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Little Genetic Differentiation Within the Dominant Forest Tree, *Eucalyptus marginata* (Myrtaceae) of South-Western Australia

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Summary

Genetic diversity in the continuously distributed *Eucalyptus marginata* Donn ex Smith from south-western Australia was investigated using nuclear RFLP loci. Diversity was assessed in 15 populations from across the range of *E. marginata,* including the three morphologically recognised subspecies, and one population of the closely related *E. staeri.* Moderate levels of genetic diversity (H_T = 0.345) were detected in *Eucalyptus marginata* but there was little genetic structure and low differentiation between populations (θ = 0.034). Isolation by distance was observed, but there was differing influence of gene flow and drift over different spatial scales. There was no genetic support for the recognition of three subspecies, or the informal division of northern and southern forms recognised by foresters. Some populations showed a high fixation index most likely due to neighbourhood structure within populations. The lack of genetic structure and low population differentiation indicates that collection of germplasm for rehabilitation of forest sites following mining or *Phythophthora cinnamomi* infection, may be made at a regional scale.

Key words: Eucalyptus marginata, population differentiation, genetic diversity, gene flow.

Introduction

Eucalyptus marginata Donn ex Smith (jarrah) is a long-lived forest tree with a widespread distribution in the mesic areas of the south-west of Western Australia, where rainfall is greater than 600mm per annum (Dell and Havel, 1989). It occurs from 100km north of Perth to the south coast (*Fig. 1*), and outliers to the main distribution occur where there is increased water availability as runoff from large granite rocks, e.g. at Jilakin Rock, or by impeded drainage in the lower soil horizon, such as at Katanning and Mt. Lesueur (Churchill, 1968). The species probably had a much larger distribution during wetter climatic periods in the past, and if rainfall was increased by 75 mm per annum the main distribution could be extended to include all the present outliers (Churchill, 1968). *Eucalyptus marginata* lives for 300–400 years (Dell and Havel, 1989), and the ability to survive in the lignotuberous stage for up to 20 years (Van Noort, 1960) assists in survival through occasional summer droughts. It also has an extensive root system for survival in a nutrient poor environment, and has adventitious

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buds that can reshoot after drought or fire (DELL and HAVEL, 1989).

Eucalypt species can be morphologically diverse and there are many species where morphological variation is not completely defined and in these cases taxa are usually recognised as subspecies. There is some morphological variation within *Eucalyptus marginata* and three subspecies have been recognised (BROOKER and HOPPER, 1993). *Eucalyptus marginata* ssp. *marginata* occurs through the majority of the distribution and is typically a tall, upright tree with dark green leaves. *Eucalyptus marginata* ssp. *thalassica* has a weeping habit with blue/green leaves and occurs in the northern part of the distribution in the Darling Range. *Eucalyptus marginata* ssp. *elegantella* is a small, compact tree with small, narrow, olive/green leaves, that is found on the Swan coastal plain, often on granitic clays (*Figure 1*). Foresters also recognise an informal distinction between the northern and southern forests (*Figure 1*), on the basis that the southern trees have thicker, dark green leaves and a more upright habit. However, morphological difference is not always a good indicator of genetic differentiation at lower taxonomic levels and the degree of morphological and genetic difference between taxa is not always congruent. Investigations of genetic relationships between subspecies in two eucalypt species with widespread distributions in Western Australia, *E. kochii* and *E. loxophleba,* have shown little genetic differentiation between the taxa (BYRNE, 1999; HINES and BYRNE, 2001). In contrast, assessment of genetic relationships in a species complex with restricted distribution showed differentiation between two subspecies that was more consistent with species rank for these taxa (ELLIOT and BYRNE, 2004).

Eucalyptus marginata is harvested for its durable, fine-grained timber. Management after harvesting is generally through natural regeneration, although some seeding may be undertaken. Bauxite mining is also carried out in the forest and mining sites are rehabilitated using seed sources. *Eucalyptus marginata* is susceptible to the root pathogen *Phytophthora cinnamomi*, which affects many species in the forests of the south west of Western Australia. Resistance to the pathogen has been observed in *E. marginata* and a genetic basis to the resistance has been identified (MCCOMB *et al.,* 1994; STUKELY and CRANE, 1994). Clonal lines with *P. cinnamomi* resistance have been selected and used to establish seed orchards to provide a source of resistant seed. This seed will be used for regeneration of jarrah forest in areas that have been badly affected by the pathogen, and in rehabilitation of sites following bauxite mining.

Sustainable management of forest ecosystems is informed by knowledge of the level and structuring of genetic diversity within species. Breeding and selection of *P. cinnamomi* resistant clones of *Eucalyptus marginata* aims to capture a broad base of genetic diversity to ensure that they are representative of the species. Deployment of seed in rehabilitation and revegetation also needs to account for patterns of genetic diversity within the species. This study investigated the level and structuring of genetic diversity within the nuclear genome of *E. marginata* in order to provide a basis for the informed breeding and deployment of genetically improved seed. In addition the genetic relationships and degree of differentiation between the three subspecies of *E. marginata* were assessed.

Materials and Methods

Plant material

Fifteen natural populations were selected from throughout the range of *E. marginata*, including two outlying populations (*Figure 1*). A population of a closely related species, *E. staeri* (Maiden) Kessell & C. A. Gardner, was also included from the Mount Frankland locality. Leaf samples from 10 individuals from each population were collected. Individuals selected were usually regrowth from earlier logging, thus most were old trees, with the minimum requirement being a diameter at chest height of 30 cm. The DNA was extracted as outlined in BYRNE *et al.,* (1998), with the addition of sodium sulfite in the extraction buffer (BYRNE *et al.,* 2001). Samples were then digested with two restriction enzymes, *Bgl*II and *Eco*RV, Southern blotted, and hybridised with 30 RFLP probes that have been selected from the eucalypt genetic linkage map (c092, c113, c115, c116, c135, c136, c170, c238, c299, c333, c395, c411, c451, g059, g067, g086, g095, g099, g142, g154, g174, g183, g195, g233, g243, g250, g256, g261, g425, g474) (BYRNE *et al.,* 1995). Restriction digestion and hybridisation were conducted according to BYRNE and MORAN (1994), and the probe plasmids were amplified and labelled with ^{32}P by the random priming method.

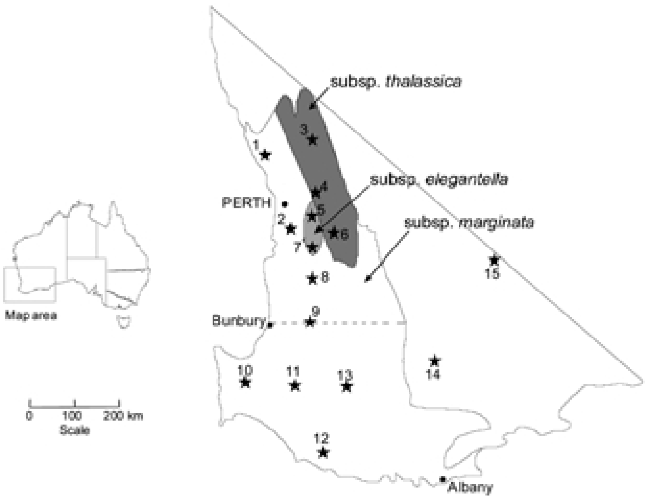

Figure 1. – The distribution of *Eucalyptus marginata* in south-western Australia showing the collection locations and distribution of subspecies. Numbers correspond to populations listed in *Table 1.* Dotted line indicates separation of northern and southern forest areas.

Data Analysis

The banding patterns were interpreted as loci according to a Mendelian multi-allelic model. Allelic diversity parameters were calculated using GDA (LEWIS and ZAYKIN, 2001) and gene diversity parameters according to NEI (1973) were computed using FSTAT (GOUDET, 2001). Inbreeding and genetic differentiation measures were calculated according to WEIR and COCKERHAM's (1984) estimates, which are unbiased for small sample sizes, using FSTAT (GOUDET, 2001). Confidence intervals were estimated by bootstrapping over all loci 1000 times. A hierarchical cluster analysis was carried out using UPGMA based on unbiased genetic distances (NEI, 1978) using PHYLIP (FELSENSTEIN, 1993) with the significance of nodes assessed by bootstrapping with 100 replications. A phylogenetic analysis was undertaken using the continuous character maximum likelihood (contml) program in PHYLIP (FELSENSTEIN, 1993) and a consensus tree following bootstrapping over loci 100 times was produced. Isolation by distance was assessed by a regression of population differentiation against geographical distance and tested for significance using a Mantel randomisation test (MANTEL, 1967). The residuals of the regression were also tested for significant association with geographic distance.

Results

RFLP analysis of genetic diversity in *E. marginata* produced interpretable fragment patterns that were scored using an allele/locus model for 26 out of the 30 probes. The majority of loci were polymorphic, but four loci were monomorphic across all populations. Within single populations up to ten loci were monomorphic. The number of alleles detected at single loci ranged from one to 16, with the maximum number of alleles in any one population sample being 10. The distribution of alleles showed similar proportions of common and rare alleles. The proportion of rare alleles (frequency < 0.1) was similar between populations, ranging from 26.5 to 38.3%, except for the Jilakin Rock population that had a lower proportion of rare alleles (15%). The proportion of common alleles (frequency > 0.5) ranged from 25.9% to 32.4%, except for the Jilakin Rock population where the frequency was slightly higher (38.3%). All populations except Jarrahdale and Welshpool Road contained unique alleles, and the Nannup population contained the most unique alleles (5.9%). All unique alleles were also rare alleles.

Allelic diversity measures were moderate in *E. marginata* (mean A = 2.7, mean P = 67.1%; *Table 1*). The values for the mean number of alleles per locus (*A*), and the mean number of polymorphic loci (*P*) were similar across populations with the highest diversity occurring at Nannup, and the lowest at the outlier, Jilakin Rock. Observed heterozygosity (mean H_o = 0.298), and Hardy-Weinberg expected panmictic heterozygosity (mean H_e = 0.332) were also moderate and generally similar across populations (*Table 1*). The inbreeding co-efficient (F_{IS}) was variable across populations. In some populations, such as Yanchep and Dwellingup, it was not significantly different from zero, while in others, such as Katanning and Mundaring State Forest, the inbreeding co-efficient was high and the standard errors indicated they were significantly different from zero (*Table 1*). In populations that had high inbreeding co-efficient (F_{IS} > 0.1) the F_{IS} values were heterogenous across loci, with high values occurring in 22–48% of loci per population, except for the small outlying population of Katanning in which 65% of loci had F_{IS} values greater than 0.15.

Table 1. – Descriptive genetic diversity parameters for *Eucalyptus marginata*. *P*, mean number of polymorphic loci (0.99 criterion); *A*, number of alleles per locus; H_e, expected heterozygosity; H_o, observed heterozygosity; F_{IS}, Wright's inbreeding co-efficient. Standard errors in parentheses.

Population	A	P	H_e	H_o	F_{IS}
1. Yanchep	2.6 (0.3)	68	0.337 (0.055)	0.339 (0.062)	-0.008 (0.054)
2. Beeliar	3.0 (0.4)	68	0.328 (0.056)	0.289 (0.050)	0.124 (0.032)
3. Julimar Forest	2.6 (0.3)	68	0.330 (0.057)	0.293 (0.056)	0.117 (0.042)
4. Mundaring State Forest	2.4 (0.3)	64	0.319 (0.057)	0.257 (0.052)	0.203 (0.062)
5. Welshpool Road	2.6 (0.3)	72	0.341 (0.057)	0.321 (0.057)	0.062 (0.037)
6. Jarradale	2.9 (0.3)	75	0.341 (0.051)	0.293 (0.047)	0.148 (0.042)
7. North Dandalup	2.9 (0.3)	68	0.347 (0.055)	0.321 (0.059)	0.078 (0.038)
8. Dwellingup	2.8 (0.3)	68	0.339 (0.057)	0.326 (0.058)	0.041 (0.031)
9. Collie	2.8 (0.3)	64	0.331 (0.057)	0.321 (0.059)	0.032 (0.028)
10. Nannup	3.0 (0.3)	75	0.352 (0.056)	0.293 (0.048)	0.175 (0.033)
11. Bridgetown	2.9 (0.3)	64	0.348 (0.058)	0.335 (0.062)	0.039 (0.033)
12. Mount Frankland	2.8 (0.4)	64	0.327 (0.063)	0.294 (0.059)	0.106 (0.033)
13. Perup Nature Reserve	2.9 (0.4)	68	0.329 (0.056)	0.282 (0.053)	0.149 (0.037)
14. Katanning	2.6 (0.3)	61	0.318 (0.058)	0.254 (0.049)	0.211 (0.047)
15. Jilakin Rock	2.2 (0.2)	61	0.291 (0.053)	0.258 (0.050)	0.121 (0.045)
Mean	2.7 (0.06)	67	0.332 (0.004)	0.298 (0.007)	0.106 (0.016)

Table 2. – Genetic diversity parameters for *Eucalyptus marginata* and six other eucalypt species assayed using nuclear RFLP analysis. *F*, overall inbreeding co-efficient; *θ*, co-ancestry co-efficient; *f*, degree of inbreeding within populations; H_T, total heterozygosity; G_{ST}, percentage of genetic diversity maintained between populations. 95% confidence intervals in parentheses.

Species	*F*	*θ*	*f*	H_T	G_{ST}(%)
E. marginata	0.137	0.034	0.107	0.345	3.1
	(0.081, 0.196)	(0.024, 0.043)	(0.046, 0.172)		
E. kochii[1a]		0.045	0.044	0.514	2.2
E. loxophleba[2a]		0.089	0.046	0.418	3.8
E. nitens[3a]			0.044	0.445	16.2
E. camaldulensis[4]	0.095	0.078	0.019	0.53	
	(0.068, 0.123)	(0.060, 0.100)	(-0.002, 0.038)		
E. angustissima	0.235	0.136	0.114	0.371	10.6
ssp *angustissima*[5]	(0.151, 0.315)	(0.089, 0.184)	(0.039, 0.192)		
E. occidentalis[6]	0.088	0.065	0.025	0.373	5.9
	(0.045, 0.132)	(0.050, 0.081)	(-0.014, 0.067)		

1. BYRNE (1999), 2. HINES and BYRNE (2001), 3. BYRNE *et al.* (1998), 4. BUTCHER *et al.* (2002), 5. ELLIOT and BYRNE (2004), 6. ELLIOT and BYRNE (2003).
a. $\theta = F_{ST}$, $f = F_{IS}$.

Total gene diversity at the species level was also moderate (H_T = 0.345) and the majority of this diversity was maintained within populations (Gst = 3.1%). The level of differentiation between populations was also low (θ = 0.034), but was significantly different from zero. An analysis of population structure using an UPGMA showed strong support (bootstrap value 100%) for the distinction of *E. marginata* from its closest relative, *E. staeri* (D = 0.16, *Figure 2*). There was little structuring within *E. marginata* and no separation of populations from the northern and southern forest regions. For each of the subspecies *elegantella* and *thalassica*, the populations were clustered together, but neither subspecies was distinct from the main subspecies *marginata* (*Figure 2*). The genetic distance between populations of the *elegantella* and *thalassica* subspecies and the remaining populations was low (D < 0.035). All nodes within the *E. marginata* clade had low bootstrap support. The phylogenetic analysis (*Figure 3*) also showed little structuring within *E. marginata* with weak bootstrap support for all nodes. Again the *elegantella* subspecies and some of the *thalassica* subspecies populations clustered together.

Regression analysis (*Figure 4*) showed a significant correlation between population differentiation (θ) and geographic distance as assessed by a Mantel Test (r = 0.568, g = 6.42, p = 0.01). Regression analysis of the residuals against geographical distance was not significant (r = –0.071, g = –0.0315, p = 0.05).

Discussion

In comparison with other eucalypt species where diversity has been assessed using RFLP loci, *E. marginata* showed moderate levels of total genetic diversity. It had less total genetic diversity than comparable continuously distributed species such as *E. loxophleba* (HINES and BYRNE, 2001) and *E. kochii* (BYRNE, 1999), but the level of diversity was similar to species with localised, more discontinuous population systems such as

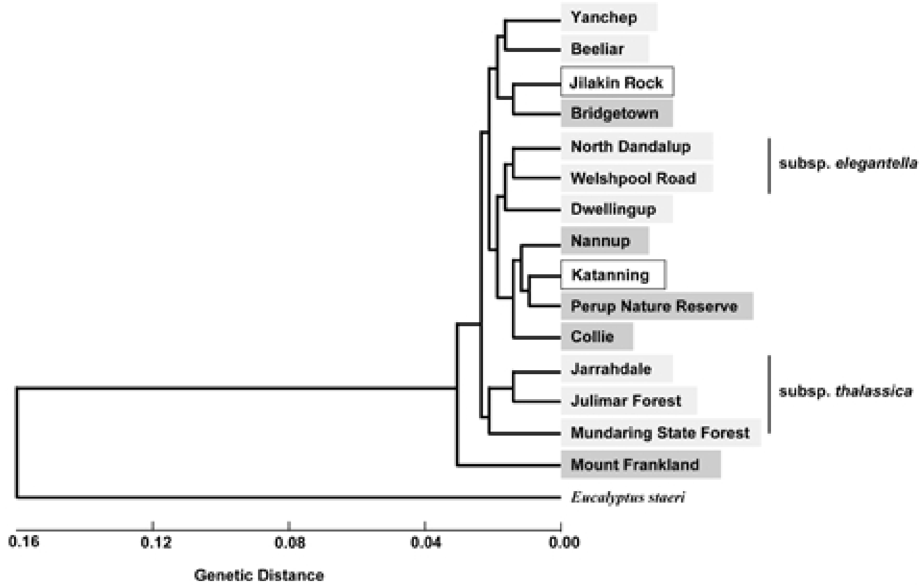

Figure 2. – UPGMA phenogram of populations of *Eucalyptus marginata*.
▩ Denotes Southern populations, ░ denotes Northern populations, ☐ denotes outlying populations.

E. occidentalis (ELLIOT and BYRNE, 2003) and *E. angustissima*, (ELLIOT and BYRNE, 2004). The higher levels of diversity in the other continuously distributed species appear to be due to high levels of rare alleles, and the allele frequency distribution in these species is skewed towards rare alleles. In contrast, in *E. marginata* allele frequencies had a U-shaped distribution, with the frequency of rare alleles being roughly equivalent to the frequency of common alleles. This pattern was also found in a species with similar moderate levels of genetic diversity, *E. occidentalis* (ELLIOT and BYRNE, 2003), and is characteristic of allele frequency distributions commonly found in allozyme studies (CHAKRABORTY *et al.,* 1980) where diversity is generally lower than that found using RFLP loci.

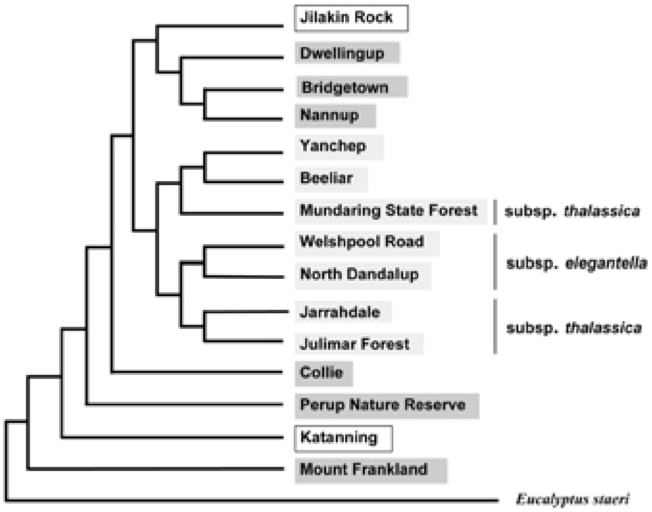

Figure 3. – Phylogenetic tree of populations of *Eucalyptus marginata* using continuous character maximum likelihood method.
▩ Denotes southern populations, ░ denotes northern populations, ☐ denotes outliers.

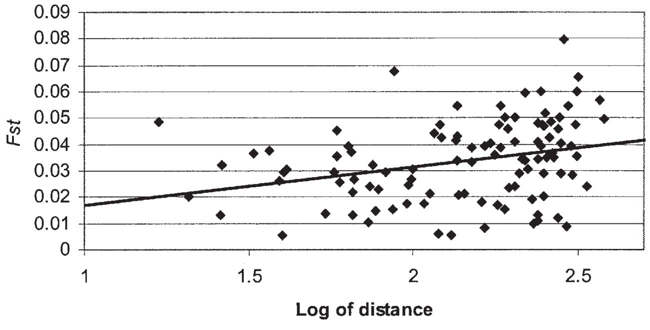

Figure 4. – The relationship between population differentiation (F_{ST}) and geographic distance (log transformed) for populations of *Eucalyptus marginata.*

Within *E. marginata* the level of genetic diversity was similar between populations, except for the outlying population at Jilakin Rock, that showed lower (although not significant) diversity than the other populations. The Jilakin Rock population also had fewer rare alleles compared to all other populations. Across the species the level of population differentiation was low, although it was significantly different from zero, and the majority of the diversity was maintained within populations with only 3.1% between populations. Although differentiation between populations was low, there was a significant correlation between population differentiation and geographic distance indicating isolation by distance. However, there was no correlation between the residuals and geographic distance. This indicates that *E. marginata* has had stable conditions for long enough for isolation by distance to develop but not long enough for an equilibrium between gene flow and drift to become established (HUTCHISON and TEMPLETON, 1999).

The low differentiation between populations of *E. marginata* was also reflected in the low genetic distance across the species. The phenetic analysis clearly distinguished *E. marginata* from its' closest relative, *E. staeri*, but showed little genetic structuring within *E. marginata* itself. The populations of the two restricted subspecies (*thalassica* and *elegantella*) were most closely related to each other than to any other population, but neither of the subspecies showed any separation from subspecies *marginata*. Similar lack of distinction between subspecies was found in studies of *E. kochii* (BYRNE, 1999) and *E. loxophleba* (HINES and BYRNE, 2001). There was no clear separation between the northern and southern forest regions, and the two outliers, Jilakin Rock and Katanning, showed no differentiation from populations in the main distribution of *E. mar-*

ginata. This lack of differentiation was also seen in the phylogenetic analysis.

High genetic similarity within species is unusual in the flora of south-western Australia. Many studies of genetic diversity in species have shown significant genetic structuring between populations (COATES, 2000; BYRNE *et al.,* 1999; BROADHURST and COATES, 2002; BYRNE *et al.,* 2003). Most of these species have distributions through the transitional rainfall zone between the high rainfall mesic environment in the extreme south-west corner and the dry arid environment in the north and east, whereas *E. marginata* occurs in the mesic region. Lack of population structuring and low level of population differentiation could be a result of recent range expansion, or could be due to high levels of gene flow throughout the distribution. The low level of differentiation in *E. marginata* is not due to recent range expansion since isolation by distance would not occur in a species with recent range expansion (SLATKIN, 1993; SCHAAL and OLSEN, 2000). Isolation by distance arises through long periods of environmental stability that enable a balance between drift and gene flow to become established (HUTCHINSON and TEMPLETON, 1999). The south-west of Australia is an ancient subdued landscape that has not been subject to geological activity or glaciation (HOPPER, 1979). While there has been periods of climatic instability in the transitional rainfall zone due to cyclic expansion and contraction of the mesic and arid regions, the high rainfall area has remained relatively climatically stable. This environmental stability would allow isolation by distance to develop within the continuous population system of *E. marginata*. While a significant correlation between genetic distance and geographic distance is evidence of isolation by distance through structured patterns of gene flow, the wide scatter around the regression line at greater distances indicates the influence of drift. Applying the theoretical model of HUTCHINSON and TEMPLETON (1999) the pattern of correlation in *E. marginata* reflects differing roles of gene flow and drift over differing spatial scales, with gene flow having greater influence over shorter distances and drift more influential at greater distances. This suggests that the time frame of climatic stability has not been long enough for an equilibrium between drift and gene flow to become established across the whole distribution (HUTCHINSON and TEMPLETON, 1999), probably due to the influence of climatic cycles in the longer term. Equilibrium between drift and gene flow was identified in the arid region in the sandalwood species *Santalum spicatum* (BYRNE *et al.,* 2003), as might be expected since the arid region has been subject to longer periods of environmental stability than the mesic region.

Outlying populations that are isolated from the main range of a species distribution can occur through long distance gene flow, or through range contraction leaving behind remnant populations. The range of *E. marginata* was likely to have been greater prior to the onset of aridity at the end of the Pleistocene, and rainfall would only have to be 75 mm higher for all of the current outlying populations to be included in the main distribution (CHURCHILL, 1968). The similarity of the outlying populations to those from the main range suggests that contraction of the range of *E. marginata* to its current distribution has occurred relatively recently without sufficient time for genetic differentiation of the outliers to develop. Range contraction since the end of the Pleistocene would not allow sufficient time for differentiation to develop in long lived trees since SLATKIN and BARTON (1989) estimate that it could take 500 generations for differentiation to reflect cessation in gene flow. Although the outlying populations were not divergent from the other populations, the furthest population at Jilakin Rock did show some evidence of the influence of drift, or possibly a bottleneck effect, as it had lower genetic diversity and fewer rare alleles than other populations.

Provenance trials have been conducted in *E. marginata* and seven-year-old seedlings have been assessed for height, diameter at breast height, form, mortality, health, vigour, insect damage, and fruit and flower status (ALCOA AUSTRALIA LTD., unpublished data). The trial found little difference in performance between populations from the main range of the distribution, although the populations from the coastal plain showed poor performance for most traits. There was a trend for families from southern provenances to have lower survival suggesting some adaptation at a regional scale. However, these differences were not consistent and there was variation in survival across families from both northern and southern provenances. Outlying populations from the eastern edge of the distribution did not show significant differences in performance, but a northern outlier (Mt. Lesueur) showed poorer performance than other provenances. The similar performance of provenances from the main range and the outlier provenances is consistent with the low differentiation and high levels of gene flow identified in this study.

Wright's fixation index was significantly higher in *E. marginata* than in comparable eucalypt species, even those with small isolated populations (ELLIOT and BYRNE, 2003, 2004). Eucalypts have a mixed mating system and generally show high levels of outcrossing. The mating system of *E. marginata* has been investigated and the mean outcrossing rate was 81% (MILLAR *et al.,* 2000). It is unlikely that the high fixation indices detected in many of the *E. marginata* populations sampled here are indicative of high levels of inbreeding since in most of these populations the F_{IS} values were heterogenous across loci. However, the high fixation index in the Katanning population is likely to be due to inbreeding since the majority of loci in this population showed high F_{IS} values, and it is a small outlier population where inbreeding would be expected. Species with continuously distributed population systems often show some subpopulation structure where mating between individuals within the same subpopulation is more likely than mating between individuals from different subpopulations (SLATKIN, 1985). Although the level of outcrossing was high, the mating system study did detect significant correlated paternity and biparental inbreeding, indicating non-random pollination and/or neighbourhood structuring within populations (MILLAR *et al.,* 2000). *Eucalyptus marginata* tends to have sporadic flowering and variation in pollen viability between trees within populations (WHEELER, 2003), both of which will lead to non-random pollination within populations. The excess of homozygotes detected in many populations in this study is also consistent with non-random pollination and neighbourhood structuring within populations. However, gene flow between populations appears sufficient to balance the effects of drift and prevent differentiation of populations.

This study has identified moderate levels of genetic diversity and low levels of differentiation within *E. marginata*. It does not support the division of *E. marginata* into the three subspecies, or the informal north/south separation recognised by foresters. The low differentiation implies that germplasm for regeneration and mine-site rehabilitation can be selected from a wide range within the main distribution, thus maintaining genetic diversity in the rehabilitated areas. However, some isolation by distance does occur and some degree of adaptation expressed in differential survival between northern and southern forest regions has been observed. Therefore seed collection zones should be at a regional scale rather than species wide.

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Source Variation in *Albizia chinensis* (Osbeck) Mer.: Seed and Seedling Characteristics

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Summary

Effect of source (altitude as well as geographic) variation in seed morphological characteristics, germination percentage and various seedling traits among and within 13 seed sources of *Albizia chinensis* were examined. The middle elevation sources showed consistent variation in different seed traits. Manan and Palampur populations had biggest and heaviest seeds among all the sources. Various seedling traits differed significantly among the sources. Higher shoot length was recorded in Augastyamuni and Tuneta (lower altitudinal populations) sources. Significant differences were also found between and among various sources for all the root-shoot attributes. Root length was observed greater in lower and middle altitude sources (Moolgarh and Tallimari) but root biomass was found higher in high altitude sources (Kundhla and Palampur). Significant positive correlations were found

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