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Genetic Diversity of an Australian *Santalum album* Collection – Implications For Tree Improvement Potential

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Abstract

The Forest Products Commission of Western Australia manages a sandalwood (*Santalum* spp.) core germplasm collection at Kununurra in the states far north. This collection serves as a significant seed source for sandalwood plantations in the area and remains an important resource for ongoing research. The collection contains *S. album* trees sourced from Indian arboreta, along with a few trees from West Timor, Indonesia. Also present are representatives of *S. macgregorii* from Papua New Guinea and *S. austrocaledonicum* from Vanuatu and/or New Caledonia. Despite the apparently diverse seed origins, the genetic background of many of the accessions remains vague. In this study, diversity and relatedness was assessed by nuclear and chloroplast RFLPs and a phylogeny was inferred. Nuclear RFLPs revealed very low levels of genetic diversity for a tree species, with an observed and expected heterozygosity (H_o and H_e) of 0.047. Nineteen genotypes were identified within the 233 *S. album* individuals sampled, with only one tree known to have originated from Timor being differentiated from Indian material. Other trees thought to have come from Timor grouped with those believed to be from India, indicating they were either incorrectly labelled or sourced from heavily modified populations. Despite the poor sample size, chloroplast RFLP analysis revealed no genetic distinction between the Timorese and Indian *S. album*, which supports the theory of human mediated seed dispersal from Timor to India. The structure of the phylogeny and associated relatedness has assisted in the establishment of seed orchards, designed to ensure maximum diversity is maintained through limiting the proximity of highly related trees. Finally, in light of these and other findings, a hypothesis concerning the evolution of *S. album* is proposed.

Key words: Sandalwood, *Santalum*, genetic diversity, phylogeny, evolution.

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Introduction

Indian sandalwood, *S. album* (Santalaceae) refers to a small root-parasitic tree renowned for its fragrant heartwood. The ground wood may contain as much as 9% essential oil (JONES et al., 2006) and is used in incense and joss stick manufacture, while the oil is used as a fix-

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ative in many high-end perfumes. *Santalum album* has been widely cultivated throughout much of south-east Asia for centuries. It is reportedly native to the Banda Sea islands of Flores, Sumba and Timor (McKinnell, 1990) and occurs widely throughout India (Rai, 1990). A small stand of *S. album* also grows on the north coast of the Northern Territory (NT) of Australia (McKinnell, 1990) although its origins are uncertain. Because of a long history of human interference, the precise evolutionary origin of S. album remains obscure (Rai, 1990). The biogeographic origins of the Pacific and eastern Australian *Santalum* species have been discussed in a recent phylogenetic study (Harbaugh and Baldwin, 2007). The authors conclude that Polynesian species of *Santalum* were the result of long-range dispersal events out of eastern Australia, and that the centre of origin for the species is most likely Australian. Unfortunately no Indonesian *S. album* was included in this study, so sound conclusions on the origins of the species could not be made. While the precise origin of *S. album* remains unclear, it seems highly unlikely that *S. album* evolved on the Indian continent in isolation from the other closely related members of the genus (Harbaugh and Baldwin, 2007).

The high value and relative scarcity of sandalwood has lead to sustained pressure on natural stands in India and Timor, threatening their survival. In an effort to reduce wild-harvest demand and develop a local industry, commercial plantations have been established in Australia in far northern Western Australia, the Northern Territory and Queensland. The Forest Products Commission of Western Australia (FPC) manages field trials of sandalwood in the Ord River Irrigation Area, Kununurra, WA. The collection is mostly *S. album* growing on a wide variety of host species, but specimens of *S. austrocaledonicum* (New Caledonia and Vanuatu) and *S. macgregorii* (Papua New Guinea) are also present in some trials. *Santalum album* growing in the FPC Kununurra collection originates mostly from families and populations across India, with a few accessions reported to have originated from Timor and the NT. Since the collection is the result of several efforts over many years, the precise origin of many accessions remains uncertain. Effective seed orchard design, breeding and selection for commercially important traits requires a good knowledge of the genetic diversity of the core collection. This study sought to address these issues using an appropriate molecular marker.

DNA based molecular markers are an effective means of determining genetic relatedness and provide estimates of genetic diversity (Parker et al., 1998). DNA mutations in the nucleus occur relatively frequently compared to chloroplast or mitochondrial DNA, and are subject to recombination during meiosis. This makes nuclear DNA markers, particularly RFLPs and microsatellites or short sequence repeats (SSRs) valuable tools for studying mating systems and population structure, whereas chloroplast DNA is best suited to studying ancient lineages (Newton et al., 1999). At the time of commencing this study, RFLPs were the only nuclear DNA markers available, although SSR primers have since been developed for *S. austrocaledonicum* (Bottin et al., 2005a) but have proved to have limited transferability to *S. album* (C. Jones, unpublished data). As the collection contains *S. album* accessions from several geographical regions, chloroplast RFLPs were employed to identify ancestral lineages and determine whether Indonesian *S. album* is genetically distinct from Indian material. These results are discussed in light of what is known on the biogeography of the region throughout late Neogene period.

Materials and Methods

Collection of plant material

Santalum album was sampled in spring 2004 when leaf samples were taken from 233 *S. album* trees within the FPC Kununurra collection. The vast majority of the sampled trees were derived from an Indian core collection, while seven of the sampled trees were grown from seed which is reported to have originated from Timor. One of the sampled *S. album* trees was from seed collected from the NT. As the arboretum was established with a primary interest in *S. album*, germplasm from Timor or the NT was particularly limited. Five *S. austrocaledonicum* trees (New Caledonia and/or Vanuatu, labelled Sau42 to Sau46) and 21 *S. macgregorii* (Papua New Guinea) were also included in the study for comparison. Five individuals of *S. spicatum* (south-western Western Australia) were used as a distant out-group since this species is the most ancestral of the genus (Harbaugh and Baldwin, 2007). These trees were from a collections included in the study by Byrne et al. (2003b) and were sampled from the northern populations of Cue (Cu) and Wiluna (W), as well as the southern populations of Nyabing (Ny), Hyden (Hy) and Dumbleyung (D).

RFLP analysis

DNA was extracted from leaf material using a CTAB extraction method as described previously (Byrne et al., 2003b) with sodium sulphate (0.1%) added to the extraction buffers to prevent DNA degradation. Variation in DNA was assayed using RFLP markers developed for *S. spicatum* (Byrne et al., 2003b). DNA was digested with *Bgl*II and *Eco*RV, subjected to 0.8% agarose gel electrophoresis and transferred to membranes by Southern blotting (Southern, 1975). Hybridisation was carried out with 21 RFLP probes (s3B, s9B, s38B, s41E, s43E, s44B, s47E, s51E, s57B, s59B, s62E, s64B, s66E, s75B, s77E, s80E, s83E s84B, s90B, s93E and s95E) which were derived from a genomic library of *S. spicatum* (Byrne et al., 2003b). The probes were hybridised to each filter and visualised using ^{32}P dCTP and autoradiography. B or E annotation indicates which enzyme (*Bgl*II or *Eco*RV) was used in combination with each probe.

Chloroplast DNA analysis of Santalum spp.

Genetic differences between Indian, Timorese and the NT *S. album*, as well as phylogenetic relationships between *S. macgregorii*, *S. austrocaledonicum* and *S. spicatum* were assessed using chloroplast probes from tobacco (Shinozaki et al., 1986; Sugiura et al., 1986) and *Petunia hybrida* (Systma and Gottlieb, 1986). Genomic

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DNA was digested with three more restriction enzymes (*Bcl*I, *Dra* and *Xba*) and Southern blotted using the method described above. These filters were combined with the filters previously made using *Bgl*II and *Eco*RV, and hybridised with labelled (^{32}P dCTP) chloroplast probes; p1, p4, p3, p6, p8, p10 from *Petunia* and PtBa1 from tobacco as per BYRNE et al. (2003a), however probe PtBa1 was only used in combination with filters using *Eco*RV and *Bgl*II. Autoradiographs were exposed for up to a week at –80 °C depending on the intensity of the signal.

Statistical analyses

Nuclear RFLP banding patterns were interpreted based on the Mendelian multi-allelic model. *Santalum macgregorii* consistently showed four bands for most nuclear probes trialled, indicating it was tetraploid. Owing to the difficulty in analysing a combination of diploids and tetraploids, *S. macgregorii* was not included in the nuclear RFLP analysis. Genetic diversity parameters, H_e and H_o, and genetic distance matrices were determined using GenAlEx version 6 (PEAKALL and SMOUSE, 2006). No further parameters could be determined as the trees did not represent true populations. Phylogenetic relationships were inferred using the Phylip package (FELSENSTIEN, 1989). A phylogenetic tree based on Euclidean distance was constructed using NEIGHBOUR and unweighted paired genetic mean analysis (UPGMA). Chloroplast RFLP data were scored for absence or presence of specific mutations. Haplotypes were determined based on these mutations and a phylogenetic tree was inferred from the haplotype data using PAUP v4.0. Branch support strength was determined by bootstrapping 10000 times.

Results

Nuclear RFLP analysis

Of the 21 nuclear probes trialled, 20 were able to be scored as co-dominant markers using classic Mendelian principles. Probe s83E was not scored as it revealed multiple bands per lane, indicative of multiple loci. Only three loci were polymorphic in the entire *S. album* collection; these yielded two (s59B and s38B) or three (s95E) alleles per locus. *Santalum austrocaledonicum* was considerably more polymorphic, with 7 loci being variable across the 5 individuals studied. *Santalum spicatum* was the most variable of the species, with variation detected across all probes. *Santalum macgregorii* was not included in the nuclear study due to tetraploid banding patterns, however considerable diversity of alleles was seen across the 15 specimens.

Variation within the *S. album* collection was very low, with heterozygosity being well below all other previous findings for the genus (*Table 1*). Only 19 different genotypes were present in the 233 individuals sampled. In comparison, the five individuals sampled from *S. austrocaledonicum* and *S. spicatum* were all represented by different genotypes. Analysis of genetic relationships by UPGMA showed genotypes clustering into groups representing the three species (*Fig. 1*). Some of the sampled trees reportedly from Timor were not resolved from the rest of the Indian material, yet were represented by genotypes 4 and 5. Of the seven reported Timorese trees only one, represented by genotype 19, was clearly distinguished from the group. Few *S. album* trees possessed rare alleles. Genotypes 18 and 19 were differentiated from the other *S. album* trees by two rare alleles at loci s90B and s64B. Genotype 19 is a tree known to have been grown from seed collected in Timor, while genotype 18 is a younger tree grown from seed collected on site at Kununurra. No record of its parent exists. It is likely that genotype 18 is progeny of 19, however several more polymorphic loci would be needed for reliable parental testing.

Chloroplast RFLP analysis

Analysis of the banding patterns revealed 38 site and length mutations in the chloroplast genome of the four species used in this study (*Table 2*). These mutations were associated with three chloroplast haplotypes in the 233 *S. album* individuals. The majority of *S. album* pos-

Table 1. – Mean heterozygosity statistics of *Santalum* spp. from the Kununurra arboretum compared with those of natural populations.

Species	n	Method	H_o	H_e	Reference
S. album (arboretum)	233	RFLP	0.047	0.047	This study
S. austrocaledonicum (arboretum)	5	RFLP	0.140	0.137	This study
S. spicatum (selected individuals)	5	RFLP	0.30	0.21	This study
S. album (natural)	100	Isozyme	0.126[a]	0.08[a]	(SUMA and BALASUNDARAN 2003)
S. austrocaledonicum (natural)	431	SSR	0.45	0.66	(BOTTIN, et al. 2005b)
S. spicatum (23 natural pops.)	100	RFLP	0.201	0.210	(BYRNE, et al. 2003b)

a = arithmetic mean of 5 population values.

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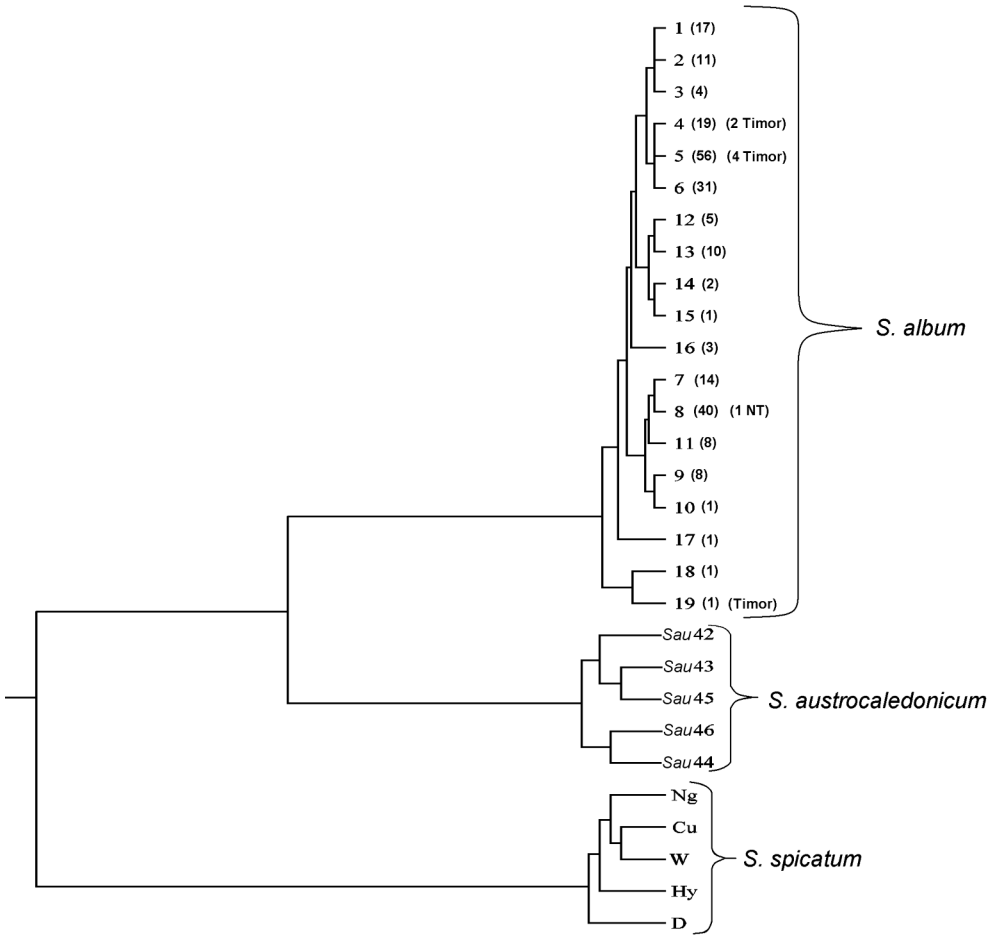

Figure 1. – UPGMA tree of the 19 genotypes of *S. album* and five genotypes of *S. austrocaledonicum* identified in trees from the FPC Kununurra collection. Number of trees within each group shown in parenthesis. Groups containing individuals sourced from Timor and the Northern Territory (NT) are also indicated. All other seed was sourced from India. *Santalum spicatum* serves as a distant out group, with five individuals from northern and southern populations of Western Australia.

sessed a single haplotype. The other two haplotypes were represented by *S. album* individual 9, and individuals 16 and 158 respectively. Similarly, there was little haplotype diversity in *S. macgregorii* with all 15 individuals bearing one haplotype. In contrast, both *S. austrocaledonicum* and *S. spicatum* showed greater variation, with three haplotypes present in the five individuals of each species. Relationships between haplotypes are shown in *Figure 2*, using *S. spicatum* as an out-group. The haplotypes are clustered according to species, except for one *S. album* haplotype which clustered with the three *S. austrocaledonicum* haplotypes. This haplotype was represented by two individuals.

Bootstrap values indicate strong support for the separation of *S. album* haplotypes (98%), and the *S. austrocaledonicum* clade (96%). Strong support was also found for *S. spicatum* (100%). The relationship between *S. album*, *S. austrocaledonicum* and *S. macgregorii* shows a polytomy, and remains unresolved in this analysis.

Discussion

The level of genetic diversity of *S. album* in the FPC Kununurra collection was remarkably low for a tree species and considerably lower than previously reported figures for natural stands of *S. album* in India (SUMA and BALASUNDARAN, 2003). Nuclear RFLP analysis showed genetic differentiation of only one Timorese tree with all others undifferentiated from material collected from India, indicating they were either mislabelled or collected from a heavily modified sample population. With few exceptions, differentiation between *S. album* families was not seen using chloroplast RFLPs. A selection of five *S. austrocaledonicum* and five *S. spicatum* individuals showed higher levels of nuclear and chloroplast DNA variation than the entire *S. album* collection. In comparison, the *S. macgregorii* samples showed no chloroplast variation, yet possessed a highly variable tetraploid nuclear genome. These findings have important implications for the conservation, selection and deployment of germplasm from the FPC Kununurra col-

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Table 2. – Mutations detected in the chloroplast genomes of 4 *Santalum* species.

Probe / enzyme	Mutation	Size (kb)	Species / Individuals
1. p1+p4 *Bcl*	Length	11→10	*S. spicatum*
2. p1+p4 *Bcl*	Length	9.4→9	*S. spicatum* (Wiluna)
3. p3 *Bgl*II	Site gain	7 → 5+2	*S. album*
4. p3 *Bgl*II	Length	7 → 9	*S. spicatum*
5. p3 *Bgl*II	Site gain	3.5 → 2 + 1.5	*S. spicatum*
6. p3 *Eco*RV	Length	14→13	*S. macgregorii*
7. p3 *Bcl*	Length	8 → 5.5	*S. spicatum*
8. p3 *Bcl*	Length	1.5 → 1.6	*S. spicatum*
9. p3 *Dra*	Length	2 → 1.8	*S. album* 16 and 158
10. p3 *Dra*	Site gain	8 → 5.6 + 2.4	*S. macgregorii*
11. p3 *Dra*	Length	1.7 → 1.6	*S. macgregorii*
12. p3 *Dra*	Site gain	7.4→2.9 + 4.5	*S. spicatum*
13. p3 *Dra*	Length	2 + 0.2 → 2.2	*S. spicatum*
14. p3 *Xba*	Length	5 → 4	*S. austrocaledonicum* 42 and 46
15. p6 *Eco*RV	Length	1.1 → 1.2	*S. album*
16. p6 *Bcl*	Site loss	5 + 4 → 9	*S. album* 16 and 158
17. p6 *Bcl*	Site gain	7 → 4 + 3	*S. spicatum*
18. p6 *Dra*	Length	4.3 → 5	*S. macgregorii*
19. p6 *Dra*	Site loss	4.5 + 1 → 5.5	*S. spicatum*
20. p8 *Bgl*II	Site loss	5 + 1 → 6	*S. macgregorii*
21. p8 *Eco*RV	Length	9.5 → 9.3	*S. macgregorii*
22. p8 *Eco*RV	Length	9.5 → 8	*S. spicatum* (Nyabing)
23. p8 *Bcl*	Site gain	8 → 5 + 3	*S. spicatum* (Kokerbin, Dumbelyung, Hyden)
24. p8 *Dra*	Length	1.4 → 1.2	*S. austrocaledonicum, S. album* 16 and 158
25. p8 *Dra*	Length	1.4 → 1.35	*S. album* 16 and 158
26. p10 *Xba*	Length	16 → 12	*S. album*
27. p8 *Dra*	Site gain	10 → 3 + 7	*S. spicatum*
28. p8 *Xba*	Site loss	6 + 5 → 11	*S. album*
29. p8 *Xba*	Length	5 → 4.8	*S. spicatum*
30. p8 *Xba*	Site loss	1 + 0.8 → 1.8	*S. album* 16 and 158
31. p8 *Dra*	Length	1.4 → 1	*S. album* 9
32. pTBa1 *Bgl*II	Length	6 → 6.4	*S. album*
33. pTBa1 *Bgl*II	Site loss	4 + 2 → 6	*S. austrocaledonicum* 45
34. pTBa1 *Bgl*II	Length	6.4 → 6.3	*S. spicatum*
35. pTBa1 *Eco*RV	Length	3.5 → 6	*S. macgregorii*
36. pTBa1 *Eco*RV	Site loss	8 + 4 → 12	*S. album*
37. pTBa1 *Eco*RV	Length	3 → 4	*S. austrocaledonicum*, *S. album* 16 and 158
38. pTBa1 *Eco*RV	Site loss	3 + 8 → 11	*S. macgregorii*

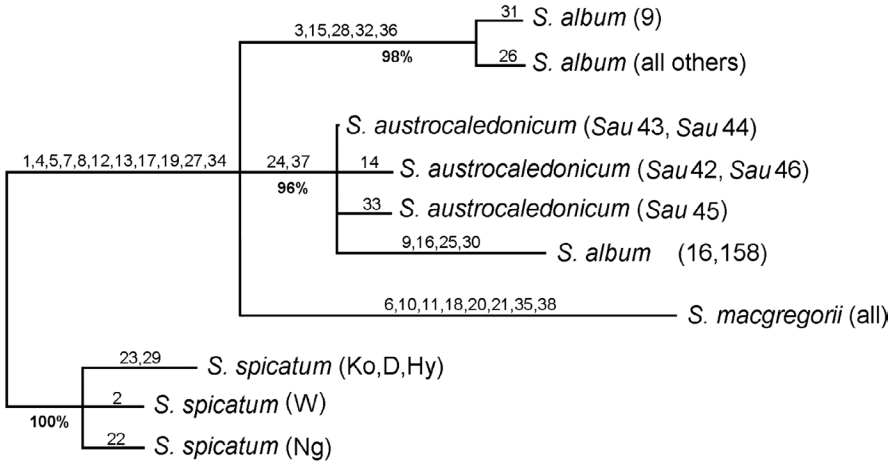

Figure 2. – Phylogenetic parsimony tree of *Santalum* haplotypes, generated from PAUP v4.0. Numbers above branches represent individual mutations listed in *Table 2*. Numbers below the line are the bootstrap support values.

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lection. Also, despite not having access to a geographically diverse collection, the low genetic diversity and lack of differentiation between a known Timorese tree and the Indian germplasm adds evidence that *S. album* was recently moved from Timor to the west in fairly recent times. Moreover, *S. album* has an Australian centre of origin (HARBAUGH and BALDWIN, 2007), low genetic diversity as well as the relative ancestry of the Timorese tree, supporting the hypothesis that *S. album* is the result of a dispersal event out of Australia from a northern progenitor.

Genetic diversity of the S. album collection

The *S. album* germplasm captured in the Kununurra collection is highly homozygous (H_o = 0.047, H_e = 0.047, SE ± 0.026) compared to other members of the genus (*Table 1*). While SUMA and BALASUNDARAN (2003) found that isozyme variation in *S. album* growing naturally in India was more diverse than that seen in the FPC Kununurra collection, the H_e and H_o values quoted are still rather low for a tree species (AUSTERLITZ et al., 2000). Isozymes are generally less likely to reveal polymorphisms than RFLPs (ALDRICH and DOEBLEY, 1992). Indeed, comparisons in eucalypts show that RFLPs generally detect twice the level of variation compared to isozymes (BYRNE, 2008). The low variation detected here in *S. album* would suggest that either the RFLPs probes used were not detecting as many polymorphic loci as possible, or more likely, the FPC collection is not representative of the species diversity seen in India and Indonesia. A molecular marker which detects higher levels of polymorphism may be more appropriate. The probes used in this study brought up sufficient variation in the species from which the library was constructed (*S. spicatum*) (BYRNE et al., 2003b) with over 60% of loci being polymorphic and also showed similar levels of polymorphism in *S. austrocaledonicum*, however this was not the case for *S. album*. Microsatellite loci have been shown to give estimates of heterozygosity nearly twice that of RFLPs in soybean (POWELL, et al., 1996) and in eucalypts (BYRNE, 2008) and may prove to be considerably more variable than RFLPs in *S. album*. Tests of microsatellite loci developed for *S. austrocaledonicum* (BOTTIN et al., 2005a) revealed very limited transferability (data not shown).

Low heterozygosity in the FPC Kununurra collection may not be entirely due to incomplete seed sourcing. If a population is subjected to significant gene flow restriction (i.e. reduction of the effective breeding population) and nearest-neighbour crossing is common, homozygosity increases rapidly over subsequent generations (TURNER et al., 1982). Bottlenecks can occur in nature through climatic or geographical changes, or through founder events. Dispersal events would result in similar circumstances, with very small populations becoming established (MICHAUX, 2001). These scenarios are well described in several species, including *Acacia mangium* (BUTCHER et al., 1998) and *Thuja plicata* (GLAUBITZ et al., 2000). In the most extreme cases, founder populations from very recent dispersal events, or severe population decline and bottlenecks can result in no detectable genetic variation (PEAKALL et al., 2003, WATERS and SCHAAL, 1991). Given what is now known about the chloroplast and ribosomal genetic diversity of the *Santalum* genus (HARBAUGH and BALDWIN, 2007) and the complementary results presented here, it is possible that *S. album* is the result of a founder population on the island of Timor. This would result in a very narrow genetic base which experienced limited or no gene-flow since establishment. The island of Timor is believed to have been formed 3 to 5 million years ago (ANONYMOUS, 2003). Climatic oscillations during the Pleistocene ice ages resulted in significant fluctuations in sea level (OUTLAW and VOELKER, 2008) interrupting gene flow between populations in northern Australia and Timor. These climatic conditions are also likely to have caused the extirpation of the ancestral form in northern Australia. The restricted populations of *S. album* currently present in northern Australia may be the result of recolonisation from Timor (HARBAUGH and BALDWIN, 2007) but is more likely to have been a very recent introduction from Indian seed in the last 50 years. More samples from this population must be included in a comprehensive regional investigation of the species.

Santalum album trees 16 and 158 had chloroplast haplotypes which clustered with *S. austrocaledonicum* (*Fig. 2*). As the same RFLP membranes were used with chloroplast probes a sample mix-up is impossible, indicating a chloroplast capture event has occurred. The phenomena is commonly detected in broad phylogenetic studies where the ancestral origins are the subject of interest (RIESEBERG et al., 1990; SMITH and SYTSMA, 1990; SOLTIS et al., 1991; WOLFE and ELISENS, 1995). Some seed growing in the FPC Kununurra collection is derived from arboreta in India and it is possible that a cross between *S. austrocaledonicum* and *S. album* has occurred within these collections. Trees 16 and 158 are more likely to be advanced generation backcrosses rather than F_1 hybrids derived from crosses within the FPC trials since they clustered with other *S. album* genotypes, and were not intermediate with *S. austrocaledonicum* in the nuclear RFLP study.

Selection, breeding and deployment

Despite the low genetic diversity, substantial phenotypic variation in heartwood content and essential oil yield exists in *S. album* captured in the FPC trials. Selection for superior trees may still be undertaken and maintenance of some genetic diversity would be important (MCDONALD et al., 2003). It should also be noted that heartwood content and essential oil yield are likely to be heavily influenced by the growing environment, and hence the extent to which this occurs must be determined. With respect to environmental effects, all species in the *Santalum* genus and almost all members of the family Santalaceae are root hemi-parasites (HEWSON and GEORGE, 1984). Even if identical soil and climatic conditions are chosen, the varying levels of haustorial connectivity will influence access to water and nutrients. Sandalwood trees in the FPC Kununurra collection are growing on a wide variety of host species at different planting densities, which simply compounds this environmental effect. Statistical analysis of host species, planting density and sandalwood growth characteristics

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in the FPC Kununurra collection is currently being undertaken.

Since clonal propagation of *S. album* is difficult at a production scale, seed will continue to be the most convenient mode of deployment. Therefore, in order to maintain genetic diversity, orchard design is critical and panmixic pollination is highly desirable as it helps to limit homozygosity in the progeny (OLSSON et al., 2001). This issue is highlighted by a recent study of a family trial for the Western Australian native sandalwood, *S. spicatum*, where the trial established was representative of the genetic diversity in the southern populations of the species. The resulting progeny of the trial were less diverse, apparently due to lack of synchronous flowing among all families (MUIR et al., 2007). Similarly *S. album* growing in the FPC Kununurra collection is at high risk of further homogenisation if random mating is not promoted. *Santalum album* is a preferential outcrossing species, however it will yield viable seed as a result of self-crosses (SINDHU VEERENDRA and ANANTHAPADMANABHO, 1996). Inbreeding depression, or the fixation of deleterious genes could occur through continued selfing. Also of concern to breeders is the risk of pollen introduction from nearby plantations. Well over 1000 Ha of *S. album* has been planted in the Ord River Irrigation Area (DONE et al., 2004) much of which is within 5 km of the FPC collection. It will be essential then to limit the flow of pollen into a breeding population through well designed orchards and possibly the creation of pollinator/wind breaks, particularly if the surrounding plantations arose from a narrow genetic base. As *S. album* is predominantly insect pollinated (SINDHU VEERENDRA and ANANTHAPADMANABHO, 1996), the foraging behaviour of pollinators must be factored into orchard design. Bees have a tendency to forage close to their starting point (FRANKIE et al., 1976) and move from tree to tree along a line of least resistance (PLOWRIGHT and GALEN, 1985). Assuming bees and other flying insects utilise a similar foraging strategy in the Kununurra seed orchards, it would seem wise to arrange more genetically divergent trees along open rows, while more genetically similar trees may be planted across alternate rows. Unlike *S. spicatum*, there is also no risk of affecting the genetic diversity of natural populations, since *S. album* is not endemic to the Kimberly region of Western Australia.

From the results of this study, it is clear that more collections of *S. album* from different areas of its distribution are required. Regardless, information on the relatedness of individuals will help with seed orchard design, so as to minimise crossing between closely related families. Alternative molecular markers such as microsatellites may reveal higher genetic diversity than the techniques used here.

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