CORE
Metadata, citation and similar papers at core.ac.uk
Provided by Research Online

UNIVERSITY
OF WOLLONGONG
AUSTRALIA

University of Wollongong
Research Online

Faculty of Science, Medicine and Health - Papers
Faculty of Science, Medicine and Health

2014

Patterns of genotypic diversity suggest a long history of clonality and population isolation in the Australian arid zone shrub Acacia carneorum

Eleanor K. O'Brien
University of Wollongong, eleanoro@uow.edu.au

Andrew J. Denham
University of Wollongong, adenham@uow.edu.au

David J. Ayre
University of Wollongong, dja@uow.edu.au

Publication Details

O'Brien, E. K., Denham, A. J. & Ayre, D. J. (2014). Patterns of genotypic diversity suggest a long history of clonality and population isolation in the Australian arid zone shrub Acacia carneorum. Plant Ecology, 215 (1), 55-71.

Research Online is the open access institutional repository for the University of Wollongong. For further information contact the UOW Library: research-pubs@uow.edu.au

Patterns of genotypic diversity suggest a long history of clonality and population isolation in the Australian arid zone shrub Acacia carneorum

Abstract

For plants capable of both sexual and clonal reproduction, the relative frequency of these reproductive modes is influenced by genetic and ecological factors. Acacia carneorum is a threatened shrub from the Australian arid zone that occurs as a set of small, spatially isolated populations. Sexual reproduction appears to be very rare: despite regular flowering, only two populations set seed. It is not known whether this reflects an ancient pattern, or results from rapid land use changes following arrival of Europeans in the region 150 years ago. We assessed genotypic variation throughout the range of A. carneorum using AFLP markers, to elucidate the relative importance of clonal and sexual reproduction in this species' history. Clonal diversity (CD) within populations ranged from 0 to 0.820 (mean CD = 0.270, SE = 0.094), but the relative abundances of genets were typically highly skewed. On average, the two fruiting populations had higher CD (mean CD = 0.590, SE = 0.265) than non-fruiting populations (mean CD = 0.179, SE = 0.077) ($t = 2.315$, $p = 0.049$), but most populations contained multiple genets. All genets were population-specific, and there was substantial divergence among populations (Φ ST = 0.690), implying a long history of isolation. We conclude that clonality has predominated in A. carneorum populations, with occasional sexual recruitment, and that current failure of most populations to set seed likely reflects both a long history of asexual reproduction and effects of habitat disturbance. Conservation of this species may benefit from translocations to increase genotypic diversity within populations.

Keywords

acacia, shrub, zone, arid, australian, isolation, carneorum, population, patterns, clonality, history, long, suggest, diversity, genotypic

Disciplines

Medicine and Health Sciences | Social and Behavioral Sciences

Publication Details

O'Brien, E. K., Denham, A. J. & Ayre, D. J. (2014). Patterns of genotypic diversity suggest a long history of clonality and population isolation in the Australian arid zone shrub Acacia carneorum. Plant Ecology, 215 (1), 55-71.

This journal article is available at Research Online: http://ro.uow.edu.au/smhpapers/1356

Patterns of genotypic diversity suggest a long history of clonality and population isolation in the Australian arid zone shrub *Acacia carneorum*

Eleanor K. O'Brien[1,2*], Andrew J. Denham[1,3] & David J. Ayre[1].

1. Institute for Conservation Biology & Environmental Management, School of Biological Sciences, University of Wollongong, Northfield Ave., Wollongong NSW 2522 Australia.

2. Current address: School of Biological Sciences, University of Bristol, Woodland Rd, Bristol BS8 1UG, United Kingdom. Ph: +44 117 928 7470

3. Office of Environment and Heritage, Department of Premier and Cabinet, PO Box 1967, Hurstville BC NSW 1481, Australia.

* Corresponding author.

Authors' email addresses:

Eleanor O'Brien: eleanor.obrien@bristol.ac.uk

Andrew Denham: Andrew.Denham@environment.nsw.gov.au

David Ayre: dja@uow.edu.au

Running title: Long-term failure of sexual reproduction in *Acacia carneorum*

Key words: AFLP, arid zone plants, clonality, grazing, mating system, population fragmentation

Word count: 5826

1 table

5 figures

2 appendices

44 references

Abstract

For plants capable of both sexual and clonal reproduction, the relative frequency of these reproductive modes is influenced by genetic and ecological factors. *Acacia carneorum* is a threatened shrub from the Australian arid zone that occurs as a set of small, spatially isolated populations. Sexual reproduction appears to be very rare: despite regular flowering, only two populations set seed. It is not known whether this reflects an ancient pattern, or results from rapid land use changes following arrival of Europeans in the region 150 years ago. We assessed genotypic variation throughout the range of *A. carneorum* using AFLP markers, to elucidate the relative importance of clonal and sexual reproduction in this species' history. Clonal diversity (CD) within populations ranged from 0 to 0.820 (mean CD = 0.270, SE = 0.094), but the relative abundances of genets were typically highly skewed. On average, the two fruiting populations had higher clonal diversity (mean CD = 0.590, SE = 0.265) than non-fruiting populations (mean CD = 0.179, SE = 0.077)($t = 2.315$, $p = 0.049$), but most populations contained multiple genets. All genets were population-specific and there was substantial divergence among populations ($\Phi_{ST} = 0.690$), implying a long history of isolation. We conclude that clonality has predominated in *A. carneorum* populations, with occasional sexual recruitment, and that current failure of most populations to set seed likely reflects both a long history of asexual reproduction, and effects of habitat disturbance. Conservation of this species may benefit from translocations to increase genotypic diversity within populations.

Introduction

For the many plant species that are able to reproduce both sexually and clonally, the relative frequency of these life history strategies is typically strongly associated with levels of genotypic diversity within, and genetic divergence among, populations (Beatty et al 2008; Eckert et al 2010). In populations where reproduction is predominantly clonal, there is little opportunity for the introduction of new genotypic variation through gene flow or recombination, and clonal diversity is likely to decline over time due to selection (including effects of inter-clonal competition) and random loss (Honnay & Bossuyt 2005; Weeks & Hoffmann 2008). As a result, clonal populations are expected to be less genotypically diverse than sexual populations of the same species, and to exhibit greater among-population genetic divergence (Silvertown 2008; Vandepitt et al 2010). However, genetic diversity within populations can remain high, since heterozygosity is preserved within clonal lineages (Balloux et al 2003). It has been demonstrated that in species capable of both sexual and clonal reproduction, even low levels of sexual recruitment can be sufficient to maintain levels of genotypic diversity equivalent to those found in obligately sexual populations (Ellstrand & Roose 1987; Balloux et al 2003; Richards et al 2004; de Witte et al 2012). Current distributions of genotypic variation can therefore be extremely useful for inferring historic frequencies of different modes of reproduction within species.

Clonality may be favoured in situations where there is a trade-off between individual survival and seed production (for example due to resource limitation), or if disturbance

events frequently prevent flowering or fruiting (e.g. Eckert 2002; Kleijn & Steinger 2002; Evju et al 2011). While it can facilitate rapid colonisation and population persistence through periods when conditions are unfavourable for sexual reproduction, prolonged clonal reproduction may result in permanent failure of sexual reproduction, due to a decline in clonal diversity (and therefore in the number of compatible mating partners in self-incompatible species), a change in ploidy, or the accumulation of mutations that reduce sexual fertility (Dorken & Eckert 2001; Eckert 2002; Honnay & Bossuyt 2005; Eckert et al 2010, Gross et al 2012). Furthermore, because exclusively clonal populations typically have low genotypic diversity, they may be less able to adapt to novel environments than sexually reproducing populations, making them more vulnerable to extinction when faced with threats such as habitat loss, invasive species and climate change (Beatty et al 2008; Sgro et al 2011). Identifying the factors affecting the relative frequency of different reproductive modes within species is therefore important for designing appropriate strategies for their conservation.

In the arid zone of eastern Australia, a suite of important habitat-forming species in the genus *Acacia* exhibits apparently high rates of clonality, and significant spatial and temporal variation in the frequency of sexual reproduction. The environment in this region has been modified considerably since agriculture commenced in the 1860s, through land-clearing and the introduction of domestic and feral grazing animals including sheep, goats and rabbits (Auld 1993; Auld & Denham 2001; Denham & Auld 2004). Grazing has been found to be a major factor limiting recruitment in plant communities from this region (Auld 1993; Auld & Denham 2001) and may contribute to the dominance of asexual reproduction by inhibiting seed set and seedling survival.

Grazing has previously been shown to increase rates of clonal reproduction within herbaceous plant populations (e.g. Kleijn & Steinger 2002; Evju et al 2011), but to our knowledge there are no published examples of increased rates of asexual reproduction in shrubs or trees attributable to grazing. Increased habitat fragmentation that has occurred as a result of land clearing may also contribute to a change in the dominant reproductive mode by limiting effective pollen transfer between isolated stands (Hall et al 1996; Young et al 1996). It is not known whether the high rate of asexual reproduction now typical of these arid *Acacias* is a result of grazing pressure and increased habitat fragmentation, or whether it reflects an ancient pattern.

Acacia carneorum Maiden is a shrub occurring in arid and semi-arid regions of south-eastern Australia (Tame 1992) (Figure 1), and is listed as a nationally vulnerable species (Auld & Denham 2001). It has a naturally fragmented distribution, restricted to dune crests and ephemeral water courses, and occurs in small patches that may be isolated from the next nearest population by several hundred kilometres (Auld 1993). The most frequent floral visitors of *A. carneorum* are wasps, native bees, flies and butterflies, suggesting these are the most important pollinators (Gilpin et al, submitted). Seeds of *A. carneorum* are large, with a prominent orange aril, which is believed to be an adaptation to promote seed-dispersal by birds (Auld 1993). Most species of *Acacia* are at least partially self-incompatible (Kenrick 2003), but it is not known whether this is true of *A. carneorum*. While *Acacia carneorum* is able to propagate sexually, seedling recruitment is currently extremely rare (Auld 1993). Despite regular flowering over a 20-year period (A. Denham personal obs.), there are only two populations where seed set is known to occur in most years. Consequently, the vast majority of new plants are

produced via vegetative suckering (Auld 1993). The establishment and survival of young plants (whether seedlings or suckers) in populations of *A. carneorum* is very low, and this has resulted in an age structure within populations that is heavily skewed towards older plants (Auld 1993). Carbon dating of a sample of mature plants found that they ranged in age from ~120 yrs – 330 yrs, suggesting that there has been little or no replacement in these populations since the introduction of grazing animals in the 1860s (Auld & Denham 2001).

We examined the distribution of genetic and genotypic variation throughout the range of *A. carneorum*, and compared the genotypic diversity present within different populations, including both fruiting and non-fruiting populations. We use these data to evaluate whether the current predominance of clonal reproduction in *A. carneorum* populations reflects an ancient pattern, or whether it has arisen in the recent past as a consequence of increased habitat fragmentation and grazing by introduced herbivores since the arrival of Europeans in Australia.

If populations of *A. carneorum* have been maintained exclusively by clonal reproduction in the long term, we expect that genotypic diversity within populations will be very low, genotypes will be confined to single populations, and genetic divergence between populations will be high, because clonal reproduction does not facilitate gene flow between populations. By contrast, if recruitment via sexual reproduction was more common up until the land use changes that occurred with the arrival of Europeans, we expect greater genotypic diversity within populations, because individual *A. carneorum* plants can live for over 300 years (Auld & Denham 2001), and

grazing is expected to result in random loss of plants across clones. Furthermore, if there was previously greater gene flow between populations via seed or pollen dispersal, we expect a pattern of isolation by distance at the landscape scale. We also compare genotypic diversity within populations that set seed with those that don't, to investigate whether current sexual reproduction is associated with higher genotypic diversity, which would be expected if either (a) genotypic diversity is a pre-requisite for sexual reproduction (due to self-incompatibility) or (b) sexual recruitment increases genotypic diversity within populations. Finally, we discuss the conservation implications for *A. carneorum*, and evaluate the potential for controlled movement of genetic material between populations to increase genotypic diversity and provide a 'genetic rescue' effect.

Materials and Methods

Sample collection

We collected phyllodes for AFLP genotyping from 10 populations of *A. carneorum* (10 – 15 plants per population, 124 plants in total) across the geographic range of the species (Figure 1), including the only two populations in which fruit set has been observed (Table 1). Typically, *A. carneorum* populations show marked size variation, consisting of a small number of large (height >3 m) plants, each surrounded by a large number of small (height<0.5 m) suckers (personal obs.). In an effort to maximise the proportion of the genotypic diversity present within populations captured in each

sample, we (a) sampled across the full spatial extent of each population and (b) deliberately targeted large plants that appeared unconnected to each other, avoiding small suckers that were clearly the result of clonal reproduction. The mean distance between sampled plants was 212 m (SE = 73.13 m) (Table 1).

AFLP fingerprinting and scoring

DNA was extracted from freeze-dried leaf material at the Australian Genome Research Facility (AGRF). The Nucleospin Plant II system (Machery-Nagel GmbH & Co, Düren, Germany) was used according to manufacturer's instructions, with the SDS buffer set option.

We used a modified version of the method of Vos *et al.* (1995) to generate AFLP fingerprints for each sample. The Invitrogen Core Reagent Kit was used to perform the digestion and ligation steps, following manufacturers' instructions except that we left samples for 16 hours at room temperature to facilitate ligation. We used the Invitrogen preamplification primer mix to amplify a subset of digested fragments, but performed reactions in ¼ of the volume specified. We performed a selective PCR step to further reduce the number of fragments. Selective PCR reactions were carried out in 20 µl volumes, containing 1 x PCR buffer, 0.2 mM each dNTP, 4 mM $MgCl_2$, 0.25 µl MseI selective primer, 0.05 µl EcoR1 selective primer, 0.5 U *Taq* DNA Polymerase and deionised water up to 17 µl, with 3 µl diluted preselective PCR reaction. We used three pairs of primers labelled with different fluorescent dyes to permit detection by the

sequencer. PCR conditions were denaturing at 94 °C for 2 mins, followed by 30 cycles of: denaturing at 94 °C for 20 s, annealing for 30 s at 66 °C in the first cycle, reducing by 1 °C in each of the subsequent nine cycles, then continuing at 56 °C for the remaining 20 cycles, and extension at 72 °C for 2 mins. We ran selective PCR products, with a LIZ size standard, on an ABI3130 automated capillary sequencer (Applied Biosystems) to separate the fragments. We generated replicate AFLP fingerprints for at least 20% of samples from each population and used independent DNA extractions to check the repeatability of AFLP scoring and choose appropriate thresholds for detection of markers.

We used GeneMapper v3.7 (Applied Biosystems) to size fragments by comparing their electrophoretic migration with fragments in the LIZ size standard. We initially identified markers between 50 and 500 base pairs using Genemapper v3.7, with a minimum peak threshold of 40 relative fluorescence units (RFU) and no normalisation procedure. We then imported the table of peak heights into the R package AFLPScore (Whitlock et al 2008). This program normalises peak intensities using the mean intensity for each marker and run to account for intensity variation across samples, and generates phenotype tables (presence/absence of each marker in each sample) for a user-specified range of marker detection and peak-calling thresholds. It also calculates the error rate from replicate fingerprints for each combination of thresholds, enabling determination of thresholds that maximise the number of markers used, while minimising the error rate. We set an E1 error rate (the probability of calling a marker absent when it is present, calculated using the Bayesian method of Hadfield et al 2006; Hadfield 2008) of 5% as the maximum allowable error rate. Normalisation, error-rate

analysis and phenotype scoring were undertaken for each pair of primers separately, due to variation in the intensity of the different fluorescent markers. Results for the three primer pairs were then combined for subsequent analyses.

Clone assignment

Caution is required in the use of molecular markers to identify clones (genets) because sampled stems (ramets) belonging to the same clone may have non-identical genotypes due to (a) genotyping errors and (b) somatic mutation. Likewise, genetically distinct individuals may exhibit identical or near-identical genotypes at the loci scored if they are closely related (Douhovnikoff & Dodd 2003). It is therefore necessary to establish a threshold level of genetic distance among individuals, below which they are considered to be the result of clonal reproduction. A threshold that is too high or low will result in underestimation or overestimation of the number of genets respectively.

To determine an appropriate threshold for identifying genets, we examined the frequency distribution of genetic distances among all genotyped individuals. The genetic distance metric used was the Euclidean distance, calculated as a count of the number of AFLP loci differing between each pair of individuals. In clonal plants, this distribution is expected to be multimodal, with a peak at the lower end of the range of genetic distances representative of genetic dissimilarity among ramets within the same genet (due to somatic mutation and genotyping error), and one or more peaks at greater genetic distances indicating differentiation among genets. Such a distribution has been demonstrated in a previous study of AFLP variation in the clonal plant *Salix exigua*,

where the identification of genets using this method was independently verified by including known clone-mates within the sample (Douhovnikoff & Dodd 2003). In the absence of an independent means of determining clonal identity, we set the threshold level of genetic distance for genet identification as the point in the frequency distribution where the tails of the first two peaks overlapped. One caveat was that the threshold value had to be at least 5%, given that we demonstrated this level of dissimilarity among replicate extractions from the same individual.

In most cases, this threshold resulted in clearly defined clusters of individuals that differed from all other individuals in the cluster by a value less than the threshold, implying that they represented a single genet. However, there were a few cases where assignment was ambiguous because ramets differed from some ramets within a cluster by less than the threshold genetic distance, but others by more. In these cases, we calculated the mean genetic distance between this ramet and all others within the cluster and compared this value to the threshold to determine whether to assign it to the same genet. To assess the consequences of varying this threshold value on estimates of within-population diversity, we calculated the clonal diversity within each population for increasing threshold values.

To estimate the probability that any of the n individuals assigned to a particular clone could instead have been produced by random mating within each population, given allele frequencies at the AFLP loci used, we calculated P_{sex} according to the method outlined in deWitt et al (2012):

$$P_{sex} = \sum_{x=n}^{N} \frac{N!}{x!(N-x)!} (P_{dgen})^x (1 - P_{dgen})^{N-x}$$

where N = the number of ramets sampled, $P_{dgen} = \prod_{i=l}^{L} p_i$, p_i = the frequency of band presence within the population at the *i*th locus and L = the number of polymorphic loci in the population. This method estimates the probability that unrelated individuals will share the same genotype by chance. Given the likelihood that even sexually reproducing plant populations will include clusters of related individuals due to limited seed dispersal, we also calculated the more conservative statistic, P_{IDsib}, which estimates the probability that two related individuals within a population will share the same multilocus genotype under random mating (deWitt et al 2012):

$$P_{IDsib} = 1\text{-}\{(3/2p)(q^2)\}$$

Where p and q represent the frequencies of band presence and absence respectively within the population. Only loci that were polymorphic among genets were used in these analyses, therefore these statistics were estimated only for populations where multiple putative genets were detected.

Genetic diversity within populations

Clonal diversity (CD) within populations was calculated as the proportion of stems with unique genotypes, according to the method of Ellstrand & Roose (1987):

$$\mathrm{CD} = \frac{G - 1}{N - 1}$$

Where G = the number of unique genotypes (genets) according to our threshold criteria and N = the total number of stems (ramets) genotyped. Thus, it has a value between 0 (monoclonal populations) and 1 (all ramets have a unique genotype).

In addition to clonal diversity, we used two measures to quantify genetic and genotypic diversity within populations: the proportion of loci that were polymorphic (*PLP*), and Shannon's Information Index (*H'*), which provides a measure of both diversity and evenness of the distribution of ramets between genets (Arnaud-Haond et al 2007). This was calculated as $H' = -\sum_{i=1}^{G} p_i \log p_i$ where p_i is the frequency of the *i*th genet, and G is the number of genets in the population. *PLP* was calculated for individual ramets sampled in each population, rather than for unique genotypes, to provide a measure of diversity that was independent of our estimate of clonal diversity based on a threshold genetic distance. Hence, this may include variation within putative genets due to somatic mutation and genotyping error, in which case it will be greater than 0 even in populations that consist of only a single genet by our measure of clonal diversity. Finally, to provide a measure of evenness that is independent of clonal diversity, we calculated Pielou's Evenness Index *J'* (Pielou 1975). This was calculated as $J' = H'/H'_{max}$, where $H'_{max} = \log G$.

Testing for associations between genetic diversity and population size and isolation

We used Spearman's rank correlations to test for associations between the measures of genotypic diversity (clonal diversity, *PLP*, *H'*) and (a) population size and (b) population isolation. Population area has been shown to be a strong predictor of the number of stems (N) in *A. carneorum* populations according to the function: $N=13.34+43.53A+0.3068A^2$ where *A* = population area in ha (A.J.D., unpublished data) and so was used to estimate population size. A full description of how this equation was derived is provided in Appendix 1. Isolation was measured as the straight-line distance to the nearest neighbouring *A. carneorum* population (in km).

Analysis of spatial genetic structure

We used a Principal Coordinates Analysis (PCA) to provide a visual representation of the distribution of variation in our AFLP markers within and among populations of *A. carneorum*. A matrix of the genetic distances among individual samples was used to generate eigenvalues and eigenvectors, and the two coordinates that explained the largest proportion of the variance were plotted.

We used an Analysis of Molecular Variance (AMOVA), implemented in GENALEX v6.4 (Peakall & Smouse 2006), to partition variation in AFLP genotypes within and

among populations of *A. carneorum*. We included only unique genotypes in this calculation, and calculated percentage variation within and among populations based on a matrix of Euclidean genetic distances between pairs of genotypes. The significance of genetic differentiation among populations was evaluated using 999 permutations of the data.

We conducted a Mantel test to examine whether there was evidence for isolation by distance across the sampled range, by comparing the matrix of genetic distances among populations with that of their geographic separation. We also used the “multiple distance class” option in GENALEX v6.4, which calculates the genetic autocorrelation (r) among pairs of populations at progressively increasing geographic distances, to test for spatial autocorrelation between populations. We used distance classes from 50 km up to 450 km (the greatest geographic separation between sampled populations). We generated 95% confidence intervals for estimates of r using 999 bootstraps. We used 999 random permutations to evaluate whether r was significantly different from zero. For both of these tests, we used mean Euclidean distances (calculated as a count of the number of AFLP loci differing between individuals) between populations instead of Φ_{ST}, because Φ_{ST} cannot be calculated when a population consists of a single genet.

Results

Clone assignment

The three primer pairs resulted in 85 AFLP markers that met our strict repeatability criteria and could be amplified cleanly and reliably. Of these, 56 (66%) were polymorphic across the whole sample, with an average frequency of 0.632 and variance 0.076.

From the distribution of genetic distances among individuals, we set our threshold for clone identification at 5% (Figure 2). The consequences of varying this threshold for clonal diversity estimates within each population are presented in Appendix 2. On the basis of the 5% threshold, we identified 38 putative genets among the 124 individuals genotyped. Of these, 25 were represented by a single ramet (Figure 3, 4). All genets were population-specific. That is, no genet was found in more than one population.

Our P_{sex} analysis revealed that the probability of two unrelated individuals sharing identical multilocus genotypes at this set of AFLP loci by chance was extremely low (<0.001) in all populations where multiple genets were observed (Table 1). The probability that related individuals would display identical genotypes by chance (P_{IDsib}) was also low (<0.01) in four of the most diverse populations (BD, HD, MCAMP and QUANDONG), but higher (0.02 – 0.15) in the remaining populations (BIMB, KOON, MULY and TW), where clonal diversity was generally lower (Table 1). Values of P_{IDsib} of less than 0.01 are recommended for estimating the number of genetically unique individuals (Waits et al 2001), therefore it is not possible to rule out the possibility that apparently clonal individuals in these populations were sexually produced siblings on the basis of the AFLP genotypes alone.

Genetic diversity within populations

Clonal diversity varied considerably across the 10 populations. There were two populations (LA and SBH) where all sampled ramets belonged to the same putative genet, while the highest clonal diversity was in one of the fruiting populations (BD), where the 12 ramets sampled included 10 unique genets (Table 1). Likewise, the other measures of genetic diversity (*PLP* and *H'*) were also highest at BD (*PLP* = 41.2%, *H'* = 0.979)(Table 1). The other known fruiting population (MCAMP) was also among the most diverse (*CD* = 0.42, *PLP* = 28.2%, *H'* = 0.477), but not as diverse as HD, which is not known to produce fruit (Table 1). On average, fruiting populations had significantly higher clonal diversity (mean *CD* = 0.590, SE = 0.265) than non-fruiting populations (mean *CD* = 0.179, SE = 0.077) (independent samples t-test assuming equal variances: $t = 2.32$, $df = 8$, $p = 0.049$). Clonal diversity was highly correlated with both *PLP* (Spearman's rank correlation $r_s = 0.948$, $p < 0.001$) and *H'* ($r_s = 0.900$, $p < 0.001$), and both of these measures of genotypic diversity were similarly higher in fruiting populations than non-fruiting populations (data not shown). *PLP* and *H'* were also highly correlated with each other ($r_s = 0.982$, $p < 0.001$).

Within populations where we identified multiple clones, the distribution of ramets among genets was highly skewed. Typically, the majority of ramets within a population sample typically belonged to a single genet, with small numbers from one or more additional genets (Figure 3). Some deviation from this pattern was seen at BD, where most genets were represented as a single ramet and two were represented by two ramets.

This is reflected in the evenness index, which was again highest (excluding the monoclonal populations) at BD (*J'* = 0.979).

Both the mean and maximum distance between ramets within the same genet were lowest in the three most genotypically diverse populations, BD, HD and MCAMP, which had a mean distance between ramets of 69 m, 41m and 41m respectively (Table 1). This pattern reflects the fact that where multiple genets were detected within a population, ramets belonging to the same genet tended to be spatially clustered (Figure 4). By contrast, the mean distance between ramets was greatest at LA and SBH, the two populations where only a single genet was detected (150m and 232m respectively; Table 1).

Associations between genotypic diversity and population size and isolation

Given the very high correlation between the three measures of genotypic diversity, we have presented only the correlation of clonal diversity with population size and isolation. Results for the other measures of genotypic diversity were qualitatively identical.

Neither population area, nor population isolation (measured as the straight-line distance to the nearest adjacent *A. carneorum* population) showed a significant association with clonal diversity (Spearman's rank correlation: area r_s = -0.143, p = 0.67; isolation: r_s = 0.030, p = 0.93), and there was very large variation in diversity among populations of

equivalent size/isolation. The two largest populations (BD and SBH) were respectively the most and least diverse populations sampled. Likewise, populations separated by up to 3 km from the next-nearest population showed a tenfold difference in clonal diversity, ranging from a single genet at LA to 10 different genets at BD (Table 1).

Spatial genetic structure

There was very strong genetic structuring across the species' range, as illustrated by the PCA plot (Figure 5). An AMOVA revealed that the majority (69%) of AFLP variation occurred between populations, with the remaining 31% within populations. The mean difference in AFLP phenotypes among individuals was 23.7% (SD 3.83%) between populations, substantially greater than that between different clones within populations (mean of 13.9%, SD 5.05%).

There was little evidence for spatial structuring of genotypes beyond the scale of the population patches. We did not find a significant relationship between geographic and genetic distance between populations across the sampled range (Mantel test: R^2 = 0.0022, p = 0.396), nor was there significant spatial autocorrelation among populations over any distance interval (results not shown).

Discussion

We have shown that despite annual flowering, but as predicted from demographic monitoring, populations of *Acacia carneorum* are typically highly clonal and lacking in genotypic diversity. The low clonal diversity within, and strong differentiation among, populations of *A. carneorum* are consistent with published observations for plants with predominantly asexual reproduction and a highly fragmented distribution (Ellstrand & Roose 1987; Hamrick & Godt 1989, 1996). This pattern suggests that the localised proliferation of successful clonal genotypes has been the dominant reproductive mode for *A. carneorum* in the past, and that populations have a long history of genetic isolation, which most likely pre-dates land use changes that accompanied the arrival of Europeans to the area 150 years ago. These results have important implications both for the processes that have shaped diversity within populations of *A. carneorum*, and the resilience of this threatened species in the face of environmental change.

Distribution of genotypic diversity

Although genotypic diversity was generally low within the *A. carneorum* populations sampled, most populations contained multiple genets. This is a common observation in clonal plant species (Ellstrand & Roose 1987), and is generally attributed to rare episodes of sexual reproduction (Eckert 2002). A key question is whether the genotypic diversity present in *A. carneorum* populations is the result of past episodes of sexual reproduction within populations (including many that do not currently set seed), or whether there has been dispersal of seed from other populations. Three main features of this data set suggest the former: (1) all genets were unique to individual populations, (2) genetic divergence between populations was substantial, and much greater than that

between genets within populations, and (3) there was no spatial structuring of genotypic variation beyond the level of the population, which would be expected if seed dispersal declined with distance. It therefore seems likely that occasional sexual recruitment, or possibly the accumulation of somatic mutations, within populations of *A. carneorum* accounts for present patterns of genotypic diversity.

A striking characteristic of the distribution of clonal diversity in most of the multiclonal *A. carneorum* populations examined was the dominance of a single genet, with remaining genets typically represented by one or very few ramets. Similar patterns have been observed in a diverse range of clonal plant species (e.g. Alberto et al 2005; Torimaru & Tomaru 2005; for review, see Vallejo-Marín et al 2010). One proposed explanation for such a pattern is that sexual reproduction is successful in generating new genotypes, but that the majority of these have poor clonal propagation (Alberto et al 2005). Alternatively, it has been suggested that the skewed distribution of genet sizes within populations of clonal plants reflects variation in the timing of establishment, and therefore in the resources available for clonal growth, or the outcome of interclonal competition (Torimaru & Tomaru 2005).

In populations of *A. carneorum* where multiple genets were detected, ramets belonging to the same genet tended to be spatially clustered, particularly in the populations with the highest genotypic diversity (Table 1, Figure 4). A spectrum of growth forms, ranging from 'phalanx' to 'guerilla' has been used to describe the mode of spread in clonal plants based on the spatial distribution of ramets (Lovett Doust 1981, Ye et al 2006). The phalanx growth form involves short connections between ramets, resulting

in tight spatial clustering, while the guerilla form describes genets with greater radial growth and wider spacing between ramets (Lovett Doust 1981). The phalanx growth form is believed to be beneficial for exploiting locally abundant resources, and excluding other genets, whereas the guerrilla form enables genets to 'seek out' resources over a greater distance and escape poor habitat (Lovett Doust 1981, Ye et al 2006). The close spatial clustering of ramets within genets of *A. carneorum* ('phalanx' type growth form) may therefore reflect the dominance of favourable microsites by individual genets, which may also account for the skewed distribution of genet sizes if resource availability varies substantially across populations. That is, variation in the relative abundance of genets within a population may reflect variation in the quality of microsites where genets first become established. However, little is currently known about variation in environmental conditions within these populations.

Comparison of fruiting and non-fruiting populations

Populations of *A. carneorum* in which fruiting is currently observed (BD and MCAMP) were, on average, more genotypically diverse than those in which reproduction appears to be exclusively clonal. This is consistent with predictions and may reflect either a requirement for genotypic diversity in order to set seed (e.g. due to self-incompatibility), or the role of sexual reproduction in producing new genotypic variation, or both. However, several populations exhibited apparently contradictory patterns of genotypic diversity. In particular, the HD population (non-fruiting) exhibited higher genotypic diversity than that seen at MCAMP (fruiting). As discussed already, the data suggest that sexual recruitment may have occurred in most of the sampled

populations, and the reasons for the current lack of seed set in many of these remains unclear. Self-incompatibility (SI) is common in Australian *Acacia*s (Kenrick 2003; Gibson et al 2011), therefore a lack of genetically compatible mates provides one plausible explanation for the current lack of seed set in most populations. However, it is not known whether *A. carneorum* is self-incompatible. Future studies should experimentally transfer pollen within and between genets to test for SI as a potential cause of reproductive failure. Alternatively environmental factors, including grazing by introduced feral and domestic animals, may mean that current conditions are unsuitable for sexual reproduction in most populations. Many studies have demonstrated a reduction in the relative frequency of sexual reproduction as a result of environmental disturbance, including grazing (Kleijn & Steinger 2002; Evju et al 2011) and trampling by humans (Rusterholz et al 2009). Experimental tests of the impact of known threats, such as grazing, on seed set in *A. carneorum* should therefore be a focus of future studies.

Limitations of AFLP markers

AFLP markers have great utility for identifying genotypic diversity because they allow a large number of polymorphic loci to be examined. However, the main limitation of dominant markers such as AFLPs is that they cannot distinguish heterozygotes from the dominant homozygote. Assessment of heterozygosity can be useful in studies of clonal plants for a range of applications, including estimating clone age (Ally et al 2008) and identifying heterozygote excess, which is common in clonal populations that lack sexual reproduction because there is no recombination (Eckert 2002). In *A. carneorum*

populations with lower genotypic diversity, we were unable to completely rule out the possibility that individuals displaying identical genotypes at the suite of AFLP loci we examined were not siblings produced by sexual reproduction. While we consider this to be unlikely given independent evidence for substantial clonal propagation in these populations, the use of more variable genetic markers could confirm this. Future studies should therefore employ co-dominant markers (e.g. microsatellites) to investigate these issues in *A. carneorum*.

Implications for conservation of A. carneorum

The maintenance of genetic diversity within species and populations is increasingly recognised as critical to allow adaptation to environmental change, particularly that expected under future climate change (Mace & Purvis 2008; Hoffmann & Sgro 2011; Sgro et al. 2011). Therefore, it is important to consider the implications of the very low levels of genotypic diversity present within *A. carneorum* populations for the long-term persistence of this species.

Although it appears that clonality has been the dominant mode of reproduction in *A. carneorum* in the long term, the present study highlights several features of this species that point to a need for active conservation management: Significant genetic divergence and the lack of any shared clones between populations means that for effective conservation of genetic diversity (and therefore adaptive potential) in *A. carneorum*, it is important to preserve as many populations as possible, because each population

captures only a small proportion of the overall genetic variation present within the species. Furthermore, these findings confirm that gene flow (seed or pollen dispersal) between isolated remnants is extremely rare and therefore highly unlikely to augment genotypic diversity in existing populations, or to found new ones. This, combined with the apparent lack of recruitment in populations within the last 120 years (Auld & Denham 2001), suggests that populations will go extinct without intervention to replace the inevitable loss of older ramets over time. Finally, the environment in which *A. carneorum* exists has undergone rapid change over the past 150 years due to the impacts of agriculture, including land clearing, changed water regimes and the introduction of grazing animals (Auld 1993; Auld & Denham 2001)) and of climate change, meaning clonal genotypes that have evolved under past conditions may not be well-adapted to this new environment.

Given this set of circumstances, we recommend the transfer of genetic material between populations of *A. carneorum* to increase genotypic diversity and maximise adaptive potential (genetic rescue). The translocation of plants or cuttings could be used to provide an immediate increase in genotypic diversity within populations, and to test for adaptive differentiation between populations. However, we also recommend that attempts be made to stimulate seed set through the controlled movement of pollen between genets, given that seed production could provide an ongoing source of novel genotypic variation within populations, and seed dispersal is the only means by which *A. carneorum* can colonise new sites. Such a strategy has previously been shown to increase fitness within small, isolated populations of other plant species (e.g. Severns 2003; Holmes et al 2008).

A common concern about genetic rescue is that deliberate outcrossing between genetically divergent populations will result in outbreeding depression due to production of less well-adapted genotypes, or breakup of coadapted gene complexes (Edmands 2007; Hedrick & Fredrickson 2010). We therefore recommend that genetic rescue be attempted using crosses between clones spanning a range of genetic distances, to determine the scale at which benefits are maximised. For populations found to contain more than one clone, this should include within-population crosses, in case local adaptation is a significant cause of among-population divergence.

Finally, we emphasise that where environmental factors (e.g. grazing) are limiting seed production and seedling survival, the success of a genetic rescue program will be contingent upon the use of complementary strategies such as protection from grazing, and possibly *ex situ* conservation measures.

Acknowledgements
We thank J. McDonald and R. Playfair for providing phyllode samples from South Australia and M. Fletcher for facilitating collections on properties in NSW. Thanks also to the Gall family who allowed us to sample phyllodes from the populations at Langawirra. We thank two anonymous reviewers for their helpful comments, which greatly improved this manuscript.

Literature cited

Alberto F, Gouveia L, Arnaud-Haond S, Pérez-Lloréns JL, Duarte CM, Serrão EA (2005) Within-population spatial genetic structure, neighbourhood size and clonal subrange in the seagrass *Cymodocea nodosa*. Molecular Ecology 14: 2669 – 2681

Ally D, Ritland K, Otto SP (2008) Can clone size serve as a proxy for clone age? An exploration using microsatellite divergence in *Populus tremuloides*. Molecular Ecology 17: 4897 – 4911

Arnaud-Haond S, Duarte CM, Alberto F, Serrão EA (2007) Standardizing methods to address clonality in population studies. Molecular Ecology 16: 5115 – 5139

Auld TD (1993) The impact of grazing on regeneration of the shrub *Acacia carnei* in arid Australia. Biological Conservation 65: 165 – 176

Auld TD, Denham AJ (2001) Flora conservation issues at Kinchega National Park, western NSW. Cunninghamia 7: 27 – 41

Balloux F, Lehmann L, de Meeûs T (2003) The population genetics of clonal and partially clonal diploids. Genetics 164: 1635 – 1644

Beatty GE, McEvoy PM, Sweeney O, Provan J (2008) Range-edge effects promote clonal growth in peripheral populations of the one-sided wintergreen *Orthilia secunda*. Diversity and Distributions 14: 546 – 555

Denham AJ, Auld TD (2004) Survival and recruitment of seedlings and suckers of trees and shrubs of the Australian arid zone following habitat management and the outbreak of Rabbit Calicivirus Disease (RCD). Austral Ecology 29: 585 – 599

de Witte LC, Armbruster GFJ, Gielly L, Taberlet, P, Stöcklin J (2012) AFLP markers reveal high clonal diversity and extreme longevity in four key arctic-alpine species. Molecular Ecology 21: 1081 – 1097

Douhovnikoff V, Dodd RS (2003) Intra-clonal variation and a similarity threshold for identification of clones: application to *Salix exigua* using AFLP molecular markers. Theoretical and Applied Genetics 106: 1307 – 1315

Dorken ME, Eckert CG (2001) Severely reduced sexual reproduction in northern populations of a clonal plant, *Decodon verticillatus* (Lythraceae). Journal of Ecology 89: 339 – 350

Eckert CG (2002) The loss of sex in clonal plants. Evolutionary Ecology 15: 501 – 520

Eckert CG, Kalisz S, Geber MA, Sargent R, Elle E, Cheptou P, Goodwillie C, Johnston MO, Kelly JK, Moeller DA, Porcher E, Ree RH, Vallejo-Marin M, Winn AA (2010) Plant mating systems in a changing world. Trends in Ecology and Evolution 25: 35 – 43

Edmands S (2007) Between a rock and a hard place: evaluating the relative risks of inbreeding and outbreeding for conservation and management. Molecular Ecology 16: 463 – 475

Ellstrand NC, Roose ML (1987) Patterns of genotypic diversity in clonal plant species. American Journal of Botany 74: 123 – 131

Evju M, Halvorsen R, Rydgren K, Austrheim G, Mysterud A (2011) Effects of sheep grazing and temporal variability on population dynamics of the clonal herb *Geranium sylvaticum* in an alpine habitat. Plant Ecology 212: 1299 – 1312

Gibson MR, Richardson DM, Marchante E, Marchante H, Rodger JG, Stone GN, Byrne M, Fuentes-Ramirez A, George N, Harris C, Johnson SD, Le Roux JJ, Miller JT, Murphy DJ, Pauw A, Prescott MN, Wandrag EM, Wilson JRU (2011) Reproductive biology of Australian acacias: important mediator of invasiveness? Diversity and Distributions 17: 911 – 933

Gross CL, Nelson PA, Haddadchi A, Fatemi M (2012) Somatic mutations contribute to genotypic diversity in sterile and fertile populations of the threatened shrub, *Grevillea rhizomatosa* (Proteaceae). Annals of Botany 109: 331 – 342

Hadfield JD (2008) Estimating evolutionary parameters when viability selection is operating. Proceedings of the Royal Society B 275: 723 – 734

Hadfield JD, Richardson DS, Burke T (2006) Towards unbiased parentage assignment: combining genetic, behavioural and spatial data in a Bayesian framework. Molecular Ecology 15: 3715 – 3730

Hall P, Walker S, Bawa K (1996) Effect of forest fragmentation on genetic diversity and mating system in a tropical tree, *Pithecellobium elegans*. Conservation Biology 10: 757 – 768

Hamrick JL, Godt MJW (1989) Allozyme diversity in plant species. Plant population genetics, breeding and germplasm resources (eds. A.D.H. Brown, M.T. Clegg, A.L. Kahler, B.S. Weir). pp. 43-63 Sinauer, Sunderland, MA.

Hamrick JL, Godt MJW (1996) Effects of life history traits on genetic diversity in plant species. Philosophical Transactions of the Royal Society of London B 351: 1291 – 1298
Hedrick PW & Fredrickson R (2010) Genetic rescue guidelines with examples from Mexican wolves and Florida panthers. Conservation Genetics 11: 615 – 626

Holmes GD, James EA, Hoffmann AA (2008) Limitations to reproductive output and genetic rescue in populations of the rare shrub *Grevillea repens* (Proteaceae). Annals of Botany 102: 1031 – 1041

Hoffmann AA, Sgro CM (2011) Climate change and evolutionary adaptation. Nature 470: 479 – 485

Honnay O, Bossuyt B (2005) Prolonged clonal growth: escape route or route to extinction? Oikos 108: 427 – 432

Kenrick J (2003) Review of pollen-pistil interactions and their relevance to the reproductive biology of *Acacia.* Australian Systematic Botany 16: 119 – 130

Kleijn D, Steinger T (2002) Contrasting effects of grazing and hay cutting on the spatial and genetic population structure of *Veratrum album*, an unpalatable, long-lived, clonal plant species. Journal of Ecology 90: 360 – 370

Lovett Doust L (1981) Population dynamics and local specialisation in a clonal perennial (*Ranunculus repens*): I. The dynamics of ramets in contrasting habitats. Journal of Ecology 69: 743 – 755

Mace GM, Purvis A (2008) Evolutionary biology and practical conservation: bridging a widening gap. Molecular Ecology 17: 9 – 19

Peakall R, Smouse PE (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes. 6: 288 – 295

Richards CL, Hamrick JL, Donovan LA, Mauricio R (2004) Unexpectedly high clonal diversity of two salt marsh perennials across a severe environmental gradient. Ecology Letters 7: 1155 – 1162

Rusterholz H-P, Kissling M, Baur B (2009) Disturbances by human trampling alter the performance, sexual reproduction and genetic diversity in a clonal woodland herb. Perspectives in Plant Ecology, Evolution and Systematics 11: 17 - 29

Severns P (2003) Inbreeding and small population size reduce seed set in a threatened and fragmented plant species, *Lupinus sulphureus* ssp. *kincaidii* (Fabaceae). Biological Conservation 110: 221 – 229

Sgro CM, Lowe AJ, Hoffmann AA (2011) Building evolutionary resilience for conserving biodiversity under climate change. Evolutionary Applications 4: 326 – 337

Silvertown J (2008) The evolutionary maintenance of sexual reproduction: evidence from the ecological distribution of asexual reproduction in clonal plants. International Journal of Plant Sciences 169: 157 – 168

Tame T (1992) *Acacias of southeast Australia*. Kangaroo Press, Kenthurst, NSW.
Torimaru T, Tomaru N (2005) Fine-scale clonal structure and diversity within patches of a clone-forming dioecious shrub, *Ilex leucoclada* (Aquifoliaceae). Annals of Botany 95: 295 – 304

Vallejo-Marín M, Dorken M, Barrett SCH (2010) The ecological and evolutionary consequences of clonality for plant mating. Annual Review of Ecology, Evolution and Systematics 41: 193 – 213

Vandepitt K, Roldán-Ruiz I, Jacquemyn H, Honnay O (2010) Extremely low genotypic diversity and sexual reproduction in isolated populations of the self-incompatible lily-of-the-valley (*Convallaria majalis*) and the role of the local forest environment. Annals of Botany 105: 769 – 776

Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acids Research 23: 4407 – 4414

Waits LP, Luikart G, Taberlet P (2001) Estimating the probability of identity among genotypes in natural populations: cautions and guidelines. Molecular Ecology 10: 249 – 256

Weeks AR, Hoffmann AA (2008) Frequency-dependent selection maintains clonal diversity in an asexual organism. Proceedings of the National Academy of Sciences 105: 17872 – 17877

Whitlock R, Hipperson H, Mannarelli M, Butlin RK, Burke T (2008) An objective, rapid and reproducible method for scoring AFLP peak-height data that minimizes genotyping error. Molecular Ecology Resources 8: 725 – 735

Ye X-H, Yu F-H, Dong M (2006) A trade-off between guerilla and phalanx growth forms in Leymus secalinus under different nutrient supplies. Annals of Botany 98: 187 – 191

Young AG, Boyle T, Brown T (1996) The population genetic consequences of habitat fragmentation for plants. Trends in Ecology and Evolution 11: 413 – 418

Table 1. Location, size, isolation and genotypic diversity of each of the *Acacia carneorum* populations sampled. *Lat* = latitude in degrees south, *Long* = longitude in degrees east, *N* = number of stems included in AFLP analysis, *A* = area of the population in ha, *D* = mean pairwise distance between sampled stems, *Total stems* = estimate of the total number of stems in the population†, *Isolation* = distance from the nearest (and next nearest) population in km, *G* = number of unique AFLP genotypes (genets) detected at the chosen threshold, *Ave DR* = average distance (in m) between ramets belonging to the same genet, *Max DR* = maximum distance (in m) between ramets belonging to the same genet, CD = clonal diversity, calculated as (G-1)/(N-1), *PLP* = percent of AFLP loci that were polymorphic within each population, *H'* = Shannon's diversity index, *J'* = Pielou's evenness index, P_{sex} = the probability that unrelated individuals could display identical multilocus genotypes under random mating, P_{IDsib} = the probability that related individuals could display the same multilocus genotype under random mating .

Population	*Lat* (*˚S)*	*Long* (*˚E)*	*N*	*A* (*ha)*	*D (m)*	*Total stems* †	*Isolation* (*km)*	*G*	*Ave DR* (*m)*	*Max DR* (*m)*	*CD*	*PLP*	*H' (SE)*	*J'*	P_{sex}	P_{IDsib}
BD*	32.53	142.16	12	17.9	624	891	1 (2)	10	49	69	0.82	41.2	0.979	0.979	<0.001	<0.001
BIMB[††]	32.10	140.22	14	8.9	-	443	2 (10)	2	-	-	0.08	15.3	0.112	0.371	<0.001	0.151
HD	29.47	141.27	10	4.2	96	210	69 (146)	7	41	53	0.67	33.0	0.759	0.898	<0.001	<0.001
KOON[††]	32.10	139.15	14	7.4	-	367	4 (11)	2	-	-	0.07	16.5	0.112	0.371	<0.001	0.093
LA	31.42	142.18	10	3.1	150	157	3 (15)	1	150	323	0	10.6	0	1	-	-
MCAMP*	32.72	141.99	12	2.0	62	106	3 (5)	5	41	99	0.36	28.2	0.477	0.683	<0.001	0.009
MULY[††]	31.60	140.80	10	5.0	-	249	3 (8)	3	-	-	0.22	21.2	0.278	0.582	<0.001	0.024

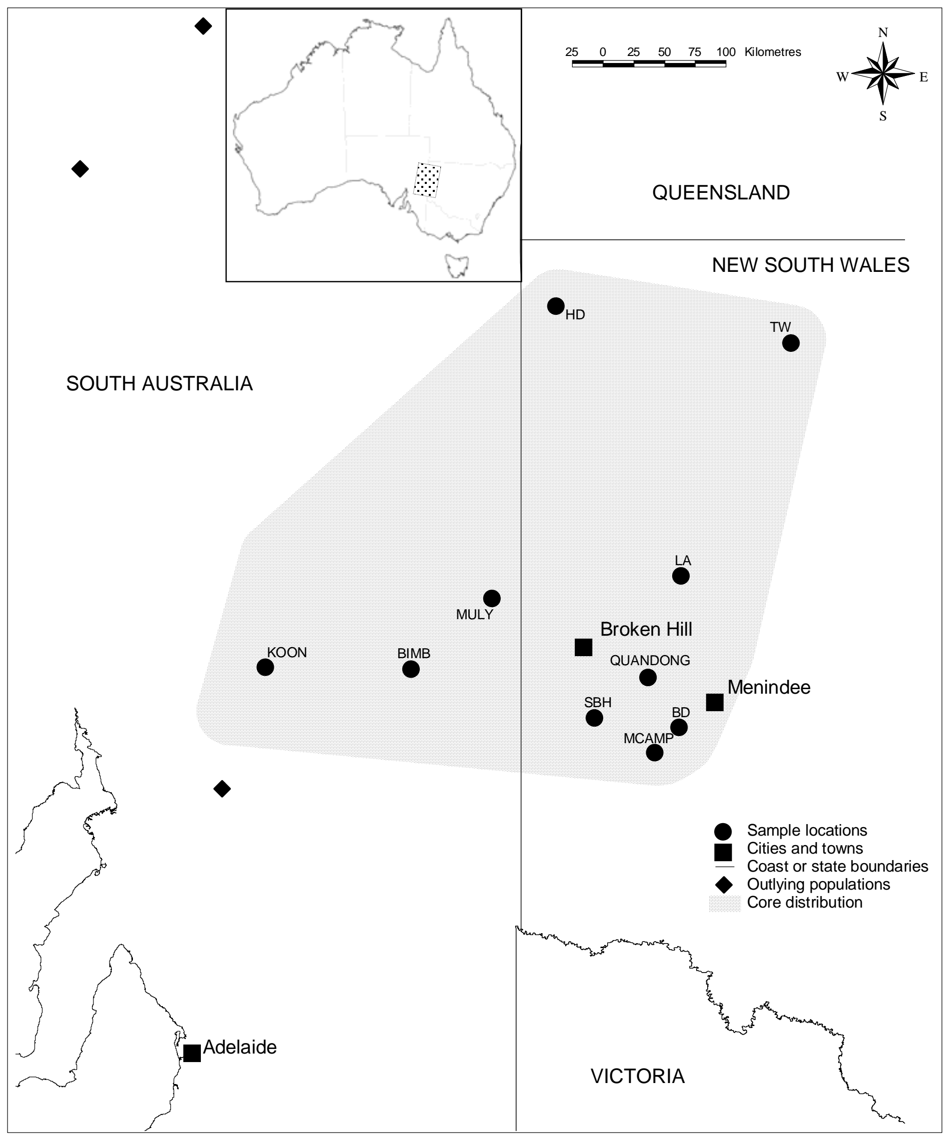
QUANDONG	32.15	141.93	15	7.2	228	357	5 (35)	3	142	388	0.14	25.9	0.349	0.731	<0.001	0.002
SBH	32.46	141.56	14	17.3	232	893	29 (33)	1	232	507	0	10.6	0	1	-	-
TW	29.73	142.97	13	5.2	92	258	139 (144)	4	105	194	0.25	21.2	0.407	0.676	<0.001	0.039

1 *Populations in which seed set has been observed.

2 †Based on the formula $N=13.34+43.53A+0.307A^2$, where A is the estimated area of the population
3 †† For populations BIMB, KOON and MULY, coordinates for individual samples were not recorded, therefore it was not possible to calculate the mean
4 distance between sampled stems, or the mean or maximum distance between ramets.
5

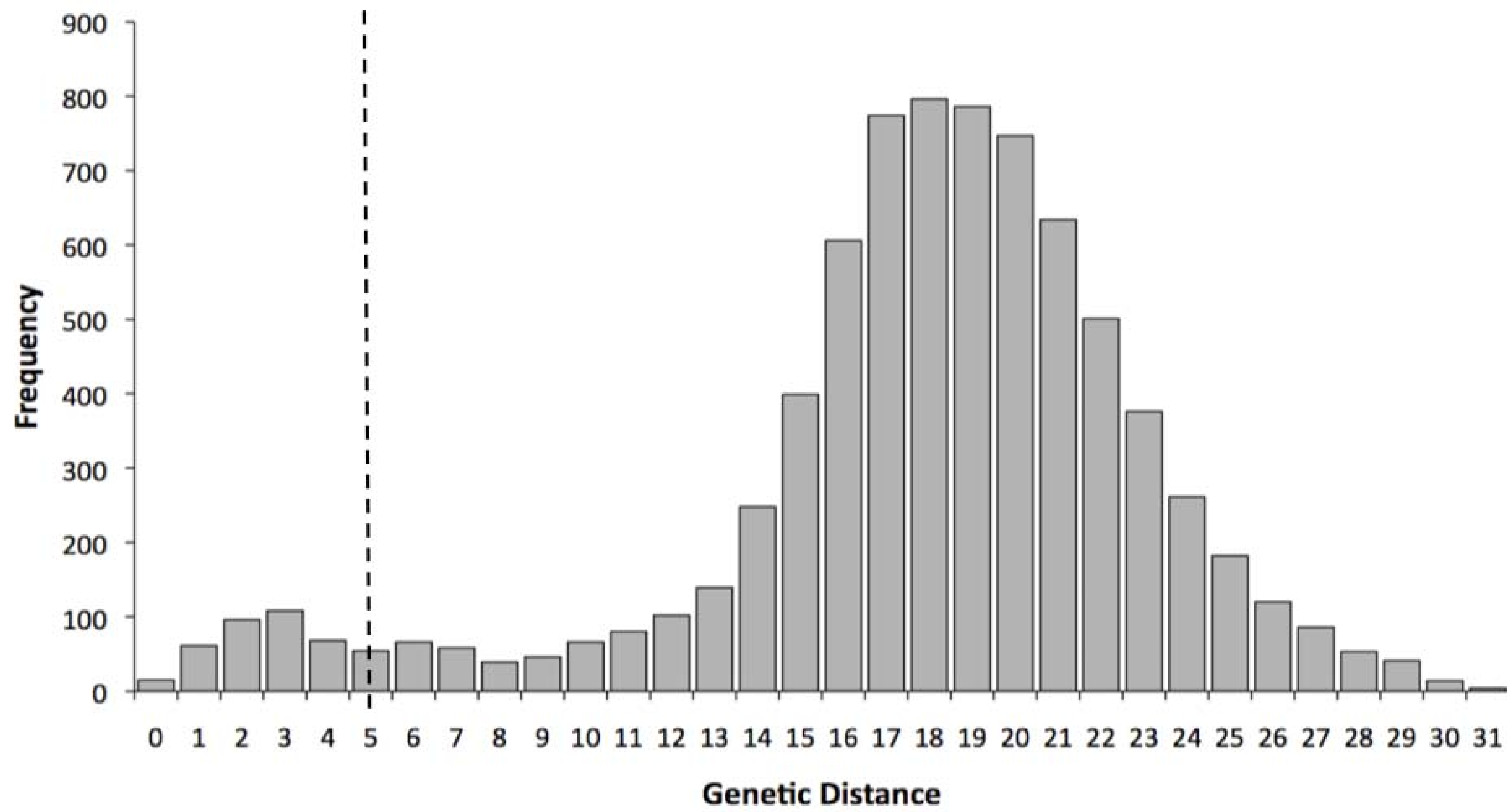
1 **Figure captions**

2 **Figure 1**. Map showing the distribution of *Acacia carneorum* and the populations
3 sampled for AFLP genotyping.
4
5 **Figure 2**. Frequency distribution of pairwise genetic distances among *A. carneorum*
6 samples included in AFLP analysis, measured as the Euclidean distance (a count of the
7 number of AFLP loci at which samples differ). The dashed line indicates the threshold
8 below which samples were considered to belong to the same genet.
9
10 **Figure 3.** Frequency histograms showing the number of ramets belonging to each genet
11 within each population.
12
13 **Figure 4.** The spatial distribution of ramets belonging to each genet within each
14 population. Different symbols indicate different genets within a population. Note
15 however that symbols are population-specific: no genets occurred in more than one
16 population, therefore the same symbol in different populations refers to different genets.
17 Diagrams for all populations are presented at the same scale to enable comparison of the
18 spatial extent of genets. Note that for populations BIMB, KOON and MULY,
19 coordinates for individual samples were not recorded, therefore the spatial distributions
20 of ramets in these populations are not presented.
21
22 **Figure 5**. Principal Coordinates Analysis illustrating clustering of populations using
23 genetic distances among individuals' AFLP genotypes. The first two axes explained
24 31% and 21% of the variation respectively.
25
26
27



1
2

3 **Figure 1.**



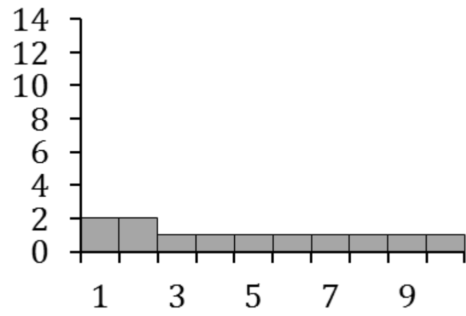
1

2 **Figure 2.**

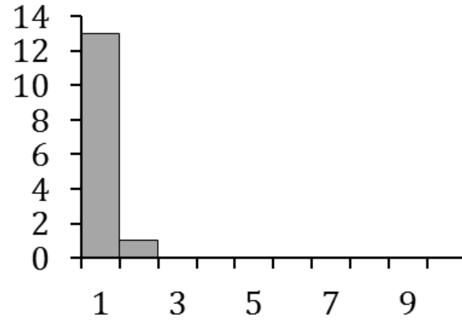
3

Number of ramets

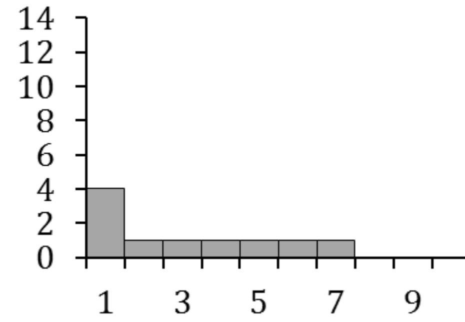
BD



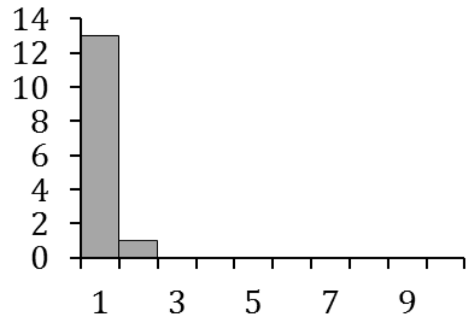
BIMB



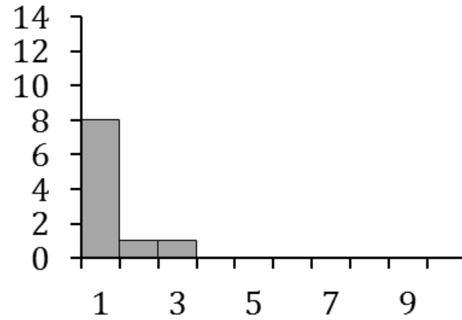
HD



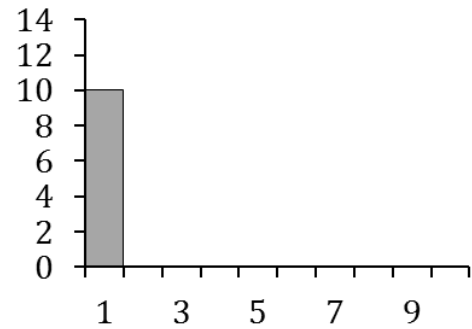
KOON



MULY

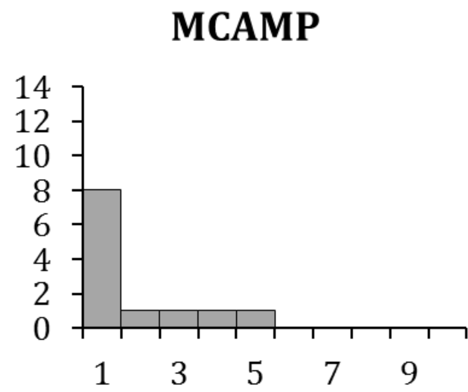


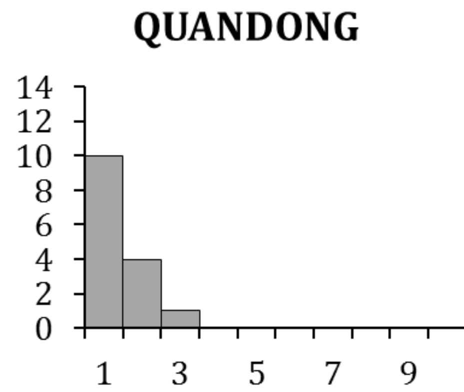
LA

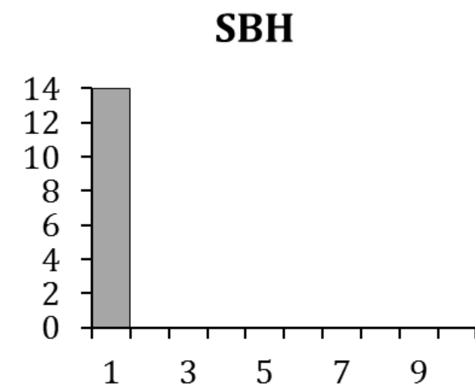


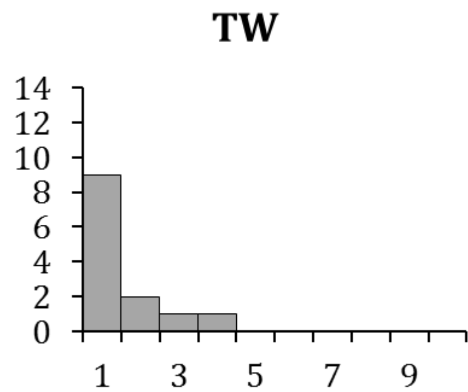
2

1

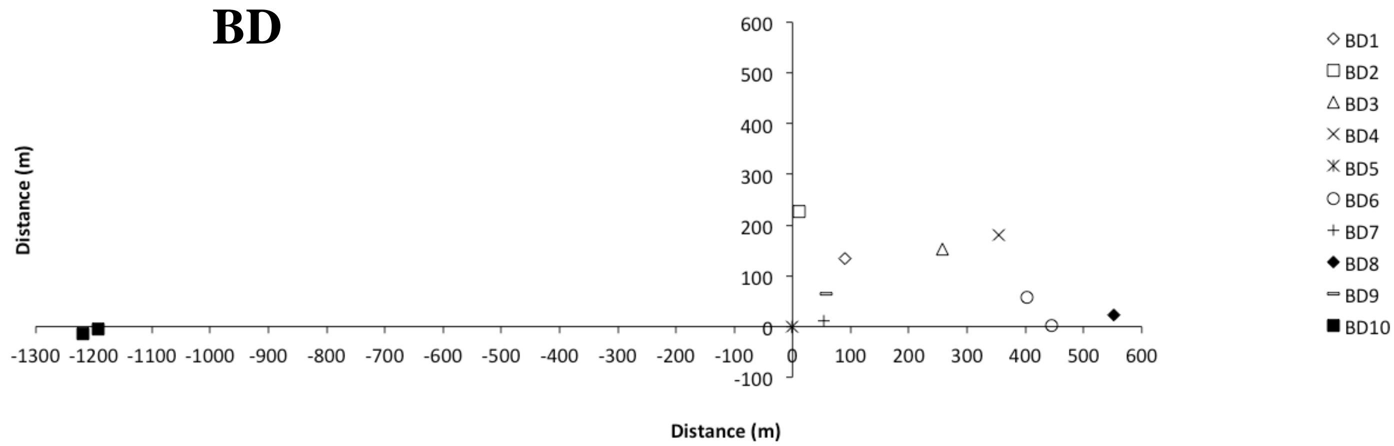






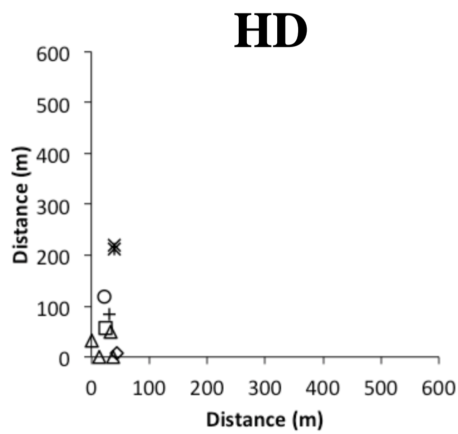


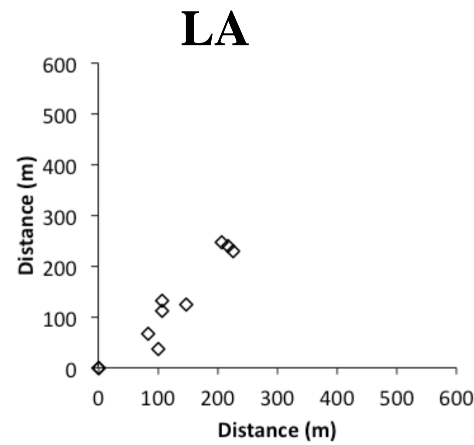
2
3
4
5 **Number of genets**
6
7 **Figure 3.**
8



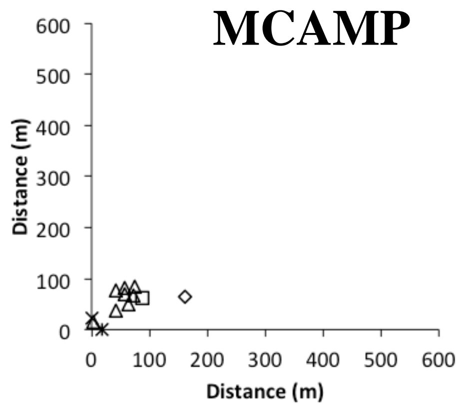
1

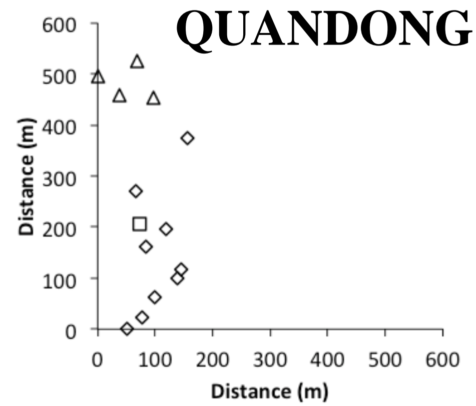
2





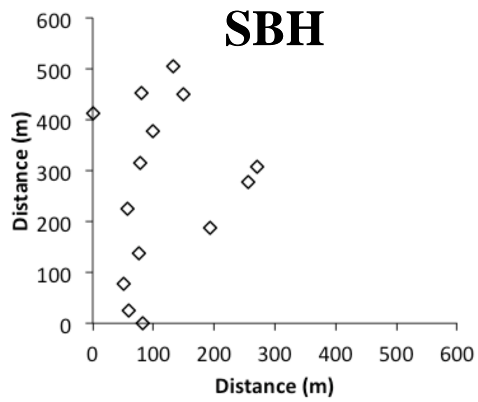
3

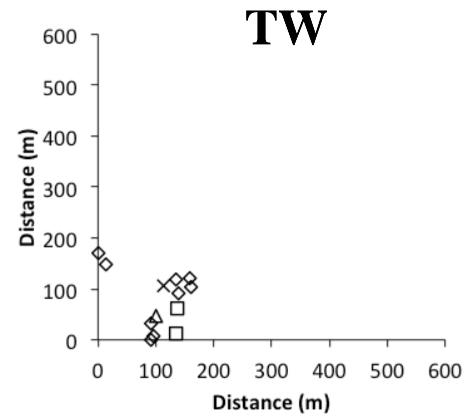




1

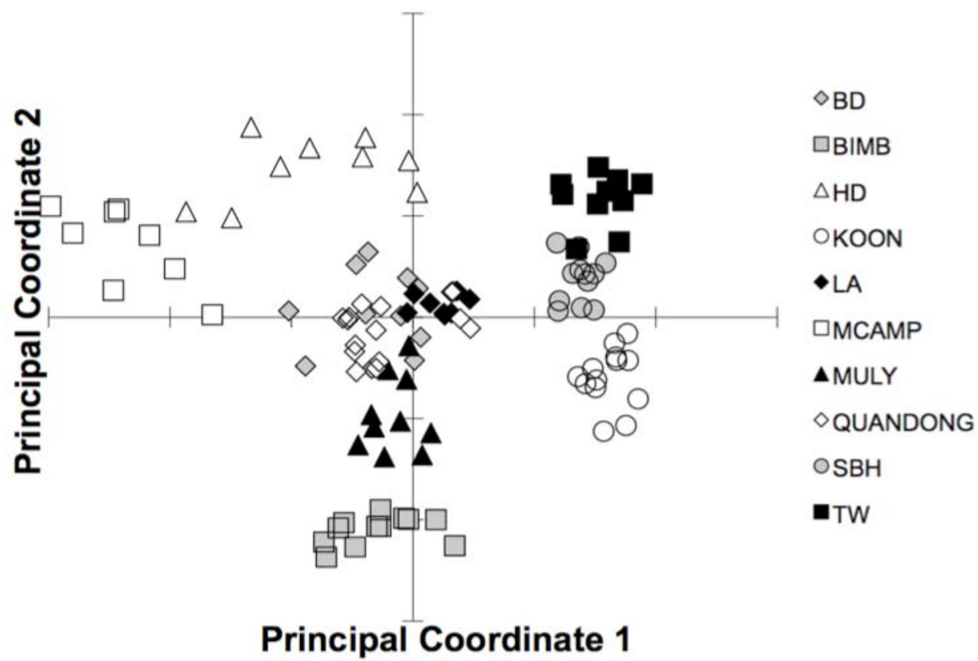
2





3

4 **Figure 4.**



1

2

3 **Figure 5.**

4

Appendix 1

Estimating the number of adult stems present in stands of *Acacia carneorum*

Resources for survey are rarely adequate to allow exhaustive counts of individual plants at many locations. For *Acacia carneorum*, which exhibits clonality via root suckering, the density of stems is often high, with clusters of smaller young suckers surrounding older stems. In order to estimate the total number of adult stems within stands, the number of adult stems (defined as those >1m tall) in one to six 5m wide transects 150-200m in length in six stands within Kinchega National Park was counted (Table 1). The number of transects depended on the size of the stand, while the length depended on its shape, with narrow stands having shorter transects. In a further three very small stands the total number of adult stems was scored (all count data courtesy of Marsh 2010). We considered each transect as an independent estimate of adult density and used an estimate of the area of occupancy of each stand to derive an estimate of the total number of adults in the stand. Thus we had several estimates of the number of adult stems from a range of stands with varying area of occupancy, ranging from 0.13 ha to over 60 ha.

We then used a curve fitting program (CurveExpert 1.4 - Hyams 2009) to find an appropriate formula for predicting the number of stems in any stand. For simplicity, we constrained curves to linear or quadratic form. We found that a quadratic curve ($y=13.34+43.53x+0.3068x^2$) provided the best fit ($r=0.9028$), while the linear fit ($y=-79.33+63.06x$) was also excellent ($r=0.9014$) (Fig. 1). At small areas of occupancy (Fig. 1b), the quadratic model is more appropriate since the linear formula predicts that populations occupying less than 1.3 ha have fewer than zero plants. In contrast, while clearly some space is required to sustain any plants, it is conceivable that >13 plants could occupy an area of much less than 1 ha, as predicted by the quadratic curve.

References

Hyams DG (2009) CurveExpert 1.4 A curve fitting program for Windows. http://www.curveexpert.net/

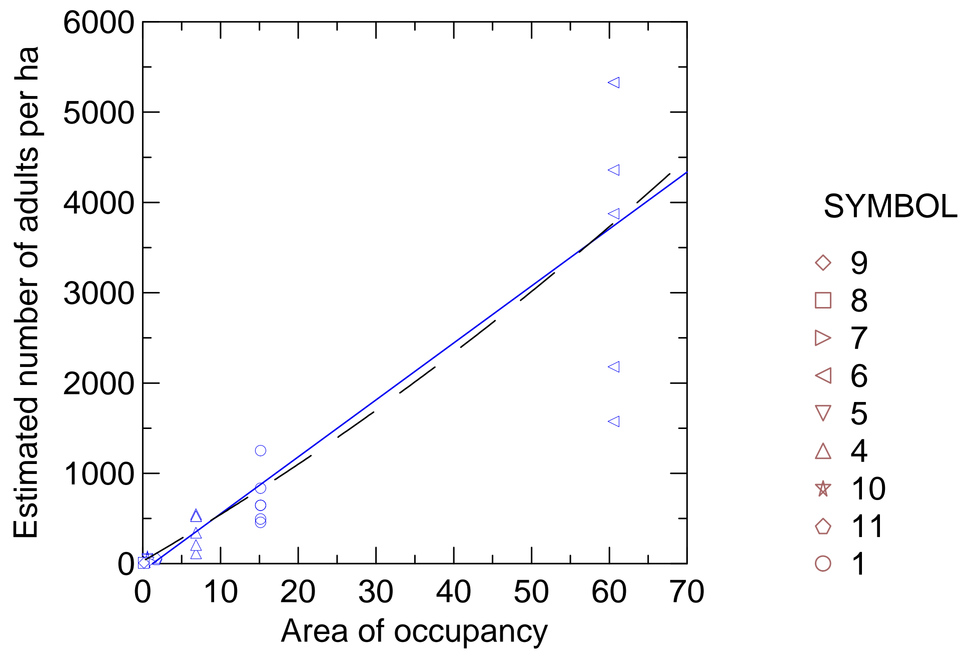
Marsh AL (2010) The likely persistence of *Acacia carneorum* in light of fragmentation in the Australian arid-zone. Honours Thesis, University of Wollongong

Table 1. Data used for estimating number of adults for a given area.

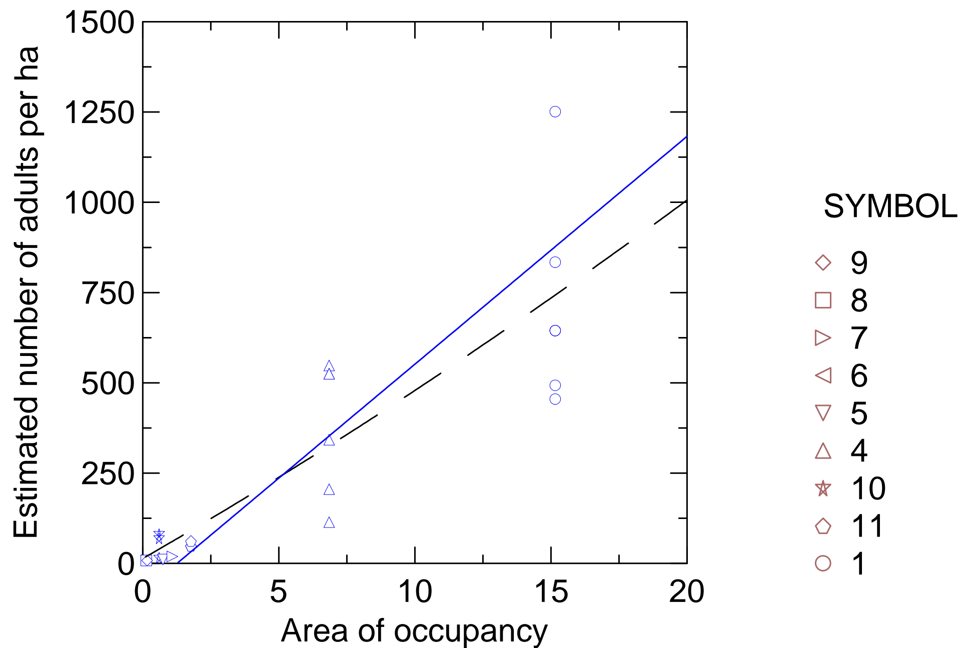
Estimated Area (ha)	Site	number of transects	Symbol in graph
15.2	BD	6	1
1.8	FR	3	11
0.6	WLC	3	10
6.9	WF	6	4
60.5	AC1	6	6
1.1	ED	n/a*	7
0.13	S5	n/a*	8
0.16	OP1	n/a*	9
0.74	S26	1	5

*Actual total counted

1



(a)



2
3 (b)
4 **Fig. 1**. Relationship between area of occupancy and estimated number of adults per population. These
5 data are derived from density estimates from nine populations at Kinchega National Park, with population
6 estimates derived from one to six 100-250 m long transects for larger areas of occupancy (different
7 symbols for different populations) and smallest areas of occupancy from a total count (data courtesy of
8 Marsh 2010). Solid line for linear curve, dashed line for quadratic curve. (a) Full data set, showing
9 estimates for up to 70 ha area of occupancy, (b) Constrained data set, showing estimates for up to 20 ha.

Appendix 2.

Effect of modifying the threshold for clone assignment on estimates of clonal diversity

The number of unique genets detected within each population for threshold genetic distances (number of AFLP loci differing) between 0 and 10. The highlighted row indicates the threshold used for clone detection.

Threshold genetic distance (no. loci)	Threshold genetic distance (%)	Number of genets within each population									
		BD	BIMB	HD	KOON	LA	MCAMP	MULY	QUANDONG	SBH	TW
0	0	12	13	9	13	1	12	10	15	12	10
1	2	12	7	9	9	1	11	10	11	2	7
2	3	11	6	9	3	1	9	7	8	1	5
3	4	10	5	7	3	1	6	6	6	1	5
4	5	10	2	7	2	1	5	3	3	1	4
5	6	9	2	7	2	1	3	3	3	1	2
6	8	9	1	4	1	1	1	3	2	1	1
7	9	4	1	3	1	1	1	2	1	1	1
8	10	4	1	2	1	1	1	1	1	1	1
9	11	3	1	2	1	1	1	1	1	1	1
10	12	3	1	1	1	1	1	1	1	1	1