in the Northern Flinders Ranges, especially those occurring in the northern limits of the species current range are probably most vulnerable to climate change. It is likely that populations in this area are already restricted to high altitude habitats and potentially characterised by low genetic diversity (Fig. 5). Any reduction in habitable area may therefore cause populations to enter an 'extinction vortex' (Shresta *et al.* 2002; Fagan and Holmes 2006; Aitken *et al.* 2008). Range fragmentation has also been predicted. If predicted range fragmentation occurs, population size and gene flow among populations may decline, which has the potential to reduce genetic diversity, increase population differentiation and reduce adaptive potential (Garant *et al.* 2007; Willi *et al.* 2006; Hoffman and Willi 2008). Reduced gene flow may also reduce population size by separating groups of interacting sub-populations (Lienert 2004; Aguilar *et al.* 2008). Small, fragmented populations tend to inbreed more (Young 1996; Lowe *et al.* 2005). Inbreeding then further reduces adaptive potential and vigour and reduces ability to cope with the challenges of a changing environment (Ellstrand and Elam 1993; Willi *et al.* 2006; Eckert *et al.* 2009).

Species distribution modelling also predicted that *Callistemon teretifolius* may experience a southerly range shift and this was most evident with the greatest climate changes predicted for 2070 (Fig. 12E). If this range shift is not possible, potentially due to limited seed dispersal and/or habitat fragmentation, the species may become restricted to the parts of its current range that remain climatically suitable (Young *et al.* 1996; Pearson and Dawson 2002). Consequently, range restriction to the Mt. Lofty Ranges may eventuate. Reserves in the Mt. Lofty Ranges should remain an effective conservation tool for this species as range loss from these reserves was not predicted (Table 12). However, the total area protected by reserves decreased under all future climate scenarios (Table 10) and range losses were predicted for all Flinders Ranges reserves (Table 12). Therefore, concerns climate change may drive species

out of reserves (Araujo *et al.* 2004; Pyke and Fischer 2005) is supported by these results. It is likely that the majority of reserves in the Adelaide Geosyncline region will not protect the species as adequately in the future. If range is lost from reserves in the Flinders Ranges it may have considerable affects both on species genetic diversity and number of surviving individuals. Reductions or loss of suitable protected area may result in loss of high diversity populations and also those that contain unique alleles (Fig. 5 and Table 6). Therefore, off reserve protection may be necessary to increase the resilience of unprotected areas and to assist dispersal between reserves (Hannah *et al.* 2007; Lindenmayer *et al.* 2010).

Conservation and management

Climate change and conservation

Conservation in a changing climate poses a number of challenges due to the complex nature of factors involved and the uncertainty surrounding both climatic changes and species responses to these (Hannah *et al.* 2002a; Dawson *et al.* 2011; Carvalho *et al.* 2011). When developing conservation strategies that accommodate climate change, factors to account for include, but are not limited to, the magnitude and direction of climate change, the position of reserves, stressors such as land clearance and habitat fragmentation and changes in community compositions (Klunderud and Birks 2002; Lindenmayer *et al.* 2010). A number of parameters have been suggested for climate change integrated conservation strategies and these include predictive modelling, an adaptive rather than static approach to management and planning for alternative future scenarios (Hannah *et al.* 2002b; Loss *et al.* 2011). It is often necessary to establish priorities as time and resources available for conservation are limited and, as such, it is important to preferentially protect some areas over others (Coppi *et al.* 2008; Carvalho *et al.* 2011). One of the major aims of conservation genetics is to preserve the maximum amount of genetic diversity and as a result management is often centred on the most diverse and/or differentiated populations or regions (Moritz and Faith 1998; Moritz 1999; Gaudel *et al.* 2000). Different conservation practices do not always align, so development of effective, climate change incorporated management strategies may require information from a range of disciplines, including population genetics, species distribution modelling and on-ground assessment (Hannah *et al.* 2002a; Scoble and Lowe 2010).

Priority populations

As time and resources are limited, it is most effective to conserve the populations of greatest conservation value (Mattner *et al.* 2002; Carvalho *et al.* 2011). Here, the use of field assessments, population genetics and species distribution models suggest that populations in the Southern Flinders Ranges should be the greatest priority for conservation. This region is a priority because it has highest genetic diversity, most rare or unique haplotypes/alleles, and was predicted to experience loss of suitable habitat under the most severe climate changes (Figs 5 and 12). Within the Southern Flinders Ranges, priority areas for management should include Dutchman's Stern CP due to large population size and the presence of high contemporary genetic diversity, the Mambray Creek Block of Mt. Remarkable NP due to the presence of unique haplotypes, and Telowie Gorge CP due to unique haplotypes and alleles and relatively large population size (Fig. 5 and Table 6). Telowie Gorge CP was the only population that was not completely fixed for the chloroplast *trnG* intron region. Therefore, management of this area will be important to try and maintain this unique variation. In Mt. Remarkable NP, three populations were found with two or less individuals, putting these populations at high risk of extinction, even when the impacts of climate change are not taken into account (Lienert 2004; Frankham 2005; Willi *et al.* 2006). Management and monitoring of these populations will be necessary to try and prevent population loss.

Populations in the Mt. Lofty Ranges and Northern Flinders Ranges are a lower priority for conservation, primarily due to lower haplotypic diversity (Fig.5). In the Northern Flinders Ranges, especially the Gammon Ranges, there was low genetic diversity and high likelihood of suitable habitat loss, indicating these populations probably have limited capacity to overcome climate change (Aitken *et al.* 2008; Gibson *et al.* 2010). There is also a lack of field based knowledge in the Gammon Ranges, so it would be difficult to determine specific areas for conservation and to better develop management strategies based on conclusive evidence. Uncertainty surrounding populations in this area means that the region should not be a priority at this time (Moilanean *et al.* 2006), but further assessment may be necessary to better support this decision. At this stage genetic analysis indicated no unique alleles in the Gammon Ranges (Fig. 5), so if population extinction occurred in this area, it should have minimal affects on overall species-level genetic diversity (Mattner *et al.* 2002).

In the Mt. Lofty Ranges, predicted suitable climate remains even with the most severe climatic changes and as such this area may facilitate population persistence with minimal

human input. Populations in this area have low cpDNA diversity, but higher contemporary diversity and as such may have the necessary variation to respond to future changes (Ellstrand and Elam 1993; Frankham 2005). The population assigned the lowest priority was the Warren CP population, due to small population size, low genetic diversity and lack of uniqueness. It is likely that this population was genetically similar to one of the Hale CP populations (Fig. 8). Consequently, loss of this population may have minimal affects on species-level genetic diversity (Mattner *et al.* 2002).

Management

Management options should be focussed predominantly on priority populations, but the majority of the species range can still be incorporated into management decisions. Management required for this region will need to be adaptive as the predicted affects of climate change varied considerably between climate scenarios in some instances, with increases in excellent area or almost complete loss of suitable climate predicted across scenarios (Fig. 12). Therefore, effective management strategies will be influenced by the degree of climate change and its effects on species distribution (Mawdsley *et al.* 2009; Dawson *et al.* 2011). There are three primary management options available and these include assisted dispersal, habitat restoration/revegetation and biodiversity corridor establishment (Mawdsley *et al.* 2009; Loss *et al.* 2011). The most suitable management option(s) will vary between population and region, depending on genetic diversity, dispersal capacity, land condition and budget (Harrison and Bruna 1999; Breinholt *et al.* 2009).

Due to the uncertainty surrounding climate change it is important to plan for a range of potential climates (Hannah *et al.* 2002b; Heller and Zavaleta 2009) and therefore, modelling a range of potential climate scenarios is beneficial. The climate scenarios modelled were at the least and most severe range of changes predicted for the Adelaide Geosyncline region, so actual changes will probably fall somewhere in between these modelled extremes. It will be important to monitor future climates and the distribution of *Callistemon teretifolius* to determine how changes compare to modelled predictions, allowing development of more conclusive management strategies. Predicted distribution during historical climatic conditions (Fig. 12F) varied from that indicated by haplotypic diversity patterns (Fig. 5). This discrepancy may be due to uncertainty surrounding past climates and limitations of species distribution models (Wiens *et al.* 2009; Buisson *et al.* 2010), but it may also indicate that the species survived to some extent, under different climates than currently occupied. If this is

true, *Callistemon teretifolius* may have some ability to respond to predicted climate change through adaptation. Therefore, maintaining genetic diversity will be important to maintain the adaptive capacity of the species (Travis *et al.* 1996; Frankham 2005; Willi *et al.* 2006).

Populations in more northern areas may be adapted to warmer and drier conditions, so movement of these genes into more southerly areas may increase adaptive potential and the speed of adaptation to environmental change in recipient populations (Breed *et al.* 2011; Sgro *et al.* 2011). It will be important to maintain connectivity between the Flinders Ranges regions to allow for gene flow and possibly a range shift across this area, in case the species cannot respond to climate change through *in situ* adaptation. MAXENT predicted declines in suitable area between the Northern and Southern Flinders Ranges, but increases in the Southern Flinders Ranges (Fig. 12). As such it is important to maintain connectivity between Wilpena Pound populations and those in the Southern Flinders Ranges, to facilitate gene flow into an area that may become more suitable for survival. Maintaining connectivity may also preserve species-level genetic diversity and population sizes (Hannah 2008; Hoffman and Sgro 2011).

Assisted dispersal

The large distance between the Mt. Lofty and Southern Flinders Ranges populations, possibly coupled with high levels of habitat fragmentation, have been effective barriers to gene flow. Due to the extent of habitat clearance and landscape alteration, restoration of this area is not a practical solution to facilitate migration across the region (Thomson *et al.* 2009). However, to get a better understanding of current dispersal ability, contemporary gene flow experiments could be conducted assessing the parentage of seeds (Sork *et al.* 1999). If these results suggest limited contemporary gene flow, then assisted dispersal may be the most feasible solution to aid gene flow across this region (Loss *et al.* 2011) and facilitate a southerly range shift. Assisted dispersal is the physical relocation of species, populations or individuals across the landscape (Loss *et al.* 2011; Weeks *et al.* 2011). Movement of individuals into the Mt. Lofty Ranges may increase resilience of populations in this area to climate change by increasing genetic diversity and introducing new combinations of genotypes as well as maintaining species-level genetic diversity (Sgro *et al.* 2011).

As a barrier to gene flow also occurs between the two Wilpena Pound populations, assisted dispersal may be the most effective option to maintain the unique haplotypes and unique combination of genotypes found in this region. Populations were only separated by 60 m, but genetically they were quite distinct (Figs 5 and 8) and as such the two populations should be managed as two separate entities, even though they occur in close geographic proximity. Little management may be necessary for WP1 as it contained no unique alleles and connectivity with the Southern Flinders Ranges was evident (Figs 5 and 9). Assisted dispersal from WP1 into the Southern Flinders and possibly Mt. Lofty Ranges may maintain the unique diversity restricted to this population, while potentially increasing the fitness of other regions (Sgro *et al.* 2011). Care needs to be taken when moving material from this population, as its uniqueness may lead to outbreeding depression and as such monitoring of recipient populations will be highly important (Mattner *et al.* 2002; Weeks *et al.* 2011). However, low species-level genetic diversity should minimise any risks of out-breeding depression (Byrne *et al.* 2011; Frankham *et al.* 2011).

Revegetation and habitat restoration

Reserve systems will be important for climate change conservation because these areas are impacted by fewer pre-existing stressors, such as habitat fragmentation (Lindenmayer *et al.* 2010). However, reserves are geographically fixed and increasingly isolated by habitat fragmentation (Hannah *et al.* 2007; Hannah 2008). Therefore, off reserve protection may become more important for climate change accommodated management plans. Restoration around parks in the Southern Flinders Ranges may be beneficial for maintenance of species-level genetic diversity and could also lead to increases in population size (Mawdsley *et al.* 2009). Seed for restoration should be collected from across *Callistemon teretifolius's* range, but predominantly from the Southern and Northern Flinders Ranges. Collecting seed from widely spaced populations (composite provenancing) should increase the genetic diversity of the region and possibly facilitate new combinations of genotypes; this has the potential to increase adaptive capacity as well as maintaining overall species-level genetic diversity (Broadhurst *et al.* 2008; Breed *et al.* 2011; Sgro *et al.* 2011).

The source populations for this type of restoration are important. Seed from small populations may be of lower quality due to inbreeding and collection may increase extinction risk of the source population (Hufford and Mazer 2003; Broadhurst *et al.* 2008). Guidelines suggest collecting seed from ten to 20 widely spaced individuals from within a population (Mortlock

2000), but this is difficult due to the restricted nature of *C. teretifolius* populations (Table 2). Therefore, collecting across a range of populations may be the most sustainable option, with a focus on collecting from genetic clusters rather than geographic populations. The most viable areas for sourcing seed include populations in Dutchman's Stern, Telowie Gorge and Hale CPs. WP1 may also be a moderate source population. Unique haplotypes occurred in populations in the Mambray Creek Block of Mt. Remarkable NP, which have very small population sizes (Fig. 5). Further field investigation of Mt. Remarkable NP would be beneficial to determine if there are any larger populations in the area that could be an alternative source population. If no larger populations are located, *ex situ* conservation efforts may be necessary to try and maintain the unique haplotypes of this region. *Ex situ* conservation may maximise chance of seedling success while minimising the amount of seed collected (Havens *et al.* 2006). These seedlings can then be incorporated into restoration projects and revegetation efforts aimed at increasing population size. To minimise chance of outbreeding depression an abundance of seeds/seedlings should be used in restoration projects, so weaker individuals may be removed through natural selection (Frankham *et al.* 2011; Weeks *et al.* 2011).

Restoration and revegetation efforts in the Mt. Lofty Ranges should be aimed at increasing population size and habitat quality. Under the most severe climate changes modelled, the Mt. Lofty Ranges remained climatically suitable and as such *C. teretifolius* may become restricted to this region if changes eventuate (Fig. 12). Management should be focussed on increasing the size of protected areas or restoration of degraded land to try and maintain or increase population sizes (Mawdsley *et al.* 2009; Lindenmayer *et al.* 2010). Population size could also be increased through revegetation projects aimed at population expansion (Mawdsley *et al.* 2009; Thomson *et al.* 2009). The Mt. Lofty Ranges may be the only region likely to facilitate survival; therefore, incorporation of genetic material from more northern areas may be important to maintain species-level genetic diversity (Weeks *et al.* 2011; Sgro *et al.* 2011). Increasing population size reduces risk of extinction, but also increases chance of a source population developing, potentially allowing colonisation into new areas (Ellstrand and Elam 1993; Jacquemyn *et al.* 2002; Aitken *et al.* 2008). Consequently, increasing populations sizes in the Mt. Lofty Ranges may help facilitate the predicted southerly range shift from this area.

NatureLinks and biodiversity corridors

NatureLinks corridors (Appendix I) are landscape-scale approaches to conserving large areas of land through management and restoration, and are in affect large-scale biodiversity corridors (DEH 2011a; 2011b). The implementation of these corridors should have positive impacts for *Callistemon teretifolius*, if they increase resilience to climate change by increasing migration and possibly adaptation. The gap between the Cape Borda to Barossa and Flinders-Olary NatureLinks may limit gene flow between the Southern Flinders and Mt. Lofty Ranges, but will not have any major consequences for other management options, as assisted dispersal is the most likely management option in this region. Smaller scale biodiversity corridors could be implemented under the NatureLinks framework. Biodiversity corridors, which link patches of isolated habitat to promote movement between patches and genetic exchange (Tewksbury *et al.* 2002; Hannah *et al.* 2005), could be implemented in the Southern Flinders and Mt. Lofty Ranges. If corridors promote animal movement, they should have indirect effects on plant populations by allowing increased movement of pollen and seed (Tewksbury *et al.* 2002). Biodiversity corridors have the potential to increase connectivity between populations and potentially increase population size. Corridors also increase resilience to climate change by allowing migration to new habitats (Smith 1997) and could therefore be used in the Mt. Lofty Ranges to help facilitate a southerly range shift.

Conclusions

The primary aim of this research was to evaluate the risk of climate change on *Callistemon teretifolius* before developing management strategies based on field assessment, population genetics, species distribution modelling and spatial analysis. *C. teretifolius* will probably be vulnerable to climate change due to small population size, limited genetic diversity and high population differentiation which may limit migratory and adaptive capacity and increase risk of genetic and demographic stochasticity. The species was characterised by small and geographically restricted populations, with low genetic diversity, which is consistent with small population size. Patterns of chloroplast haplotype diversity indicated refugia in the Southern Flinders Ranges and two subsequent expansion events into the Mt. Lofty and Northern Flinders Ranges, potentially as a result of post-glacial warming. Range reductions, fragmentations and southerly shifts were predicted for all future climate scenarios, with most severe range changes predicted for 2070 with a 4.7^{o}C temperature increase and 25% reduction in rainfall. Habitat fragmentation may contribute to the high differentiation between populations and may limit survival in areas otherwise deemed climatically suitable. It is

likely that the current reserve system may not protect the species as adequately in the future, as a result of range loss and southerly shifts.

As a result of this research, the Southern Flinders Ranges was identified as a priority for conservation, as a result of highest genetic diversity and predicted range fluctuations. Management of this species and region will need to be adaptive as uncertainty surrounds predicted climate changes and species responses to these. The Cape Borda to Barossa and Flinders-Olary NatureLinks may be important for the species' survival if resilience to climate change in the protected areas is increased. A combination of assisted dispersal, habitat restoration, revegetation and biodiversity corridor implementation may provide the most effective conservation outcomes. However, on-going monitoring will be necessary to track the success of these conservation works. The results support the use of an integrated approach when evaluating vulnerability to climate change as this can provide more conclusive results, which encompass a species past, present and potential future structure and allow management decisions to be formed around population demographics, genetics and landscape variables.

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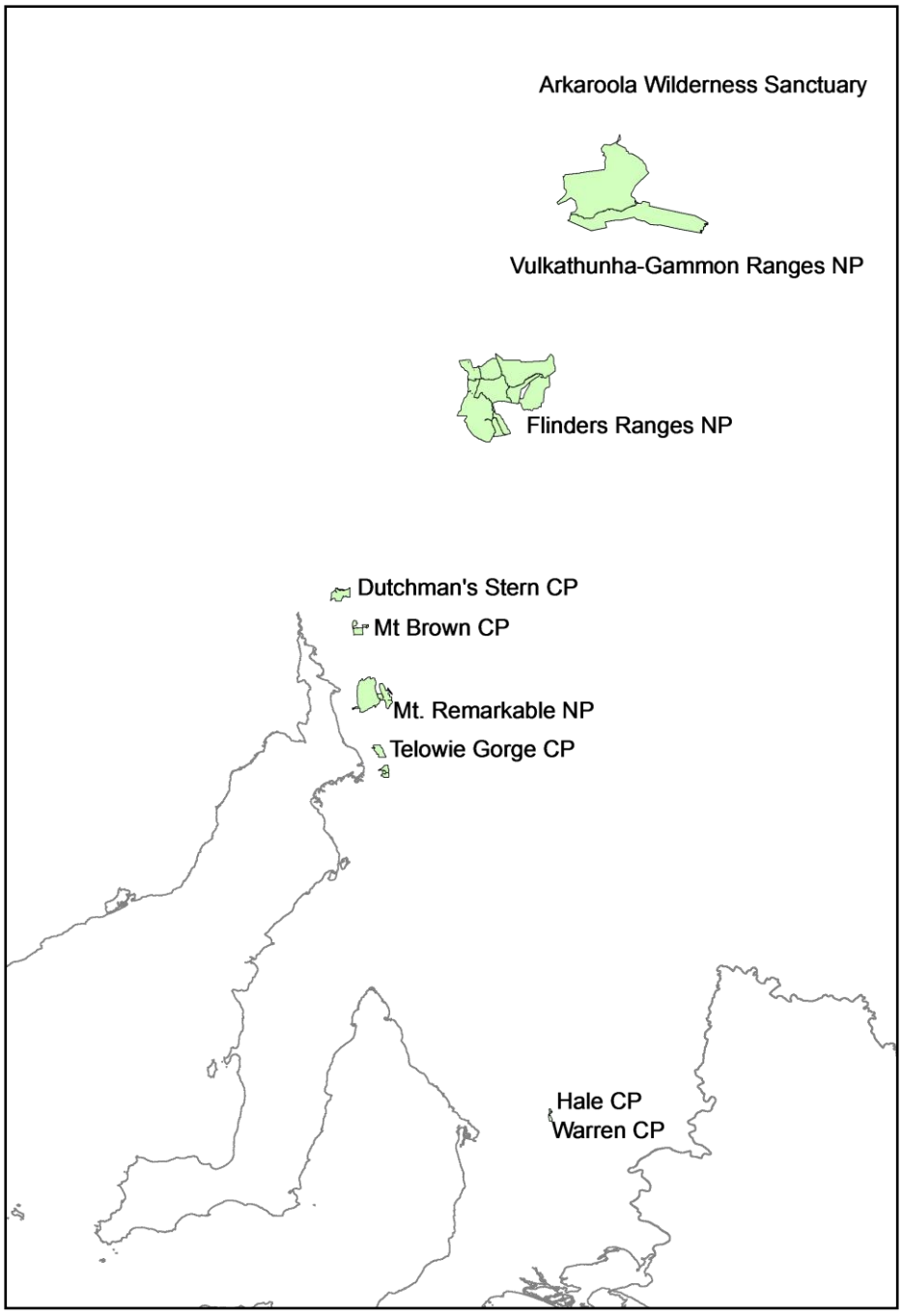
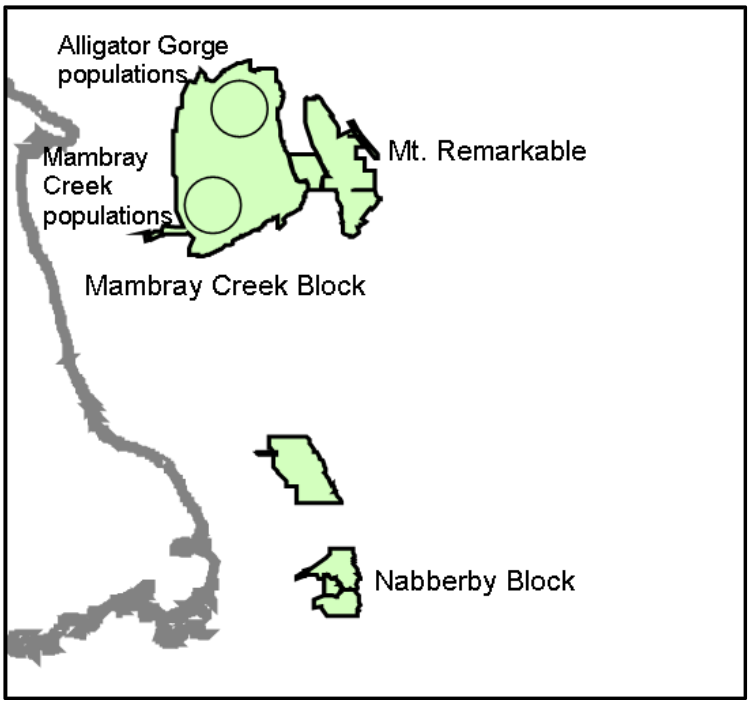


Appendix A: Location of National (NP) and Conservation Parks (CP) sampled, indicated by green polygons. Arkaroola Wilderness Sanctuary is not part of the national reserve system and as such not represented by a polygon, but location is still shown. Hale and Warren CP's occur in the Mount Lofty Ranges, Telowie Gorge, Mount Remarkable NP, Mount Brown CP and Dutchman's Stern CP are located in the Southern Flinders and the Flinders Ranges NP and Vulkathunaha-Gammon Ranges NP occur in the Northern Flinders Ranges. The Gammon Ranges are encompassed within the Vulkathunha-Gammon Ranges NP.



Appendix B: Mount Remarkable National Park, showing the three sections that comprise the park.

Appendix C: Details of herbarium voucher specimens from which leaf material was sourced.

Sample number	Voucher ID	Longitude	Latitude	Year collected
HB1	AD132306	-30.95833333	138.7247222	1999
HB2	AD133567	-30.41555556	139.1683333	1999
HB3	AD134147	-31.92833333	138.3044444	1999
HB4	AD98439551	-30.49166667	139.0833333	1980
HB5	AD98576050	-30.33333333	139.3666667	1971
HB6	AD98576041	-30.51666667	139.1	1966
HB7	AD97808274	-30.45	139.0833333	1974
HB8	AD98529020	-30.48916667	139.08	1980
HB9	AD98431475	-30.46666667	139.0666667	1980
HB10	AD97429145	-30.50194444	138.8994444	1973
HB11	AD96503127	-30.50194444	138.8994444	1964
HB12	AD97021081	-30.41055556	139.1136111	1969

Appendix D: Primers used for genotyping and sequencing

Primer Pair	Region	Amplified successfully	5'-sequence-3'	Sequenced and/or genotyped (S/G)
P370,371	ITS-1	Y	CTGTAGGTGAACCTGCGGAAGGATCC TTTTCCTCCGCTTATTGATA	S
P186,187	psaA	-	TGAAGATAATGTATTGGC ACTTCTGGTTCCGGCGAACGAA	-
1. P211, 212 ccmp1	*trnK* intron	Y	CAGGTAAACTTCTCAACGGA CCGAAGTCAAAAGAGCGATT	G
2. P213, 214 ccmp2	5' to *trnS*	Y	GATCCCGGACGTAATCCTG ATCGTACCGAGGGTTCGAAT	G
3. P215, 216 ccmp3	*trnG* intron	Y	CAGACCAAAAGCTGACATAG GTTTCATTCGGCTCCTTTAT	G
4. P217,218 ccmp4	*atpF* intron	Y	AATGCTGAATCGAYGACCTA CCAAAATATTBGGAGGACTCT	G
5. P219, 220 ccmp5	3' to *rps2*	Y	TGTTCCAATATCTTCTTGTCATTT AGGTTCCATCGGAACAATTAT	G
6. P221, 222 ccmp6	ORF 77–ORF 82 intergenic	Y	CGATGCATATGTAGAAAGCC CATTACGTGCGACTATCTCC	G
7. P223, 224 ccmp7	*atpB–rbcL* intergenic	Y	CAACATATACCACTGTCAAG ACATCATTATTGTATACTCTTTC	G
8. P225, 226 ccmp8	*rpl20–rps12* intergenic	-	TTGGCTACTCTAACCTTCCC TTCTTTCTTATTTCGCAGDGAA	-
9. P227, 228 ccmp9	ORF 74b–*psbB* intergenic	-	GGATTTGTACATATAGGACA CTCAACTCTAAGAAATACTTG	-
10. P229, P230 ccmp10	*rpl2–rps19* intergenic	Y	TTTTTTTTTAGTGAACGTGTCA TTCGTCGDCGTAGTAAATAG	G
A. P293, 294	psbM-trnG	N	AGCAATAAATGCRAGAATATTTACTT CCAT GGGATTGTAGYTCAATTGGT	-
B. P295, 296	trnS-trnG	Y	AGATAGGGATTCGAACCCTCGGT TTTTACCACTAAACTATACCCGC	S
C. P297, 298	trnFM-trns	N	CATAACCTTGAGGTCACGGG GAGAGAGAGGGATTCGAACC	-
D. P320, 321	trnT-trnS	Y	AGGTTAGAGCATCGCATTTG TTACCGAGGGTTGGAATCCCT	S
E. P372, 373	rpL 16	N	GCTATGCTTAGTGTGTGACTCGTTG CCCTTCATTCTTCCTCTATGTTG	S
F. P374, 375	trnD-trnT spacer	Y	ACCAATTGAACTACAATCCC AGGACATCTCTCTTTCAAGGAG	S
G. P376, 377	tmG intron	Y	GCGGGTATAGTTTAGTGGTAAAA GTAGCGGGAATCGAACCCGCATC	S
H. P204, 205	ndhF	Y	GGATTAACYGCATTTTATATGTTTCG CGATTATATGACCAATCATATA	S
I. P362, 363	rpoc	N	GGCAAAGAGGGAAGATTTCG CCATAAGCATATCTTGAGTTGG	S

Appendix E: **Primer and adapter sequences used for AFLPs**.

PET, NED, VIC and FAM refer to the fluorescent dye used.

PRIMER/ADAPTER	5' - sequence - 3'	Primer number
Eco RI forward adapter	CTCGTATACTGCGTACC	G773
Eco RI reverse adapter	AATTGGTACGCAGTA	G774
Mse I forward adapter	GACGATGAGTCCTGAG	G775
Mse I reverse adapter	TACTCAGGACTCATC	G776
Eco RI +A primer	TACTGCGTACCAATTCA	G783
Mse I +C primer	GACGATGAGTCCTGAGTAAC	G790
Mse I +CAA primer_1	GACGATGAGTCCTGAGTAACAA	G785
Mse I +CAC primer_2	GACGATGAGTCCTGAGTAACAC	G784
Eco RI +3 primer_1	(PET) TACTGCGTACCAATTC**AGC**	G1302
Eco RI +3 primer_2	(VIC) TACTGCGTACCAATTC**ACG**	G1303
Eco RI +3 primer_3	(NED) TACTGCGTACCAATTC**AAC**	G1304
Eco RI +3 primer_4	(PET) TACTGCGTACCAATTC**ACA**	G1305
Eco RI +AGC	(FAM) TACTGCGTACCAATTC**AGC**	G1486
Eco RI +AGC	(NED) TACTGCGTACCAATTC**AGC**	G1487
Mse 1 +CCG	GACGATGAGTCCTGAGTAACCG	G786
Mse 1 +CAG	GACGATGAGTCCTGAGTAACAG	G1488
Mse 1 +CGA	GACGATGAGTCCTGAGTAACGA	G1494

Appendix F: Twelve primers used for initialling trialling step for AFLPs.

Sequences for each are shown in the table above. E=Eco RI primer; M=Mse 1 primer. NED and VIC are the fluorescent dyes used. Primer combinations 1, 3 and 8 were used in final analysis.

Primer Pair	Forward Primer Number	Reverse Primer Number
1	E-AGC (NED) G1487	M-CAA G785
2	E-AGC (NED) G1487	M-CAG G1488
3	E-AGC (NED) G1487	M-CCG G786
4	E-AGC (NED) G1487	M-CGA G1494
5	E-ACG (VIC) G1303	M-CAA G785
6	E-ACG (VIC) G1303	M-CAG G1488
7	E-ACG (VIC) G1303	M-CCG G786
8	E-ACG (VIC) G1303	M-CGA G1494
9	E-AAC (NED) G1304	M-CAA G785
10	E-AAC (NED) G1304	M-CAG G1488
11	E-AAC (NED) G1304	M-CCG G786
12	E-AAC (NED) G1304	M-CGA G1494

Appendix G: Predicted climate scenarios

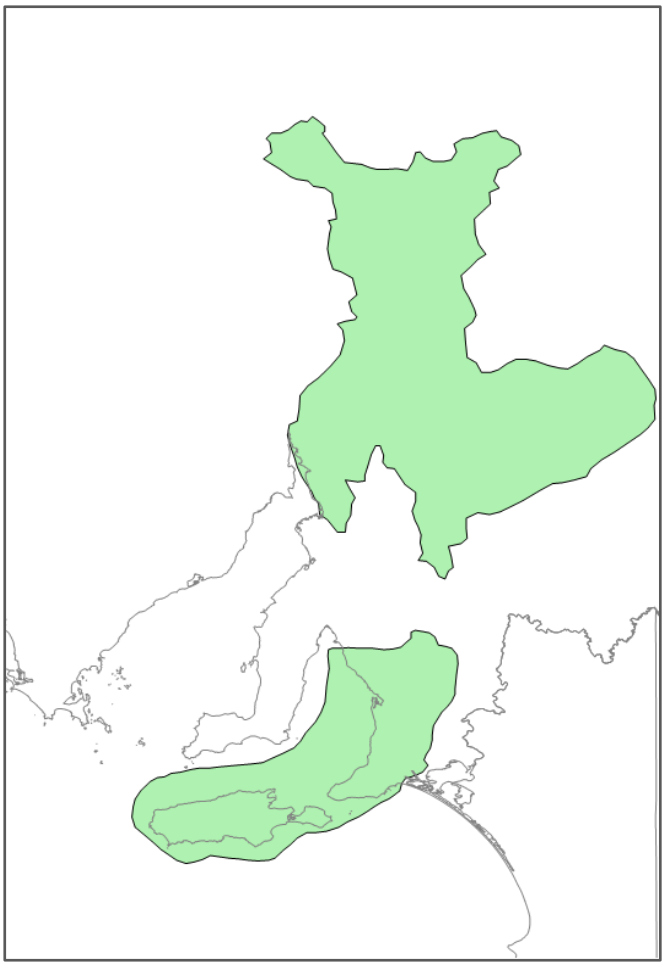
The five different climate scenarios modelled and the temperature and rainfall changes expected for each. For the future climate scenarios the each extreme scenario from the range of changes predicted was modelled.

	Temperature change (^{o}C)	**Rainfall change (%)**
2030 Low	0.5	+ 1
2030 High	1.5	-0.9
2070 Low	1.2	+4
2070 High	4.7	-25
Pleistocene	-6	-10

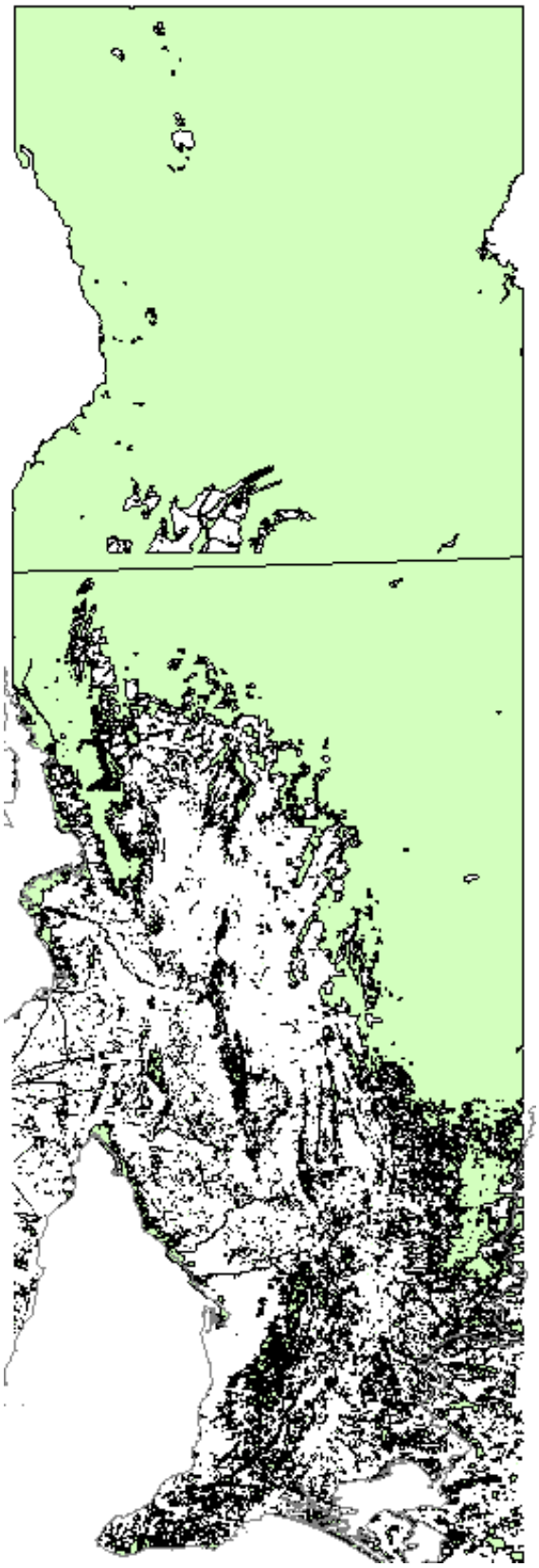
Appendix H: The 19 bioclimatic variables used in species distribution modelling.

The 10 variables that contributed 2% or more to the Maxent model were used in the final models for each algorithm.

Variable	**Description**	**Used in final models (Y/N)**
BIO1	Annual Mean Temperature	N
BIO2	Mean Diurnal Range (Mean of monthly (max temp - min temp))	Y
BIO3	Isothermality (BIO2/BIO7) (* 100)	Y
BIO4	Temperature Seasonality (standard deviation *100)	Y
BIO5	Max Temperature of Warmest Month	N
BIO6	Min Temperature of Coldest Month	Y
BIO7	Temperature Annual Range (BIO5-BIO6)	N
BIO8	Mean Temperature of Wettest Quarter	Y
BIO9	Mean Temperature of Driest Quarter	Y
BIO10	Mean Temperature of Warmest Quarter	Y
BIO11	Mean Temperature of Coldest Quarter	Y
BIO12	Annual Precipitation	N
BIO13	Precipitation of Wettest Month	Y
BIO14	Precipitation of Driest Month	N
BIO15	Precipitation Seasonality (Coefficient of Variation)	Y
BIO16	Precipitation of Wettest Quarter	N
BIO17	Precipitation of Driest Quarter	N
BIO18	Precipitation of Warmest Quarter	N
BIO19	Precipitation of Coldest Quarter	N

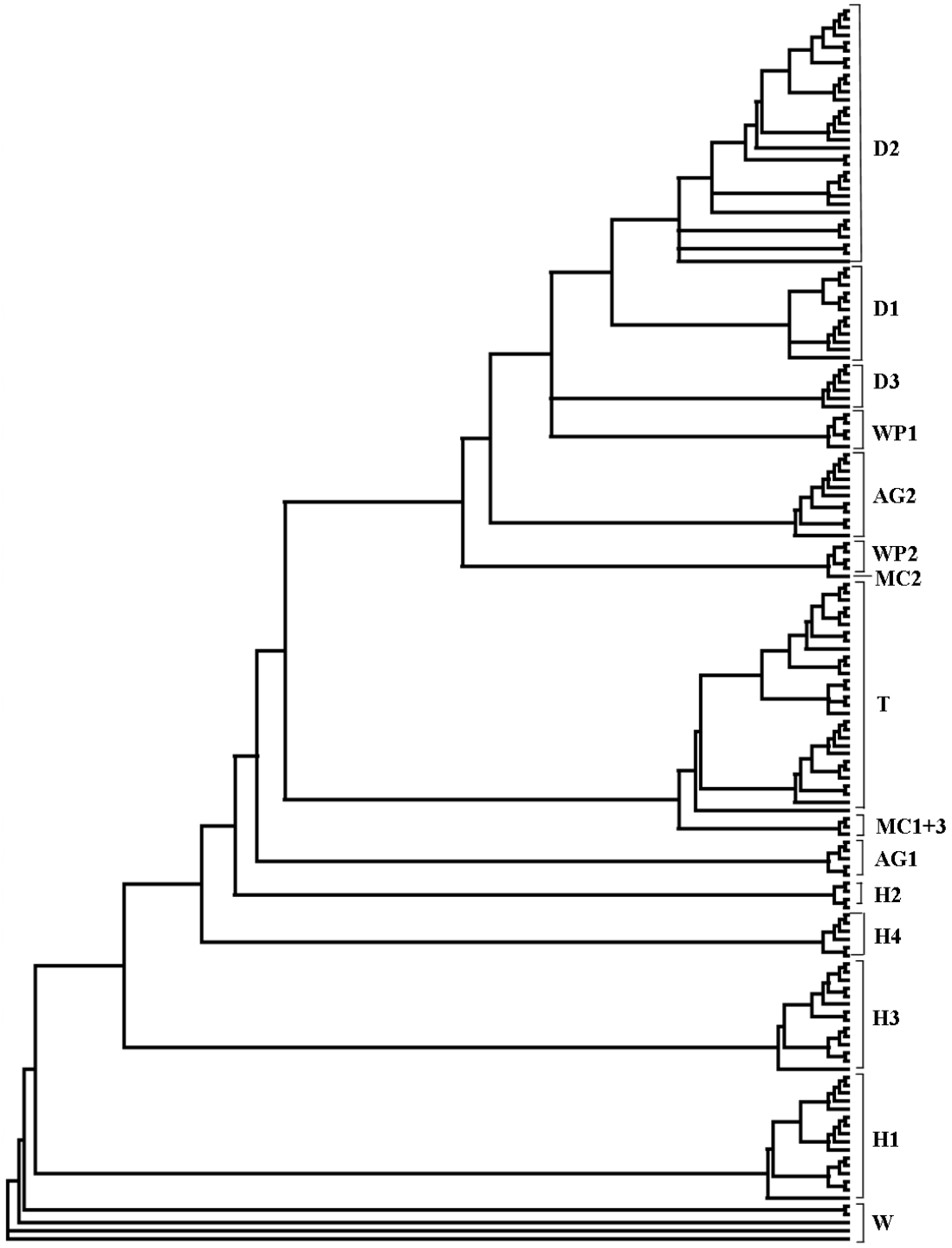


Appendix I: The position and extents of the two proposed Nature Links (NL). The Cape Borda to Barossa NL (southern) and Flinders-Olary NL (northern) are shown.



Appendix J: Native vegetation cover of the modelled area. Green represents areas of native vegetation. White areas show where land clearance has occurred. In total 61 900km^2 of the modelled area is covered by intact native vegetation.

Appendix K: On-ground assessment of *Callistemon teretifolius*. Top: 4WD track through Telowie Gorge CP, Southern Flinders Ranges. Population was found in and adjacent to disturbed area. Centre: *C. teretifolius* in the bottom left of the image, growing from a ridgeline in Dutchman's Stern CP, at approximately 600m elevation. Bottom: *C. teretifolius* growing from rock in Hale CP. All images were taken during April 2011.



Appendix L: Strict consensus of 5000 Maximum Parsimony trees showing relationships between populations. W=Warren, H=Hale, T=Telowie, MC=Mambray Creek, AG=Alligator Gorge, D=Dutchman's Stern and WP=Wilpena Pound. Results from 153 individuals are shown based on 132 parsimony informative characters.