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Reproductive biology of three commercially valuable *Santalum* species: development of flowers and inflorescences, breeding systems, and interspecific crossability

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Abstract *Santalum* (sandalwood) spp. are hemi-parasitic trees, the heartwood of which produces valuable aromatic oil. There appears to be a significant commercial opportunity for establishment of a planted sandalwood resource. However, lack of basic biological knowledge is one constraint on such development. The study reported here addresses one such constraint. Controlled pollination using 13 genotypes of *Santalum lanceolatum* was undertaken to elucidate (i) self-incompatibility (ii) intraspecific cross-compatibility in the species, and (iii) interspecific cross-compatibility with *S. album* and *S. austrocaledonicum*. *S. lanceolatum* may be considered to have a facultative allogamous (incomplete outbreeding) breeding system. This study found variation between genotypes in the level of putative self-incompatibility: some (20%) were found to set seed following self-pollination, while the remaining 80% had no seed development with such pollinations. However, a significantly greater proportion of genotypes developed seed following intraspecific cross-pollination (62%) compared with self-pollination (20%). While total geographic isolation and significant morphological divergence exists between *S. lanceolatum* with each of *S. album* and *S. austrocaledonicum* this study found no indication of reproductive barrier(s) between them, indicating potential for use of interspecific hybridization in genetic improvement, but also suggesting the potential of undesirable gene flow between native and introduced species.

Keywords Mating system · Sandalwood · Genetic improvement

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Introduction

Santalum (sandalwood) spp. are hemi-parasitic trees, occurring in India, Indonesia, PNG, Australia and the South Pacific. The heartwood of several species produces valuable aromatic oil widely used in perfumery, medicines, and incense. Sandalwood products are currently sourced unsustainably by whole-tree harvesting from natural stands, and the international price for natural sandalwood products continues to increase. There thus appears to be a significant commercial opportunity for establishment of a planted resource, which would reduce pressure on wild stands, improve consistency of product supply, and which could potentially improve livelihoods of smallholder farmers.

In the state of Queensland, Australia, native sandalwood (*Santalum lanceolatum*) has long been

commercially exploited for its powdered heartwood, used in funeral pyres and incense. Harvesting from natural stands in Cape York in the tropical far north of the state, principally to supply Chinese markets, began during the 1860s and continued until the early 1930s. Although indigenous traditional knowledge was crucial to this trade, indigenous people received few benefits beyond payments for their labour (Wharton 2009). Larger commercial stands in the Mitchell Plains and Channel Country of the southern end of the Gulf of Carpentaria began to be exploited during the 1920s and 1930s but this declined during WWII (Keenan 1996). Commercial extraction of sandalwood from central and western Queensland recommenced in the late 1980s and the Queensland Government currently exports approximately 500 m^3 annually under a commercial license (DPI&F 2004).

Santalum lanceolatum has the largest geographical distribution of any *Santalum* species, occurring naturally in all mainland Australian states. In general, the quality of oil from this species has been considered to be of lower quality than that produced from other commercial sandalwood species such as *S. album*, *S. yasi*, and *S. austrocaledonicum*. However, the recent discovery of high-quality *S. lanceolatum* in Cape York (Page et al. 2007) suggests that the species has important commercial potential, and, as a result, indigenous communities have become interested in rebuilding their heavily depleted sandalwood resources as a basis for development of community-based enterprises.

The decline of natural populations of all sandalwood species around the world has stimulated the emergence of a sandalwood plantation sector. This industry is particularly well developed in both tropical northern (*S. album*) and temperate southern (*S. spicatum*) areas of Western Australia (McKinnell 2008). More modest planting activities have also recently been recorded in China (*S. album*) (Xiaojin et al. (in press)), India (*S. album*)(Jeeva et al. 1998) and Vanuatu (*S. austrocaledonicum*) (Page et al. 2010a). Programmes also exist for development of cultivars with improved productivity and oil quality (Page et al. 2010b).

The development of *S. lanceolatum* as a significant commercial plantation species will depend on the development of high-quality genetic material, i.e., with rapid growth and high volume of heartwood containing concentrated oils with high levels of α- and β-santalol. However, development of such material will require information on the basic biology of the species, which is currently lacking in several respects. Here we present information on the reproductive biology of *S. lanceolatum*, including flower and inflorescence development, breeding system, and crossability with two other commercial species (*S. album* and *S. austrocaledonicum*).

Methods

Genetic material

The study described below was carried out using grafted plants of *S. lanceolatum* (13 genotypes collected in Cape York Peninsula), *S. album* (3 genotypes from India) and *S. austrocaledonicum* (1 genotype from Vanuatu) (pots of 300 mm diameter, soil-less potting medium, insect-proof greenhouse with drip irrigation). The genotypes of *S. lanceolatum* comprised seven from northern, three from central and one from southern Cape York. Two of the *S. album* genotypes came from Kolar district in the Indian state of Karnataka (CSIRO seedlots 19645 & 19648) and one genotype from Kununurra, Western Australia of an unknown source (CSIRO seedlot 19942). The *S. austrocaledonicum* was sourced from unselected seed from the Southern Island of Tanna. These *S. lanceolatum* genotypes represented a proportion of the variation across its range in Cape York. The *S. album* and the *S. austrocaledonicum* genotypes were used based on their good growth performance in north Queensland and their capacity to produce seed. All genotypes were grafted on to unselected seedlings of *S. album*. The premise for grafting was to produce potted plants of sexually mature individuals of known genotypes that could be easily used for controlled pollinations. While a total of eight *S. album* and five *S. austrocaledonicum* were grafted for this study, only the numbers mentioned previously developed sufficient flowers in which to undertake controlled pollinations.

Development of flowers and inflorescences

Phenological stages of flowers and inflorescences of *S. lanceolatum, S. album*, *and S. austrocaledonicum* were determined by twice daily observations of an individual inflorescence from three accessions of each species. These observations were undertaken from the period of anthesis of the first flower, to petal fall and

style desiccation of the last flower on a given accession. A single inflorescence from each species was photographed daily (Fig. 1). Phenological stages of a flower were measured relative to the day of anthesis, or the time of flower opening, which was considered to be day zero on its development scale.

Controlled crossing

Flowers were emasculated during anthesis using pointed forceps. Anthers were removed by applying light pressure to its filament in motion that pulled them away from the stigma to ensure there was no contact between them. Forceps were sterilised in 70% ethanol between handling different genotypes. Individual inflorescences were pollinated by a single pollen source by applying the pollen-shedding anther to the stigma until pollen grains had adhered to the stigma. All pollinations were carried out with anthers collected on the day of pollination. Each inflorescence was then tagged with details of the pollen donor.

Seven pollination combinations were made during three separate flowering events in September 2007, December 2007, and February 2008, in total involving 83 individual genotype combinations on 2143 individual inflorescences (Table 1). Flowers were left on the plants for approximately 8–10 weeks from pollination to fruit harvest. Fruits from each pollination combination were collected, the flesh was removed, and the seed air-dried before storing in sealed plastic containers at 4°C.

Germination and statistical analysis

Seeds were germinated in a medium consisting of medium grade perlite and vermiculite (1:1) under 50% shade, with automatic watering for 15 min per day. Crosses were considered to be successful if the seeds germinated and survived for a period of 3 months.

Table 1 Controlled crossing schemes used in pollination experiments with *S. lanceolatum*, *S. album,* and *S. austrocaledonicum*

Pollination type (*N*)	Number of unique crosses	Number of flowers combination^{-1}
SL, unpollinated (7)	7	182
SL × SL, selfed (10)	10	234
SL × SL, intraspecific (13)	13	241
SL (11) ♂ × *S. album* (3) ♀	20	820
S. album (3) ♂ × SL (11) ♀	23	430
SL (5) ♂ × SA (1) ♀	5	116
SA (1) ♂ × SL (5) ♀	5	120
Total	83	2143

N, number of genotypes per species; SL, *Santalum lanceolatum*; SA, *S. austrocaledonicum*

We looked at four response variables for each pollination type: the proportion of flowers that developed into seeds and the proportion that developed into surviving seedlings (at 3 months), and, similarly, the proportion of unique pollinations developing seed and seedlings. We tested the null hypothesis that the pollination types do not differ for these response variables using an equality test of two binomial proportions (Ott and Longnecker 2001):

$$z = \frac{(\hat{\pi}_1 - \hat{\pi}_2)}{\sqrt{\frac{\hat{\pi}_1(1-\hat{\pi}_1)}{n_1} + \frac{\hat{\pi}_2(1-\hat{\pi}_2)}{n_2}}}$$

where the two binomial populations are denoted by $\hat{\pi}_1 = (y_1/n_1)$ and $\hat{\pi}_2 = (y_2/n_2)$ and y_1 seeds/seedlings are recorded for the random sample of n_1 pollinations from population one, and y_2 seeds/seedlings are

Fig. 1 Inflorescences observed in determining floral phenology in *S. lanceolatum* (*left*) *S. album* (*centre*) and *S. austrocaledonicum* (*right*)

recorded for the random sample of n_2 pollinations from population two.

This statistical approach was used because, although a sufficient number of pollinations per pollination type were performed, in some cases a low number of replicates or genotype combinations did not permit evaluation by analysis of variance.

Results

Development of flowers and inflorescences

Flowers of all three species occur in terminal or axillary panicles consisting of >10 flowers. The corolla of each three species consists of 4-, rarely 5-tepals, which together with the anthers, alternate with the hypanthial lobes. Anther filaments are short and dorsifixed to anthers that shed pollen along longitudinal slits. Trichomes are found at the base of each anther filament extending in the floral tube and towards the back of the anthers (longer in *S. lanceolatum* and *S. austrocaledonicum*). The ovary is inferior to the floral tube and once fertilisation has been effected, the floral tube abscises from the pedicel. The ovary swells to become a single seeded drupe with a floral tube abscission scar at its top. In absence of fertilisation, flower abscission occurs rapidly approximately 1–2 days after flower closure (*S. lanceolatum* and *S. austrocaledonicum*) or floral tube desiccation (*S. album*). The tepals of both *S. album* and *S. austrocaledonicum* open more completely than *S. lanceolatum*; with the tepal tips recurving downward in the former two species. No change in stigma morphology such as swelling or evidence of exudate was observed during the experiment. The style and stigma in *S. album* changed from white to red in synchrony with the change in tepal colour (Table 2). *S. album* flower parts tend to be smaller: the stigma is smaller and less prominent compared with both *S. lanceolatum* and *S. austrocaledonicum.*

Flower longevity is distinct between species (Table 2) with

(a) *S. lanceolatum* flowers opening and closing within 12–24 h, often within morning and evening of the same day;
(b) *S. album*, flowers opening for seven to nine days (mean 8.7), and flowers begin to change colour from white to red after opening (changes starts at 19 h, complete at 53 h); and
(c) *S. austrocaledonicum* flowers typically open during the morning of a given day and close again during the afternoon of the following day (24–48 h).

Breeding system studies: unpollinated flowers

There were no signs of fruit development (e.g., floral tube abscission) in any of the unpollinated flowers. All flowers were shed towards the end of their expected 'life' (*S. lanceolatum* 12–24 h).

Breeding system studies: self-incompatibility in *S. lanceolatum*

Mean seed set per self-pollinated flower was 1.0%, which was significantly ($P < 0.05$) fewer than the 9.0% of flowers in intraspecific cross-pollination (Fig. 2). Seed set following self-pollinations occurred in accession 2 and 29 where 3.6 and 7.4% of self-pollinated flowers set seed from 55 pollinations combined. The percentages for accession 2 were not significantly ($P < 0.05$) different from intraspecific cross-pollinations involving this accession (used both as pollen donor or recipient) where 8.6% flowers set seed from 105 pollinations. No intraspecific cross-pollinations were performed using accession 29 so a similar comparison between self- and intraspecific cross-pollinations for this genotype was not possible. No seeds were set from any of the remaining eight genotypes after a total of 179 self-pollinations.

Two of ten self-pollinated genotypes (20%) set seed in this experiment, which was significantly ($P < 0.05$) lower than after intraspecific cross-pollinations, where 8/13 (62%) unique crosses developed seed and seedling (Fig. 3). Likewise the percentage of unique crosses developing seed within reciprocal interspecific hybridisations between *S. lanceolatum* with each of *S. album* and *S. austrocaledonicum* were significantly ($P < 0.05$) greater (45 and 90%, respectively) when compared with self-pollinated flowers. A similarly low-level of self-pollinated genotypes developed seedlings relative to interspecific crosses, but no significant difference was found between self- and intraspecific cross-pollinations (Fig. 3).

Table 2 Comparative floral features and phenology of *S. lanceolatum*, *S. album*, and *S. austrocaledonicum*

Floral feature	*S. lanceolatum*	*S. album*	*S. austrocaledonicum*
No. flowers per inflorescence	>10	20–40	20–40
Inflorescence life (days)	7–14	18–25	10–20
Flower life	12–24 h	7–9 days	24–48 h
Flower width (tips of each tepal)	5–7 mm	5–6 mm	5–7 mm
Anther filament length (mm)	1.0–1.5	~1.0	1.0–1.5
Anther length (mm)	1.5–2.0	1.5	1.5–2.0
Hypanthial lobe colour	Yellow	Red	Yellow
Hypanthial lobe length (mm)	1.0–1.5	0.5–1.5	1.0–1.5
Relative position of style to anthers	Above the level of the top of the anthers	At, or slightly below, the level of the top of the anthers	Above the level of the top of the anthers
Tepal colour at opening	White	White	White
Tepal colour change	No	Yes (to red)	No
Hours after anthesis in which tepals change colour	–	19–53	–

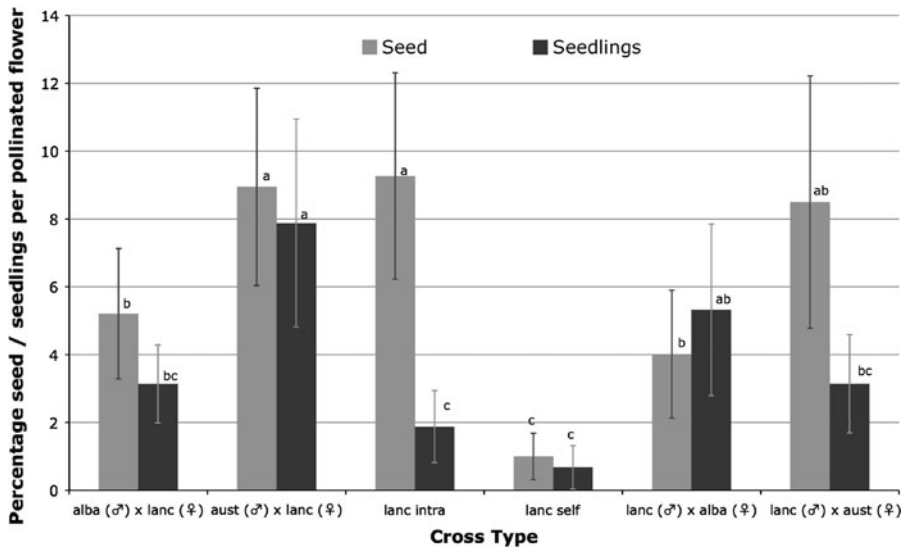

Fig. 2 Percentage seed and seedlings per pollinated flower for self and intraspecific pollinations in *S. lanceolatum* (*lanc. self* and *lanc. intra*, respectively) and reciprocal interspecific pollinations between *S. lanceolatum* with each of *S. album* (*lanc.* × *alba.* and *alba.* × *lanc.*) and *S. austrocaledonicum* (*lanc.* × *aust.* and *aust.* × *lanc.*). *Vertical bars* represent standard errors. Cross types sharing *lower case letters* are not significantly ($P < 0.05$) different within either the seed or seedling response variable

The percentage of self-pollinated *S. lanceolatum* flowers developing into seed was significantly lower than for all other pollination types. The percentage of self-pollinated flowers that developed into seedlings, however, was not significantly different from intraspecific crosses among *S. lanceolatum* genotypes despite the latter (1.8%) being triple that the former (0.6%) (Fig. 2).

The percentage of *S. lanceolatum* self-pollinated flowers that developed into seedlings was not significantly different from intraspecific crosses among *S. lanceolatum* genotypes and also between *S. album* (♂) and *S. lanceolatum* (♀) and *S. lanceolatum* (♂) and *S. austrocaledonicum* crosses (♀). Significantly greater percentage of flowers developing into seedlings was found for each of the interspecific crosses

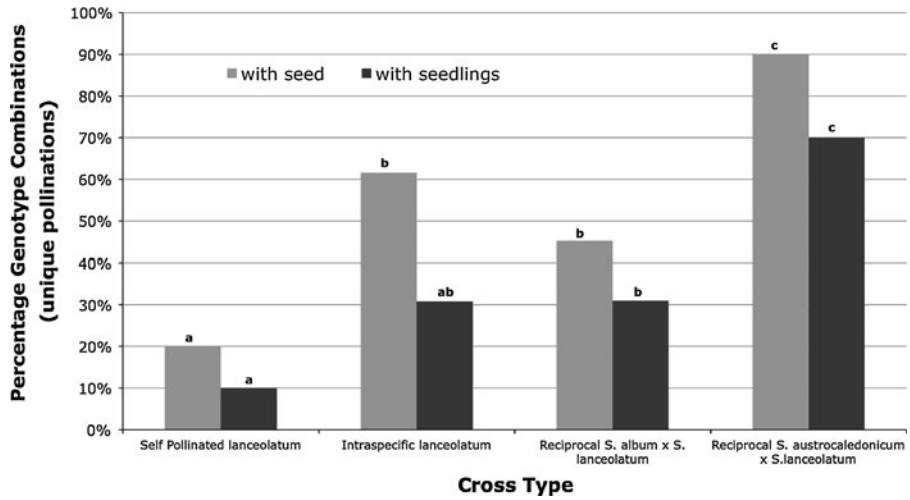

Fig. 3 Percentage of unique pollinations (i.e., different self-pollinated genotypes or different genotype combinations among cross types) with viable seed and seedlings. Cross types sharing *lower case letters* are not significantly ($P < 0.05$) different within either the seed or seedling response variable

S. lanceolatum (♂) × *S. album* (♀) and *S. austrocaledonicum* (♂) × *S. lanceolatum* (♀) compared with *S. lanceolatum* self-pollinated flowers (Fig. 2).

Breeding system studies: intraspecific cross-compatibility in *S. lanceolatum*

Of the 241 intraspecific crosses made in *S. lanceolatum* only 9.0 and 1.8% of pollinations resulted in the production of seed and seedlings, respectively (Fig. 2). For those crosses representing greater than ten pollinations, the seed set ranged from 0% in three different genotype combinations (averaging 16 pollination for each) to 14.2% in crosses between accessions 16 (♀) and 29 (♂) (totalling 14 pollinations).

Only accession 25 was used in over 50 intraspecific cross-pollinations each as a pistillate and pollen parent with at least three different genotypes. The mean percentage of seed set per pollination in this accession was not significantly different between pistillate (4.8%) and pollen (5.4%) parent. No other accession had a sufficient number of pollinations or was crossed with sufficient (>2) genotypes to permit evaluation of differences in fecundity when used as 'female' or 'male' parent for intraspecific crosses.

While the number of seed developed per pollinated flower was significantly ($P < 0.05$) greater in *S. lanceolatum* intraspecific crosses compared with self-pollination, there was no difference among these cross types in number of seedlings per pollinated flower (Fig. 2). A similar pattern was found between these two cross types for the percentage of unique pollinations that developed seed and seedlings (Fig. 3). No significant differences were found for unique crosses developing seed or seedlings between *S. lanceolatum* intraspecific and reciprocal *S. lanceolatum* × *S. album* interspecific. In contrast, a significantly ($P < 0.05$) greater number of unique crosses were found to develop seed and seedlings in reciprocal *S. lanceolatum* × *S. austrocaledonicum* compared with *S. lanceolatum* intraspecific crosses.

Interspecific cross-compatibility: *S. lanceolatum* × *S. album*

The percentage of seed set per pollinated flower varied from 0–23% (*S. lanceolatum* (♂) and *S. album* (♀)) and 0–16% (*S. album* (♂) and *S. lanceolatum* (♀)) for those crosses with > 10 pollinations. Thirty-eight percent of the seeds developing from the former interspecific cross each produced two seedlings, whereas 7.5% of the seeds from the reciprocal cross produced two seedlings. No other cross type (self, intraspecific or *S. lanceolatum* × *S. austrocaledonicum*) resulted in seed that produced two seedlings.

A significantly ($P < 0.05$) greater number of seeds per pollinated flower was found following intraspecific pollination among *S. lanceolatum* genotypes

compared with reciprocal interspecific crosses between *S. album* and *S. lanceolatum*. However no significant differences were found in the number of seedlings per pollinated flower between crosses among *S. album* (♂) × *S. lanceolatum* (♀) and *S. lanceolatum* intraspecific pollinations. Furthermore crosses between *S. lanceolatum* (♂) × *S. album* (♀) had a significantly ($P < 0.05$) greater number of seedlings per pollinated flower than for *S. lanceolatum* intraspecific pollinations.

Significantly ($P < 0.05$) fewer unique crosses were found to develop seed and seedlings in reciprocal *S. lanceolatum* × *S. album* interspecific compared with reciprocal *S. lanceolatum* × *S. austrocaledonicum* crosses (Fig. 3).

Interspecific cross-compatibility: *S. lanceolatum* and *S. austrocaledonicum*

Variation among the crosses between *S. lanceolatum* (♂) and the single genotype of *S. austrocaledonicum* (♀) was found in the percentage seed set per pollinated flower, ranging from 4 to 23% and from 0 to 18% in the reciprocal cross (*S. austrocaledonicum* (♂) and *S. lanceolatum* (♀)). No significant differences in the number of seed per pollinated flower were found between *S. lanceolatum* intraspecific crosses and each of the reciprocal interspecific crosses between *S. lanceolatum* and *S. austrocaledonicum*. Number of seedlings per pollinated flower for *S. austrocaledonicum* (♂) × *S. lanceolatum* (♀) cross was significantly ($P < 0.05$) greater than both self- and intraspecific crosses within *S. lanceolatum*. The reciprocal interspecific cross (*S. lanceolatum* (♂) and *S. austrocaledonicum* (♀)) however, was not found to differ from these self- and intraspecific crosses.

Discussion

Stages of flower development

The morphological and phenological similarities between *S. lanceolatum* and *S. austrocaledonicum* suggest that the natural pollinators of both may be similar. The substantially greater longevity of individual *S. album* flowers compared with both *S. austrocaledonicum* and *S. lanceolatum* is an important phenological distinction between them.

The onset and duration of stages in the floral development in *S. album* was found to vary substantially between flowers on an individual. In other species such as *Eucalyptus regnans* it is known that within a genotype, maturation after anthesis can vary between flowers and seasons, and is strongly influenced by mean daily temperature (Griffin and Hand 1979). It is likely that stigma receptivity in *S. album* occurs during the period of flower opening, since the stigma was observed to desiccate before floral tube abscission. Changes in stigma colour and shape after pollen shed may be used as a basis for determining the onset and duration of stigma receptivity.

Kulkarni and Muniyamma (1998) evaluated changes in stigma morphology in *S. album* and, while these authors did not directly measure stigma receptivity, reported that the presence of a shiny sugary drop on the stigmatic surface was likely to represent stigma receptivity. It was further reported that greatest proportion of the stigma with this morphological feature was consistently observed on the day after flower opening (Kulkarni and Muniyamma 1998). No observations of any stigma exudate were observed in the *S. album* accessions used in this study. However, further investigation of stigma receptivity and morphological changes may lead to visual associations between stigma receptivity and flower development stages, that could be employed in controlled pollination procedures in *S. album*. Stigma receptivity in both *S. spicatum* and *S. album* were reported to commence after the flower opens and attaining a peak 2–3 days later (Rugkhla et al. 1997). They further reported that pollen tubes grow more slowly in green compared with red flowers, where they took two and one day, respectively to reach the ovary.

Differences in the rate of flower development in *S. album* are most likely influenced by variation in environmental factors such as temperature, but further investigation is required to elucidate this. Given the brief 'life' of the flowers in *S. lanceolatum* and *S. austrocaledonicum* the phenological variation was proportionally much greater than for *S. album*, such that the life of a flower in *S. lanceolatum* could vary by as much as 100% (i.e., 12–24 h). In both species no visual changes in stigma morphology were detected. The timing and duration of stigma receptivity requires further investigation, but it is likely that these species are either slightly protandrous (pollen shed before stigma receptivity) or pollen shed and receptivity

occur simultaneously. This is proposed since pollen shed, particularly for *S. lanceolatum*, occurs throughout the period where the tepals are open and the stigma is available for pollination. Furthermore the upper part of the stigma is abscised concurrently with the floral tube, so the stigma is only available for pollination by 'large' insects during the opening of the tepals. In *Santalum* species, the most common pollinators are bees, flies, beetles, ants, butterflies, and wasps (Kulkarni and Muniyamma 1998; Jyothi et al. 1991). In this study, both ants and flies were commonly observed on the flowers of the three species for individuals not included in the insect-proof greenhouse. Ants were often found to chew and remove the style at its base, although the purpose for this behaviour was not determined. From observations in this study it is possible that small insects (such as thrips and ants) could penetrate the small openings in the tepals of *S. lanceolatum* and *S. austrocaledonicum* after the flower has closed. The frequency of such events and their influence on effecting pollination is not yet known.

Breeding system of *S. lanceolatum*

We have found that *S. lanceolatum* does not produce fruit or seed without pollination, and that, although seed set is higher under cross-pollination than selfing, some selfs do result in viable seed. Therefore, our results suggest strongly that *S. lanceolatum* is facultatively allogamous (incompletely outbreeding), with no capability for apomixis or parthenocarpy. We discuss these findings in more detail below.

In this study, no fruit or seeds were set following isolation of flowers and restricting pollination of *S. lanceolatum*. This result suggests that this species does not possess a capacity for the development of parthenocarpic fruit or clonal seed. This result is similar to that found in *S. album* in China, where no seeds were found in flowers isolated from open pollination by bags (Ma et al. 2006).

Our results demonstrate that mean seed set per pollinated flower in *S. lanceolatum* is significantly higher following cross-pollination than following selfing. Clearly, this indicates the probable presence of self-incompatibility. Rugkhla et al. (1997) proposed that both pre- and post- fertilisation self-incompatibility mechanisms were operating in *S. album* and *S. spicatum*. However, our finding that seed was set after selfing in 20% of accessions suggests that putative self-incompatibility mechanism(s) in *S. lanceolatum* may either be incomplete, or subject to genetic variation between accessions. Furthermore, two self-pollination derived seeds were successfully germinated and have continued to grow for 2 years without indication of any deleterious effects of inbreeding. Warburton et al. (2000) found little to no sexual reproduction in natural populations of *S. lanceolatum* in Victoria due to pollen sterility in one and self-incompatibility or pistil dysfunction in other populations. Each population in that study was found to consist of many ramets (derived from root suckers) of one clone that survived historical commercial exploitation. This, combined with the findings of our study give greater weight to the possibility that self-incompatibility mechanisms operate in *S. lanceolatum*, but that genetic variation in its expression exists within its natural populations. It is possible that any self-compatible genotypes present in the Victorian populations may have been removed during the period of uncontrolled harvesting.

Our results are similar to those found by Muir et al. (2007) for *S. spicatum*, where one family showed a high level of inbreeding, which was contradictory to the high mean outcrossing rate (95.2%). These authors proposed that flowering of this family was non-synchronous with many other families, resulting in higher inbreeding. This flexibility in breeding strategy would be of advantage in continental Australian species dispersing and colonizing many islands in south-east Asia and Pacific (Harbaugh and Baldwin 2007). In *S. album* Ma et al. (2006) reported 24% of flowers with geitonogamous (same plant and different flower) self-pollinated set seed.

In our study all cross types (self-, intraspecific and interspecific) were carried out on a given individual ramet. Therefore, it is possible that the reduced selfing rate recorded in this study compared with Ma et al. (2006) could be due to competitive interactions between flowers with 'outcross' and those with 'self' pollen and preferential maternal resource allocation to those most competitive. It would be of interest to evaluate the percentage seed set between these three cross types, where each type is restricted to an individual ramet of a given genotype. This would remove any interaction effects that may have been operating in the present study.

Intraspecific crossing in *S. lanceolatum*

The mean level of seed set amongst crosses of eight genotypes of *S. lanceolatum* was 7.5% of pollinated flowers. Fruit set (and thus seed set, given a fruit is generally single seeded) from open pollinated *S. album* trees was less than 2–3% in China (Ma et al. 2006) and 5.2% in India (Sindhu Veerendra and Anantha Padmanabha 1996). Rugkhla et al. (1997) reported a final fruit set of 1.3% in controlled intraspecific outcross pollination of *S. spicatum* in Western Australia. These authors also found a 10% fruit set in controlled outcrosses of *S. album*, which was similar to the 9.4% found by Kulkarni and Muniyamma (1998) in India. While Ma et al. (2006) found that 2–3% of open pollinated *S. album* flowers set seed, this was increased to 14% during artificial outcross pollinations. These results suggest that while improved seed set may be achieved using controlled pollination, several *Santalum* species produce an abundance of flowers but less than 10% of these typically develop into viable seed.

The significantly greater (i) number of seeds set per intraspecific outcross and (ii) percentage of unique intraspecific pollinations (genotype combinations) developing seed compared with self-pollinated flowers suggests a putative self-incompatibility mechanism. However, the low germination rate (40%) for intraspecific outcross derived seed resulted in no significant difference in the number of seedlings between intraspecific and self-pollinated flowers. The crossing between genotypes of *S. lanceolatum* was restricted to those with synchronous flowering, this resulted in a high proportion of crosses between individuals of the same population. The unique intrapopulation crosses represented 70% of the intraspecific crosses and 93% of 'intraspecific flowers' were pollinated with pollen from individuals of the same population. While it is possible that the degree of relatedness among intraspecific crosses may have contributed to the low germination, further replication of this study using a greater number of individuals from different populations is required to reveal the exact nature of the low germination rate among 'intraspecific seeds'.

Interspecific crosses between *S. lanceolatum* and each of *S. album* and *S. austrocaledonicum.*

Despite total geographic isolation and significant morphological divergence between *S. lanceolatum* with each of *S. album* and *S. austrocaledonicum*, our results suggest that no reproductive barrier appears to exist between them. Seed producing two seedlings were found in reciprocal crosses between *S. album* and *S. lanceolatum*, and although this is not unusual for these species, the level (7.5% for *S. lanceolatum* female and 38% for *S. album* female) was elevated compared with all other crosses in this study and with *S. album* intraspecific crosses in controlled crosses in China where the frequency was 2.5% (Ma et al. 2006).

Our study suggests that *S. lanceolatum* has a particularly high cross-compatibility with *S. austrocaledonicum*, but this result may have been confounded by the use of only a single *S. austrocaledonicum* genotype (T1). Greater numbers of unique crosses between these species would be needed to determine if the results in this study accurately reflect the cross-compatibility between them.

These results however, reflect similar findings with putative hybridisations between *S. yasi* and *S. album* in Fiji, with no apparent reproductive barrier or hybrid breakdown (Bulai and Nataniela 2005; Doran et al. 2005). Bulai (2007) further reported that spontaneous hybrids between *S. yasi* and *S. album* are now being produced in clonal seed orchards, and these hybrids appear to have higher vigour, wider environmental tolerances and are less dependent on forming host associations. The vegetative morphology of the interspecific hybrids in this study is similar to those of its parents, since similarities in leaf morphology exist between the parents used in this study. Further morphometric study is however required to determine any quantitative differences in other morphological traits and growth rates. High interspecific crossability does not however, appear to be universal in *Santalum*: Rugkhla et al. (1997) found that no seeds developed after 1,930 reciprocal controlled pollinations between *S. spicatum* and *S. album*, and reported that strong incompatibility mechanisms operated between pollen and style, and possibly in the developing zygote.

Doran and Brophy (2005) proposed that interspecific hybrids may provide the opportunity to improve the planted form of sandalwood, particularly given the high vigour of F_1 hybrids between *S. album* and *S. yasi* observed in Fiji. Hybridisation between *S. lanceolatum* and *S. album* may be used to incorporate important characters from each of these species into a cultivar for use in commercial plantations. For example, the straight form and fire tolerance of

S. lanceolatum could be combined with the typically higher heartwood oil concentration and quality (%α- and β-santalol) of *S. album*. However, given the difficulty of routine vegetative propagation of *Santalum* spp., such an approach would only be straightforward if additive genetic effects predominate in the characters of interest (general combining ability). A more complex procedure of reciprocal recurrent selection would be necessary to combine the desirable traits from both species in cultivars if non-additive gene effects predominated in the F_1 hybrids (specific combining ability).

Barriers to successful introgression were found to exist between *E. crebra* and *E. melanophloia*, where Drake (Drake 1981) found the hybrid population produced only 10% of the capsule yield of either parental species which, under natural selection, would put the hybrids at a competitive disadvantage. While segregating populations can be generated through artificial hybridisation of *Chamelaucium uncinatum* with each of *C. megalopetalum*, *Verticordia plumosa* and *V. grandis*, the resulting progeny of all crosses were infertile (Growns et al. 2002) and therefore it was not feasible to carry out further breeding. The high level of cross-compatibility between *S. lanceolatum* with each of *S. album* and *S. austrocaledonicum* indicates the likelihood that they are not widely divergent genetically and chromosomally (few chromosome structural differences) and thus the transfer of characters, even those under quantitative genetic control, would appear to be feasible from interspecific crosses. While the high cross-compatibility between these three species indicates the likelihood that they are not widely divergent genetically, it would be necessary to evaluate the fertility and seed production level of both their F_1 hybrid and F_2 progeny, because it is possible that genetic divergence between the species may not be significantly manifest until these post-hybridisation stages.

The apparent lack of interspecific barriers between *S. lanceolatum* with each of *S. album* and *S. austrocaledonicum* also has implications for the conservation of their natural stands. Given its low relative value, it is unlikely that *S. lanceolatum* would be introduced into areas of natural populations of *S. album* or *S. austrocaledonicum*. Commercial plantings of *S. album* have however, already been established in some areas of Queensland with existing natural populations of *S. lanceolatum*. It is highly possible that gene flow will occur between the *S. album* plantings and the *S. lanceolatum* populations. It is unclear, whether such hybrid progeny would have an advantage in these environments and persist beyond one or two generations. These considerations however may need to be evaluated by those responsible for both management of *S. lanceolatum* wild stands and improvement of *S. album* germplasm for commercial production.

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