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Genetic diversity, differentiation and conservation in *Araucaria bidwillii* (Araucariaceae), Australia's Bunya pine

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Abstract

Habitat fragmentation has resulted in many species becoming geographically restricted, as dispersal among subsequent isolates becomes compromised. This study investigated the effects of historical fragmentation on the genetic diversity and differentiation of Australia's Bunya Pine (*Araucaria bidwillii*) using random amplified DNA (RAPD) markers. High diversity characterises all Bunya populations sampled, regardless of population size or degree of isolation, which may be either due to similar effective population size among populations, or the lagging effects of the detection of contemporary genetic signal in long-lived conifer species. Large genetic differentiation characterises the northern and southern populations, although all sampled populations are significantly differentiated from each other. The northern population, at Mt Lewis, is responsible for the majority of variation detected among populations, and as such represents a significant genetic reservoir. The conservation of the genotypes of the Mt Lewis population may be imperative for the future of the species, given predicted models of accelerated climate change over the coming decades.

Abbreviations: ENSO – El Niño southern oscillation; ESU – evolutionary significant unit; PCA – principal coordinates analysis; PCR – polymerase chain reaction; RAPD – random amplified polymorphic DNA; UPGMA – unweighted pair group method using arithmetic averages

Introduction

Habitat fragmentation results in many species becoming geographically restricted in disjunct isolates. Dispersal represents an important mechanism for the "escape" of species from deleterious environmental conditions, including those associated with changing climatic conditions. This escape mechanism has been compromised for many plant species through habitat fragmentation, and as we enter a period of accelerated climate change (Houghton et al. 1996), the management of species within their present habitats has become of increasing concern. Any adaptive evolution *in situ* will necessarily rely on selection from pre-existing genotypes. Yet for the majority of plant species, little is known of the effects that historical climate change, and associated reductions and expansions in population size, has had on their genetic integrity.

Historical genetic patterns afford us the opportunity to examine how species have responded to previous climatic events over the timescales appropriate for detecting ecological and evolutionary processes, such as gene flow, mutation, genetic drift and natural selection (Wu et al.

1999); processes which may remain undetectable in standard ecological "snapshot" surveys. If the goals of conservation biology are to include the evolutionary potential of species (Moritz 2002), then an understanding of these processes, and quantification of the genetic diversity and divergence within species, is integral to any informed management attempt. In the past the majority of conservation reserves have necessarily been made in the absence of any genetic information.

Population size has been used as a surrogate for genetic diversity, as theory predicts a positive correlation between population size and genetic diversity (Frankham 1996; Gram & Sork 1999). Yet large differences may exist between effective population size and census size (Sherwin and Moritz 2000). Small populations are at risk of entering what has been termed an "extinction vortex" (Gilpin and Soulé 1986) or "mutational meltdown" (Lynch et al. 1995), where the deleterious genetic effects of small population size are fed back into the population/species until the population/species is driven to extinction. Of similar concern to small populations are genetic bottlenecks, which leave only a fraction of the genetic diversity available for subsequent expansion and diversification of the species (Young et al. 1996).

It is important, for any informed management effort, to determine whether extant population size reflects genetic diversity, as lowered genetic variation may reduce the opportunity for adaptation, lower population viability and lead to the above deleterious processes (Sherwin and Moritz 2000). Similarly, the identification of evolutionary significant units (ESUs: see Moritz 2002), and whether populations display significant differentiation, can further assist management strategies.

Australia's Bunya pine (*Araucaria bidwillii* Hook., Araucariaceae) represents one such species that has undergone extensive range reductions and fragmentation, now occurring in only 2 widely disjunct regions in North and South Queensland, Australia. Extant populations occur from as far north as Mt Lewis in North Queensland (16°30′ S) and as far south as the wider Brisbane area (26°15′ S–27°00′ S), with a substantial break in distribution of approximately 1000 kms (Figure 1). The northern populations are small and are suggested to be relictual while the southern populations are generally more extensive. This conifer is wind pollinated and extremely long-lived – factors which generally contribute to a low degree of population differentiation (Nybom and Bartish 2000).

The fossil record suggests this lineage was previously both more speciose and more widespread historically, occurring in both Hemispheres during the Mesozoic (Stockey 1994). The history of *Araucaria* on the Australian continent has comprised substantial shifts in distribution, presumably in response to adverse climatic conditions (Kershaw and Wagstaff 2001). More recently, pollen profiles from North Queensland suggest substantial shifts in local abundance for the genus throughout the Quaternary (Kershaw and McGlone 1995), although specific status cannot be ascertained due to generic similarity in pollen morphology. How historical changes in climate and abundance have affected the species remains obscured. Similarly, its predicted response to future accelerated climate change, which is anticipated to be of an order of magnitude faster than climate changes during the recent geological past (Houghton et al. 1996), concerningly remains obscured.

Of the 630 conifer species worldwide, the IUCN has identified the 200-odd southern conifers as being at greater risk of extinction (Farjon and Page 1999), yet, relative to their northern counter-parts, we know virtually nothing about them, ecologically or genetically. Superimposed on this is species-specific exploitation, which has further decimated and fragmented the extant populations of Bunyas over the last two centuries. The sociological importance of this tree (Haebich 2002) adds further weight to the urgency and importance of managing this iconic Australian species.

Of the molecular techniques available for investigating plant population genetic structure, RAPDs allow for the study of genetic diversity and its partitioning without prior knowledge of the genome (Williams et al. 1990). This technique is especially suited to situations when there are no genetic studies available from which to derive species-specific markers, such as microsatellites, and, thus, it is the most widely employed anonymous genetic marker in plant population studies (Nybom and Bartish 2000). Furthermore, a large number of informative loci from across the entire genome can be assessed

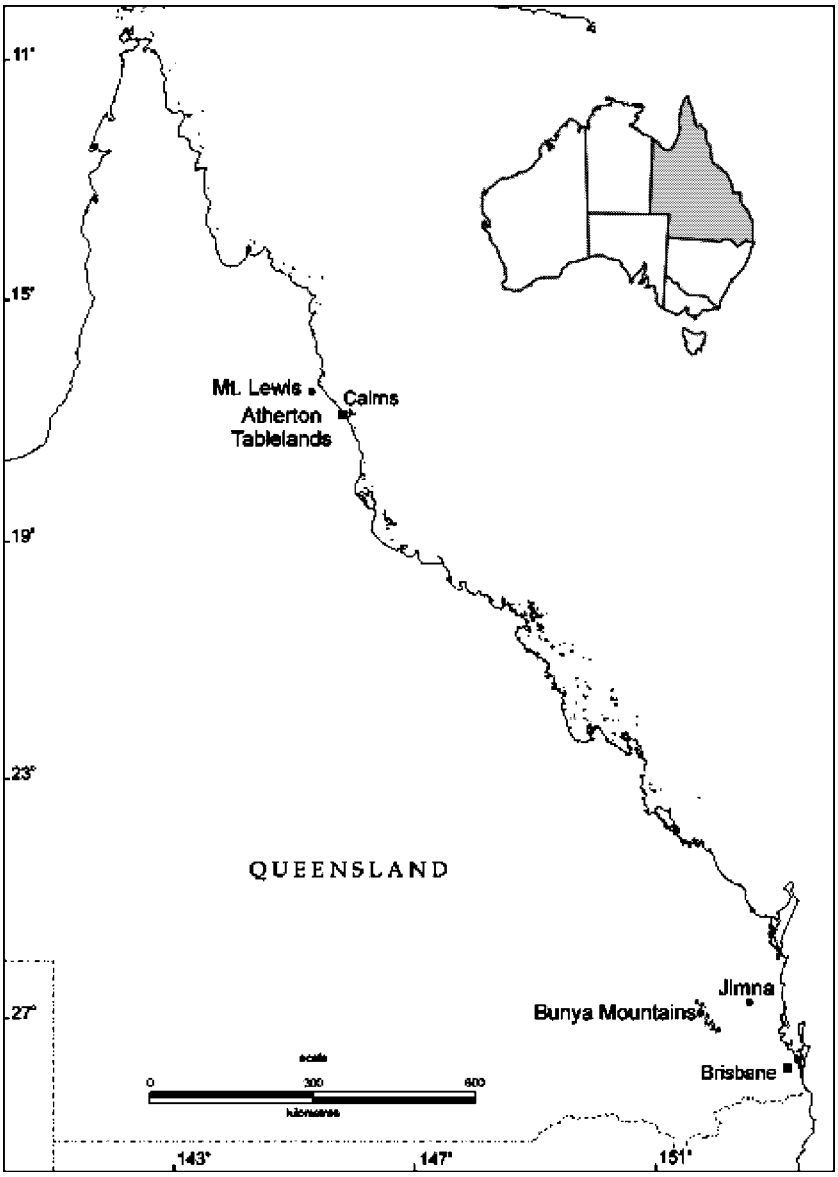

Figure 1. Localities of Bunya populations sampled for this study – Mt Lewis, north-west of Cairns; Bunya Mts, north-west of Brisbane and the Jimna/Conondale Range region north of Brisbane. These populations encompass the extant latitudinal and longitudinal distribution of the species.

with RAPDs at relatively low cost. Preliminary studies of population dynamics of southern conifers have also focussed on this methodology, making comparisons to other southern conifer genetic studies more meaningful (e.g. Allnutt et al. 1999; Allnutt et al. 2001; Bekessy et al. 2002; Allnutt et al. 2003).

The goal of the present study was to assess the genetic variation within and among populations of Bunyas throughout its natural range using RAPDs. Such information has recently been used in management strategies for the conservation of fragmented populations and to assess which populations should be prioritised for management (e.g. Bekessy et al. 2002). Specifically, the following questions were addressed: (1) Is population size positively correlated with genetic diversity in Bunyas? (2) Do populations display any evidence

of historical bottlenecks through reduced genetic diversity? (3) Do populations form a cohesive unit or does population genetic structuring exist, necessitating differing management strategies? If so, does the structuring reflect the predicted refugia of Webb and Tracey (1981), i.e. northern region, southern coastal region, and Bunya Mts?

Materials and methods

Leaf samples were collected from a total of 131 individuals from six populations across the geographic range of the species (Table 1). Trees were selected randomly from each population, but were separated as far as possible to minimise the chance of sampling closely related or genetically identical individuals. All samples included in this study were collected from mature (canopy forming) reproductive individuals. A minimum of 20 individuals per population was targeted, with only one population (Burton's Well) yielding less (17 individuals). Approximately five leaves were collected from each tree and placed into plastic sealable bags with a small amount of silica gel, to assist in the rapid drying and preservation of DNA, until they could be processed.

DNA isolation

The isolation of DNA follows the CTAB extraction protocol outlined in Pye et al. (2003). Total cellular DNA concentrations were calculated using a fluorometer (Hoefer™ DyNA Quant™ 200: Amersham Pharmacia Biotech, San Francisco, USA) and all extracts were diluted to approximately 10 ng/μl.

RAPD reactions

After optimisation of template and $MgCl_2$ concentrations, all RAPD reactions were performed under the following conditions so as to ensure maximum reproducibility. All reactions were performed in a Omn-E Thermal Cycler (Hybaid Ltd., UK), using 25 μl total reaction volume. Optimised reactions contained: approx. 50 ng template DNA, 1 μl primer (Operon), 0.5 μl 40 mM dNTPs (Promega), and 0.25 μl 0.4% bovine serum albumin, with the appropriate amount of double distilled H_2O. These constituents were then subjected to a 30 min. soak at 60 °C to remove excess proteins from the reaction. Enzyme mix, containing 2.5 μl $10 \times$ PCR buffer (Life Technologies), 1.5 μl 50 mM $MgCl_2$ (Life Technologies), and 0.1 U of *Taq* polymerase (Life Technologies), was then added before employing the following thermal cycling conditions: initial denaturation for 5 min at 94 °C, followed by 40 cycles of denaturation for 1 min at 94 °C, annealing for 1 min at 36 °C, and extension for 1 min at 72 °C. The amplification was completed by a final extension step of 2 min at 72 °C, to allow complete extension of the PCR products.

Forty primers, from primer kits OPB and OPC (Operon Technologies, Alameda, California), were screened with a subset of *A. bidwillii* samples for production of clear and variable RAPD profiles. From these, nine 10-mer primers were chosen for the analysis of the entire sample set (Table 2). RAPD products were separated on 2% agarose gels, with the addition of 1 μl/250 mL ethidium bromide, over 5 h (70 V) and visualised over UV light. A 1 Kb ladder (Life Technologies) was run alongside the RAPD products to estimate fragment size and to facilitate the scoring of bands

Table 1. Localities and descriptives of sampled populations of *A. bidwillii*

Population name	Region	Latitude	Longitude	Altitude (m)	Approx. population size	No. of individuals sampled
Jimna	Jimna State Forest/ Conondale Range	26° 42′	152° 27′/38′	500	$1000 > x > 100$	21
Westcott	Bunya Mts	26° 51′	151° 34′	1000	$1000 > x > 100$	21
Burton's Well	Bunya Mts	26° 49′	151° 33′	800	$x < 100$	17
Dandabah	Bunya Mts	26° 52′	151° 35′	1000	$x \sim 1000$	26
Paradise	Bunya Mts	26° 52′	151° 35′	1000	$x \sim 1000$	22
Mt Lewis	North Qld	16° 30′	145° 22′	1000	$x < 100$	24

Table 2. RAPD primer sequences used in the analysis

Name	Sequence 5′–3′
OPB20	GGACCCTTAC
OPB18	CCACAGCAGT
OPB17	AGGGAACGAG
OPB15	GGAGGGTGTT
OPB10	CTGCTGGGAC
OPB9	TGGGGGACTC
OPB8	GTCCACACGG
OPB1	GTTTCGCTCC
OPB6	TGCTCTGCCC

among populations. A random sample of reactions was duplicated to ensure repeatability.

Data analysis

Each RAPD product was assumed to represent a single locus and was scored as present (1) or absent (0) and entered into a binary data matrix. Bands with identical migration were considered as identical fragments, regardless of intensity. Long electrophoretic running times also minimised the scoring of non-homologous bands due to increased band separation. Only bands that appeared in size ranges between, or closely adjacent to, visible monomorphic bands were scored. While this substantially reduced the overall number of bands that could have been scored, it importantly avoided mis-scoring faint or artifactual bands. Monomorphic loci were not scored, as suggested by Nybom and Bartish (2000) in their analysis of RAPD studies, so as to make estimates of polymorphism more comparable across studies.

Genetic distance was calculated using Nei's measure of genetic distance (Nei 1973) as implemented in PopGene Ver. 1.31 (Yeh et al. 1999). Shannon's diversity estimates (Lewontin 1972) were also calculated in PopGene and used to estimate the degree of variation within each population. RAPD markers are dominant and therefore heterozygotes cannot be detected. The use of Shannon's index is recommended for dominant data since it is not sensitive to this bias (Meekins et al. 2001). The notation of Shannon's index as '*S*' and not '*H*' is used here in keeping with the suggestion of Allnutt et al. (1999) to avoid confusion with diversity measures such as heterozygosity, to which Shannon's index is not comparable. '*S*' was calculated using the following formula:

$$S = -\sum p_I \log_2 p_i$$

where p_i is the frequency of the presence or absence of each RAPD band. Total diversity across the species was estimated using the mean value of *S* over all the populations sampled. The percentage of polymorphic loci (%P) was calculated for each population in PopGene, as was the mean value across all populations.

A Euclidean distance matrix was calculated in Arlequin ver. 2.000 (Schneider et al. 2000) and used as the input file for an Analysis of Molecular Variance (AMOVA) (Excoffier et al. 1992). Variance components were estimated for (i) within populations, (ii) among populations within regions, and (iii) among regions. Regions were defined based on the extant geographical structure of populations, so the following populations were initially considered as regions: Mt Lewis, Jimna/Conondale and the Bunya Mountains. Different permutations were also examined to see which best explained the distribution of RAPD phenotypes across the species' range. Pairwise Φ_{ST} values among all populations were calculated in Arlequin and used to conduct a Principal Coordinates Analysis (PCA) in GenAlEx Ver. 5.04 (Peakall and Smouse 2001) to spatially examine the variation among populations. G_{ST} values derived from Nei's genetic distance in PopGene were also compared with Φ_{ST} estimates from the AMOVA analysis in Arlequin to examine congruence between analytical methods. Finally, an unrooted UPGMA tree was calculated from Nei's unbiased measures of genetic distance, which graphically displays the relative divergence among populations sampled.

Results

The 9 RAPD primers scored from the 40 screened primers produced a total of 50 polymorphic loci. This represents a conservative proportion of the total number of variable loci as the above scoring methodology was employed.

A minimum of three monomorphic markers was produced from each primer, but these were not included in the analysis. Two population-specific

bands were recorded for the Mt Lewis population, both from primer OPB 15 (Table 2). Additionally, five loci present in all southern populations were not present in the Mt Lewis population.

The relative degree of diversity, as indicated by Shannon's index varied across populations from 0.43 (Mt Lewis) to 0.53 (Jimna/Conondale) (Table 3), but these differences were not significant ($P = 0.37$, ANOVA). The mean population diversity was 0.46. Percent polymorphic loci varied from 74% (Mt Lewis) to 92% (Jimna/Conondale). The mean percentage of polymorphic loci across populations was 84% (Table 3).

Genetic structuring was investigated by AMOVA. The hypothesis being tested was that three separate refugia existed for the species (northern region, southeast coastal region, and Bunya Mts) based on phyto-geographical evidence (Webb and Tracey 1981). However, the variation among populations within a region was greater than the variation among regions and regional values were not significant ($P = 0.16$).

Table 3. Shannon's diversity index (S) and percent polymorphic RAPD loci (%P) for all Bunya populations sampled and mean values averaged over all populations and pooled species-level values. Standard errors are presented in parentheses

Population	S (± standard errors)	%P
Jimna/Conondale	0.5333 (0.2099)	92
Westcott	0.4751 (0.2401)	86
Burton's Well	0.4628 (0.2677)	80
Mt Lewis	0.4261 (0.2894)	74
Dandabah	0.498 (0.216)	90
Paradise	0.464 (0.2499)	82
Mean, S_{pop}	0.4755	84
Species level	0.6043 (0.0898)	100

When only two regions are considered (Table 4), corresponding to northern and southern populations, most of the variation (62%) is found within populations but a significant proportion was due to differences between regions (29%) and among populations within regions (8%) ($P < 0.00001$). This hierarchical structure best explains the distribution of RAPD phenotypes in the sampled landscape (other permutations not shown). All pairwise Φ_{ST} values between individual pairs of populations were significant (Table 5). Maximum values are recorded between Mt Lewis and all other populations sampled.

The unrooted UPGMA tree (Figure 2) graphically illustrates the relationships among populations and regions. The northern population is separated from all southern populations. A long branch leads to the Mt Lewis population, while the Jimna (coastal) population clusters with the Bunya Mts populations. The same pattern was obtained using principal coordinates analysis of pairwise Φ_{ST} values (not shown). When the analysis is restricted to southern populations only, both Φ_{ST} and G_{ST} estimates are in agreement ($\Phi_{ST} = 11\%$, $G_{ST} = 12\%$). These values are also in close agreement with those obtained from other southern conifers with similar pollination and dispersal mechanisms (*Araucaria araucana*, $\Phi_{ST} = 12\%$, Bekessy et al. 2002).

Discussion

Two striking results stand out from this analysis: firstly, the high genetic variation recorded for a "relictual" southern conifer, and secondly, the extreme population differentiation, predominantly between northern and southern populations. This is in contrast to the majority of studies that have found genetic diversity within populations to be

Table 4. AMOVA analysis of RAPD variation for 6 *Araucaria bidwillii* populations. Regions were defined as northern (Mt Lewis) and southern (the remainder) populations

	d.f.[a]	Variance component	% of total variance	p value[b]
Between regions	1	3.38847	**29.32**	0.00000
Among populations within regions	4	0.96942	**8.39**	0.00000
Within populations	125	7.19764	**62.29**	0.00000

[a]Degrees of freedom.
[b]Significance of the variance components.

Table 5. Matrix of pairwise differences between populations (lower diagonal) with significance values (above diagonal), calculated from AMOVA derived Φ_{ST} values

	Jimna	Westcott	Burtons Well	Mt Lewis	Dandabah	Paradise
Jimna	–	0.000	0.000	0.000	0.000	0.000
Westcott	0.08201	–	0.000	0.000	0.000	0.000
Burtons Well	0.07770	0.16918	–	0.000	0.000	0.000
Mt Lewis	0.38840	0.43379	0.34846	–	0.000	0.000
Dandabah	0.10947	0.12827	0.12133	0.41227	–	0.000
Paradise	0.11061	0.12320	0.15651	0.43828	0.04657	–

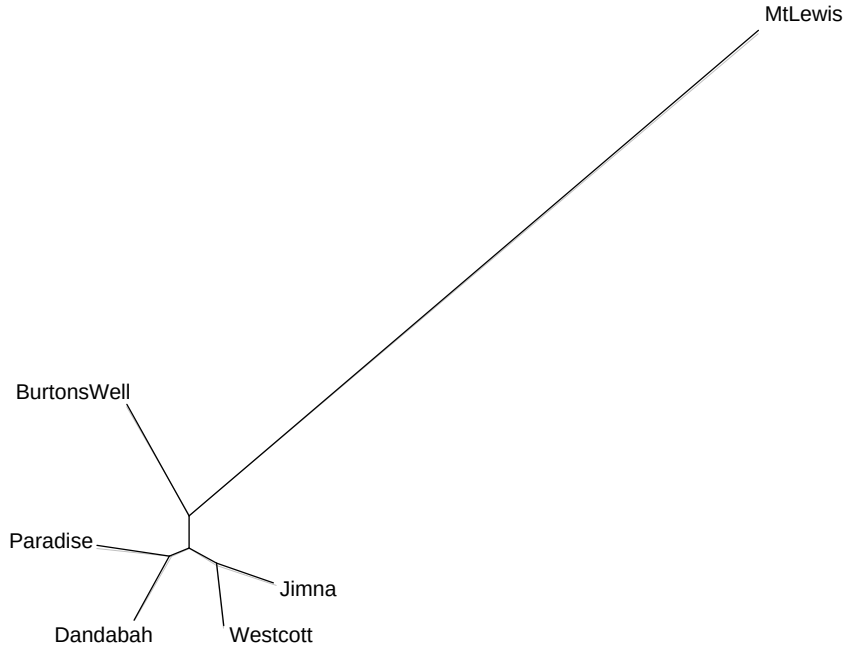

Figure 2. An unrooted UPGMA tree, derived from Nei's distance measure, showing the relative differentiation of Bunya populations. The long branch leading to the Mt Lewis population illustrates its substantial divergence from the remaining populations sampled.

negatively correlated with the degree of population differentiation (Nybom and Bartish 2000).

Genetic diversity in Bunyas

Genetic diversity in Bunyas, as revealed by Shannon's index, is comparatively high and in line with that reported in RAPD studies for other Southern conifers–*Fitzroya cupressoides*, $S = 0.54$ (Allnutt et al. 1999); *Araucaria araucana*, $S = 0.65$ (Bekessy et al. 2002). This result is in contrast to recent reports that genetic diversity within some members of the Australian Araucariaceae is generally low (Peakall et al. 2003). However, low genetic variation may not characterise the family as a whole, as Bekessy et al. (2002) also reported high variation in their study of the South American *Araucaria araucana*. The study by Peakall et al. (2003) used different methodologies in their comparisons of diversity between *Araucaria* and *Agathis* and *Wollemia nobilis*. Further molecular work on other members of the Araucariaceae may clarify this discrepancy.

Despite probable range reductions in the past, there is no evidence for loss of genetic variation, or genetic bottlenecks, in any of the populations examined. Such loss or bottlenecks would be reflected in lower diversity values in affected

populations. The mean value of "*S*" obtained for Bunyas (0.48) is only slightly lower than values reported for other southern conifers–*Araucaria araucana* 0.65 (Bekessy et al. 2002), *Fitzroya cupressoides* 0.54 (Allnutt et al. 1999), *Podocarpus salignus* – 0.69 (Allnutt et al. 2001), *Pilgerodendron uviferum* – 0.57 (Allnutt et al. 2003). There is also no significant difference in the variation found among populations, regardless of size or degree of isolation of the population (ANOVA, $P > 0.05$). This suggests that extant population size in Bunyas cannot predict the level of diversity it contains – smaller populations (< 100) may harbour equal amounts of genetic diversity as larger populations (> 1000).

The percentage of polymorphic markers was also high regardless of the population size or isolation. The Mt Lewis population recorded the lowest polymorphism and the Jimna population the highest, a result which is in agreement with estimates of genetic diversity derived from "*S*". Further comparisons across studies are not made here, due to the inherent subjectivity of primer choice to maximise polymorphic loci and the inclusion, in some studies, of monomorphic loci (see Results).

Small endemic populations may lose genetic variation by genetic drift (Frankham 1995). For conifers, the lagging effects of extremely long generation times and the indirect methodology (i.e. genetic methods) used in many studies may mean that the genetic effects of relatively recent size reductions are not yet detectable, particularly in Bunya populations. Populations that are marginal, small or geographically isolated (e.g. Mt Lewis, Burtons Well, Jimna/Conondale) are expected to be more susceptible to the effects of genetic drift due to lower effective population size and/or increased selection pressures. However gene flow may still be sufficient to negate these forces, at least among the southern populations. In any case the loss of genetic variation and its restoration are slow processes, made slower in this instance by extremely long generation times inherent in the Araucariaceae.

These results suggest that effective population size in Bunyas may be independent of actual census size. More likely however is the scenario that current population sizes are of insufficient age to allow a genetic equilibrium to be reached and therefore any reduction in genetic variability due to the effects of small contemporary population size remains undetectable at this stage. We can say that, historically, population size fluctuations do not appear to have affected the genetic diversity of any of these populations.

Regional differentiation in Bunyas

In a summary article of genetic structuring in plants, G_{ST} (analogous to Φ_{ST}) was found to be significantly correlated with breeding system, life cycle, stage of succession, and floral morphology (Loveless and Hamrick 1984). Outbreeding, wind pollination, late successional status and long life cycles are all expected to encourage gene flow and inhibit population differentiation (Hamrick and Godt 1996, Nybom and Bartish 2000). Gymnosperms are further expected to display low Φ_{ST} /G_{ST} values based on averages from previous studies (Nybom and Bartish 2000).

It is important, but rarely conducted in published population genetic studies, to test all permutations of hierarchical structure, as significant differentiation may be found in a number of differing structures. That which minimises among population within region variation and maximises regional differentiation is the best hypothesis for the variation observed.

Significant genetic structuring is predicted among refugia due to historically low levels of gene flow. From these results it is evident that the hypothesis supporting three separate refugia for the species (Webb and Tracey 1981) is not supported ($P = 0.16$). The hierarchical structure that bests explains the variation (Table 4) provides evidence for only two distinct refugia – northern and southern.

This strong genetic signal possibly relates to a major evolutionary change in the Australian flora. Increased aridity from the Pliocene onwards led to the contraction of rainforest environments along the eastern coast (Kershaw and Wagstaff 2001), and probably resulted in the contraction of Bunyas to regional refugia located approximately within the boundaries of their present rainforest distribution. Fire, associated with increased climatic oscillations during the Quaternary, or perhaps the onset of El-Niño-Southern Oscillation (ENSO), remains the most likely influence on extant population size and distribution. This is especially the case for the northern populations, as this region

reached tropical latitudes during the Quaternary for the first time in the history of *Araucaria* on the Australian continent (Kershaw and Wagstaff 2001). Increased climatic variability and burning during the late Holocene has presumably been responsible for the current reduction in size of the northern populations, as indicated by elevated charcoal deposits within the micro-fossil record (Hopkins et al. 1993). The slightly lower estimates of genetic diversity and polymorphism for the Mt Lewis population may reflect the initial genetic effects of these processes. However, in taxa with extremely long life spans any contemporary genetic effects are likely to be largely underestimated. These results further highlight the potential of members of the family Araucariaceae for paleoenvironmental reconstruction, as suggested by Kershaw and Wagstaff (2001).

Pairwise differences between populations indicate that all populations are significantly differentiated from each other (Table 5), emphasising the high degree of population structuring in Bunyas. Significant differentiation occurs between populations separated by as much as 1000 kms (Mt Lewis – Bunya Mts) or as little as 2 kms (within Bunya Mts). This is surprising for a wind-pollinated, long-lived conifer as all of these traits (breeding, system, longevity and taxonomic status – gymnosperm versus angiosperm) are expected to minimize differentiation among populations (Nybom and Bartish 2000). Northern conifers constitute the majority of population statistics relating to generalised "gymnosperm" values, so further research is also required on the enigmatic group of conifers now confined to southern latitudes, to ascertain why they are so inherently different with regard to population dynamics.

While distance alone can explain the differentiation of the Mt Lewis population, this is not the case for southern populations. Remarkably, the coastal population is more genetically alike to "core" Bunya Mts populations than some of these populations are to each other (Figure 1; Table 5). The occurrence of indigenous "festivals", concurrent with the Bunyas' masting events, raises the possibility of dispersal by human activity, at least within southern Queensland. Whether the detected genetic pattern represents historical connectivity or dispersal by humans cannot be ascertained from the present data. It seems likely from historical records that a more or less continuous Araucarian forest connected the coast to the Bunya Mts, over which a mixing of genotypes may be expected. Loss of intervening habitat and selective logging may simply have left *in situ* genotypes that are more closely related than present geographic isolation of these populations would suggest. In either case the potential for pollen-mediated gene flow would be expected to be greater among southern populations than between northern and southern populations, due to distance alone, which may explain this association.

When AMOVA analyses are restricted to southern populations only (data not shown), the values ($\Phi_{ST} = 11\%$, $G_{ST} = 12\%$) are in close agreement not only with each other but also with those shown from other southern conifers with similar pollination and dispersal mechanisms (*Araucaria araucana*, $\Phi_{ST} = 12\%$, Bekessy et al. 2002). This, together with the UPGMA figure, suggests that the Mt Lewis population is a distinct evolutionary lineage, comparable to an Evolutionary Significant Unit (ESU), (Moritz 2002).

The differentiation of the Mt Lewis population may be either due to drift, caused by small population size and low levels of historical gene flow, selection in absence of gene flow without significant loss of diversity, or simply reflect a different refugial origin. In either case, the Mt Lewis genotypes may represent a significant entity given models of global climate change that predict a warmer climate than at any time during the recent geological past (Huntley 1998), as this population represents an evolutionary lineage already exposed to higher temperatures. The presence of slight phenotypic divergence in leaf morphology (Pye pers. obs.) provides further support for this theory. Research is underway to assess current management strategies and taxonomic status of this distinct population.

The Mt Lewis population is currently protected within a Forestry Reserve within the Wet Tropics World Heritage Management Area. Current threats to the population include stochastic genetic effects associated with small population size, fire (which may ironically also promote colonisation), increased seed predation due to small population size (which lowers the effectiveness of the Bunya's strategy of mass seed production, which occurs approximately every three years), cyclones, pathogens (a significant threat identified for

Wollemia nobilis, Araucariaceae (Bullock et al. 2000)) and other stochastic environmental threats. Given the strong divergence detected here between northern and southern populations, an additional threat may be posed by outbreeding depression (Young et al. 1996). This could be brought about by pollen flow from the large number of Bunyas planted on the adjacent Atherton Tablelands over the last few centuries, which, based on leaf morphology, appear to be derived from the southern populations, as they possess pungent leaf tips; a characteristic confined to the southern populations of Bunyas. Genetic analysis of seedlings, by means of the aforementioned Mt Lewis population-specific markers, is currently being undertaken to assess this potential threat to the genetic integrity of the population. If the seedlings lack the markers associated with the Mt Lewis adult population, we can infer that inter-population pollination may be occurring, which may lead to the risks associated with outbreeding depression (Young et al. 1996).

Implications for conservation

Genetic and ecological studies complement each other with regard to the conservation of species (Sherwin and Moritz 2000), and as such ecological knowledge is required specifically for those populations of Bunyas that display the largest divergence (Mt Lewis, Burton's Well). Both these populations appear in ecotonal environments suggesting, in line with ecological theory, that selection may be stronger in these environments than in their ''core'' habitat. This divergence may also be a consequence of lower densities in these populations (see Table 1), as Gram and Sork (1999) found that populations with low densities may harbour a different suite of genotypes to denser populations. This is thought to be due to adaptive genetic variation and thus, Gram and Sork (1999) suggest a strategic conservation plan should aim to preserve a range of populations with differing densities in order to maintain adaptive genetic variation.

It is apparent from these results that both the northern and southern populations of Bunya are on independent evolutionary trajectories, which suggests that their management, and subsequent conservation, should be considered independently.

The Mt Lewis population is already subject to a different climatic regime to the southern populations; a regime which may be more similar to that expected under a global climate change scenario. Given the high degree of genetic divergence and the presence of population-specific markers, the conservation of this population may be imperative for the future of the species – acting as a genetic reservoir that may be already adapted to increased temperatures. However, as suggested by Petit et al. (1998), conservation attempts should encompass both genetic diversity and divergence, as both contribute to the total diversity of the species. *A. bidwillii* is not represented in extensive *ex situ* plantings and thus these populations have no buffer against stochastic events. Therefore, the preservation of the remaining genetic material of *A. bidwillii* should remain a priority if we are to adequately protect this economically, sociologically and biologically important southern conifer.

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