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Genetic Diversity, Mating System and Systematic Relationships in Two Red Mahoganies, *Eucalyptus pellita* and *E. scias*

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Abstract

Isozyme variation in two closely related red mahoganies (*Eucalyptus pellita* F.Muell. and *E. scias* L.A.S.Johnson and K.D.Hill) was examined in 17 populations of *E. pellita* from Australia, Papua New Guinea and Indonesia and 8 of *E. scias* (including all 3 subspecies) from south-eastern coastal Australia. Measures of genetic diversity and relationships between species and subspecies were based on 15 variable loci. Both *E. pellita* and *E. scias* had moderately high levels of genetic diversity, comparable to other similarly distributed species. Most genetic diversity within each species was found within populations (80% in *E. pellita* and 83% in *E. scias*). There were substantial allelic differences between the species at several loci; the populations clustered into groups corresponding to the two species. Yet genetic differentiation between the two species was relatively low, and the three subspecies of *E. scias* were not well separated. Outcrossing rates in *E. pellita* are variable, with low rates (< 50%) measured in populations from Irian Jaya and Cape York Peninsula, and 73% in a population from north-east Queensland.

Introduction

Eucalyptus pellita F.Muell. and *E. scias* L.A.S.Johnson & K.D.Hill are members of a group of species collectively known as the red mahoganies. Along with the related species *E. urophylla* S.T.Blake (Timor mountain gum), *E. resinifera* Smith (red mahogany) and *E. notabilis* Maiden (Blue Mountains mahogany) they form part of the informal subseries *Resiniferinae* of the subgenus *Symphyomyrtus* (Pryor and Johnson 1971). This group has been the subject of considerable research interest in recent years primarily because of the commercial importance of *E. urophylla* (Eldridge *et al.* 1993) and the potential of *E. pellita* for plantation forestry in lowland tropical areas (Werren 1991; Haines and Harwood 1992). Genetic diversity and mating system in *E. urophylla* have recently been examined by House and Bell (1994).

Until recently, *E. pellita* was the name given to all large-fruited red mahoganies from the island of New Guinea to southern New South Wales. Johnson and Hill (1990) described a new species (*E. scias*) with three subspecies (*scias*, *callimastha* and *apoda*) from what was originally *E. pellita*, based on populations in the south of the taxon's range in eastern coastal Australia. Morphologically, *E. pellita* and *E. scias* are distinguished from each other by: smaller and narrower adult leaves without pronounced mucronate drip tips in *E. scias*, and longer petioles, larger juvenile leaves and longer pedicels in *E. pellita* (Johnson and Hill 1990). The subspecies of *E. scias* are characterised by regional differences in bud and fruit morphology. *Eucalyptus pellita* remains the name for all occurrences north of the Queensland and New South Wales border and on the island of New Guinea. Johnson and Hill (1990) preface the creation of a third taxon by stating that '*E. pellita,* as now defined, is restricted to north-eastern Queensland', implying a new taxon to be named to describe Cape

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York, Papua New Guinea and Indonesian populations. A study of seedling morphology of *E. pellita*, *E. scias* and *E. urophylla* by Pinyopusarerk *et al.* (1993) showed clear groupings centred on the species as they are currently classified.

Eucalyptus pellita is a medium to tall forest tree (but can have a mallee habit when growing on infertile coastal dunes), occurring from Townsville in northern Queensland north to New Guinea (Fig. 1). Its major area of distribution is in the humid coastal strip between Townsville and Cape Flattery. It is found between sea-level and 600 m, in open-forest formation with a range of other *Eucalyptus* species (Boland *et al.* 1984). The distribution of the subspecies of *Eucalyptus scias* is a series of disjunct patches in New South Wales. *Eucalyptus scias* subsp. *apoda* occurs as a small tree (sometimes as a mallee) at high altitudes in the Great Dividing Range north and east of Tenterfield in northern New South Wales and in similar habitats in the Banda Banda Range west of Kempsey. By contrast, *Eucalyptus scias* subsp. *scias* is known from coastal and sub-coastal forests from Sydney to Cessnock. The most southerly subspecies, *E. scias* subsp. *callimastha*, is found in coastal wet

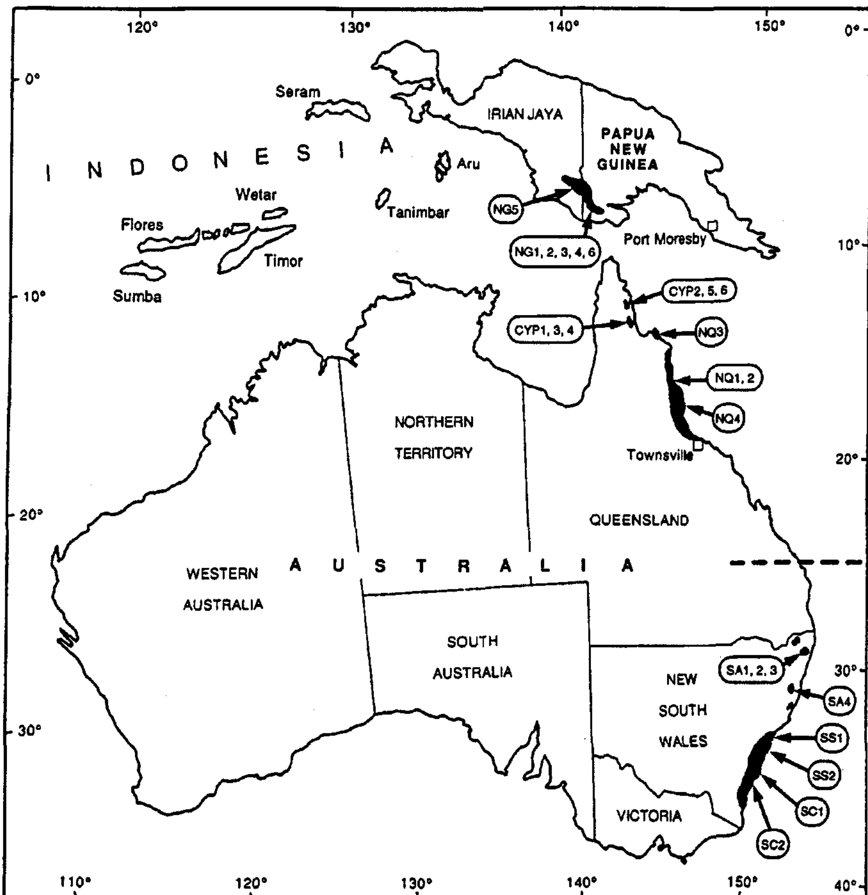

Fig. 1. Map showing the natural distribution of *Eucalyptus pellita* (above dashed line) and *Eucalyptus scias* (below line) in Australia and New Guinea, and the locations of populations used in this study. See Table 1 for population codes.

sclerophyll forests to heath scrubs from north of Wollongong south to Batemans Bay (Johnson and Hill 1990).

Isozymes have frequently been used to investigate patterns of genetic diversity within Australian tree species (Moran 1992), although their use in eucalypt systematics has been little exploited (Moran *et al.* 1990). Groupings of populations based on allelic frequencies have been shown to correspond to those generated by analyses of other traits (morphology, growth characteristics) in a number of species (e.g. the case studies of *Eucalyptus paliformis* and *E. parvifolia* by Prober *et al.* (1990)). In other instances, patterns of isozyme variation have not been congruent with groupings based on morphological features (e.g. Burgess and Bell 1983; House and Bell 1994).

The aims of the present study were, using isozymes, to describe levels and patterns of genetic diversity within each species, assess rates of outcrossing in *E. pellita*, and examine genetic differentiation and taxonomic relationships between *E. pellita* and *E. scias* and its subspecies.

Materials and Methods

Seventeen populations of *E. pellita* and eight of *E. scias* were selected to cover the total ranges of the species and subspecies (Fig. 1). Seed collections that consisted of at least 10 individual trees were chosen where obtainable, although some collections were represented by 1–3 mother trees. Only bulked seed collections were available from five of the populations. For each population, at least 50 seedlings (5 from each of 10 mother trees) were assayed for their isozyme genotypes. A minimum of 50 seedlings were also assayed from bulked seed collections. Details of the populations sampled are given in Table 1.

Isozyme procedures using starch gel electrophoresis follow those outlined by Moran and Bell (1983) and details of enzyme systems and buffers used are given in Table 2. Seeds were germinated on moist filter paper in incubators, and 7–9-day old seedlings were crushed in 50 μL of 0.05 M, pH 9.0 borate grinding buffer to which 1 mg mL^{-1} dithiothreitol and 20 mg mL^{-1} polyvinylpyrrolidone (MW = 40000) were added. Allelic bands were interpreted in accordance with Mendelian genetic principles and electrophoretic segregation patterns of progeny arrays from open-pollinated families. Banding patterns that were indistinct and uninterpretable were excluded. For each enzyme, the fastest migrating locus was designated '1', the next fastest '2' and so on. Similarly, within each locus the most anodally migrating allele was designated '1' and successive slower alleles '2', '3' etc. The distance each allele had migrated from the origin was measured to the nearest mm in order to establish allelic identity across populations. Progeny from both species were run on the same gels and alleles were coded in a complete sequence that covered all alleles identified.

Genotype arrays were analysed using the BIOSYS-1 package (Swofford and Selander 1989). Hierarchical estimates of genetic diversity (Nei 1978) were calculated based on: (1) the total data set; (2) each species; (3) zones of populations (for *E. pellita*) and subspecies (for *E. scias*) within each species; and (4) populations within the zones or subspecies; so that:

$$H_T = H_S + D_{ST}$$

and within species

$$H_S = H_P + D_{ZS} + D_{PZ},$$

where H = total genetic diversity, D = mean genetic diversity, and the subscripts are P = populations, Z = zones (*pellita*), subspecies (*scias*), S = species, and T = total dataset. Zones of populations within *E. pellita* were determined on the basis of the geographical distribution of the species (see Fig. 1 and Table 1). The degree of genetic differentiation between populations (G_{ST}) can be calculated as

$$G_{ST} = D_{ST}/H_T\,.$$

A hierarchical cluster analysis of all populations was performed using Nei's (1978) unbiased genetic distance calculated for pairs of populations, and employing the UPGMA algorithm. Error bars were added using the method of Ritland (1989). A distance Wagner analysis was carried out using Rogers'

Table 1. Details of populations of *E. pellita* and *E. scias* used in this study
E. pellita: NQ = north-east Queensland (see Fig. 1); CYP = Cape York Peninsula; NG = New Guinea; *E. scias*: SA = *E. scias* ssp. *apoda*; SS = *E. scias* subsp. *scias*; SC = *E. scias* subsp. *callimastha*. NSW, New South Wales; Qld, Queensland; PNG, Papua New Guinea; IND, Indonesia (Irian Jaya)

(*a*) *E. pellita*

Population code	CSIRO seed-lot number	Location	Lat. °S	Long. °E	Altitude (m)	*N*
NQ1	17860	SSW Kuranda, Qld	16°56′	145°36′	425	10
NQ2	17861	NW Kuranda, Qld	16°41′	145°32′	440	10
NQ3	18313	Starcke, Qld	15°05′	145°12′	30	7
NQ4	18314	El Arish, Qld	17°50′	146°03′	50	10
CYP1	13998	NE Coen, Qld	13°53′	143°19′	560	12[A]
CYP2	13999	NE Wenlock, Qld	12°43′	143°08′	100	10[A]
CYP3	14339	NE Coen, Qld	13°53′	143°17′	580	10
CYP4	17874	Lankelly Ck, Qld	13°53′	143°16′	500	10
CYP5	17875	Tozer Gap, Qld	12°44′	143°12′	100	10
CYP6	18356	Mt Tozer, Qld	12°45′	143°12′	450	7[A]
NG1	16120	Keru-Mata, PNG	8°36′	141°45′	30	9
NG2	16121	Tokwa-Kiriwa, PNG	8°30′	141°25′	45	10
NG3	16122	Goe-Kiriwa, PNG	8°20′	141°32′	50	10
NG4	16615	Keru-Kumbalusi, PNG	8°35′	141°45′	35	10
NG5	17854	Bupul-Muting, IND	7°21′	140°36′	40	10
NG6	18195	SW Komavai, PNG	7°35′	141°07′	50	2

(*b*) *E. scias*

Population code	CSIRO seed-lot number	Subspecies	Location	Lat °S	Long °E	Altitude (m)	*N*
SA1	18281	*apoda*	Girard, NSW	28°57′	152°20′	850	1
SA2	18282	*apoda*	Boonoo Boonoo, NSW	28°54′	152°09′	1040	5
SA3	18283	*apoda*	Malara, NSW	29°08′	152°18′	950	6
SA4	18295	*apoda*	Mt Boss, NSW	31°09′	152°27′	1260	6
SS1	10430	*scias*	Cessnock, NSW	32°54′	151°24′	300	3[A]
SS2	18294	*scias*	Bouddhi, NSW	33°31′	151°24′	140	10
SC1	11813	*callima-stha*	Yatteyattah, NSW	35°16′	150°26′	90	–[B]
SC2	18320	*callima-stha*	South Brooman, NSW	35 30	150 15	60	9

[A]bulked seed collection (*N* = number of mother trees); [B]unkown number of mother trees

(1972) distance measure and mid-point rooting to examine phylogenetic relationships between the two species. Multi-locus estimates of outcrossing rates in three populations of *E. pellita* were made using the procedures of Ritland and Jain (1981).

Results

Fifteen zones of enzyme activity, representing loci in 10 enzyme systems, were scored for their allozyme frequencies in both species. Indistinct zones were noted at *Gpi*-1, *Ugp*-1, *Pgm*-2, *Lap*, *Aap* and *Est*-3: these were all variable but unresolvable. No scorable, yet invariant, loci were detected.

Table 2. Enzymes and buffer systems used (the same loci were scored for both *E. pellita* and *E. scias*)

MC = morpholine citrate–8.4 gL^{-1} citric acid adjusted to pH 6.1 with N-(3-aminopropyl)-morpholine and diluted to 1 in 20; L = lithium borate–8.25 gL^{-1} Tris, 1.9 gL^{-1} citric acid, 0.225 gL^{-1} lithium hydroxide, 1.175 gL^{-1} boric acid (pH 8.2); H = histidine–10.5 gL^{-1} l-histidine adjusted to pH 8.0 with 10N NaOH and diluted to 1 in 10

Enzyme system	Gel buffer	EC code	no. loci
Aspartate aminotransferase (*Aat*)	L	2.6.1.1	3
Esterase (*Est*)	L	3.1.1.–	2
Glucosephosphate isomerase (*Gpi*)	MC	5.3.1.9	1
Glycerate dehydrogenase (*Gly*)	MC	1.1.1.29	1
Isocitrate dehydrogenase (*Idh*)	MC	1.1.1.42	1
Malate dehydrogenase (*Mdh*)	MC	1.1.1.37	2
Malic enzyme (*Me*)	H	1.1.1.82	1
Phosphogluconate dehydrogenase (*Pgd*)	MC	1.1.1.44	2
Shikimate dehydrogenase (*Sdh*)	MC	1.1.1.25	1
Uridine diphosphogluconic pyrophosphatase (*Ugp*)	MC	2.7.7.9	1

Allele Frequencies

Whilst there were no fixed allelic differences between the species at any locus (Table 3*a*, *b*), some alleles were absent from one or the other species and there were substantial differences in allele frequencies at several loci. *Eucalyptus pellita* did not have the fastest migrating alleles at *Gpi*-2 and *Est*-1, whilst the fastest alleles at *Mdh*-2 and *Pgd*-1 were absent in the populations of *E. scias* studied. With the exception of allele *Gpi*-2_1 in population SC2 (*E. scias* subsp. *callimastha*), all these alleles were at low frequencies in the species they occurred in, and absent from some populations.

In *E. scias*, *Gpi*-2 was highly polymorphic in all populations, with five of the eight alleles being the commonest in at least one population, and occurring at < 0.5 frequency in six of the eight populations. Only at *Est*-1 were allele frequencies clearly related in some way to subspecific status: subsp. *apoda* had very low frequencies of the allele (*Est*-1_2) which was the commonest allele in the other two subspecies. At *Aat*-1, three populations of subsp. *apoda* had low frequencies (< 0.25) of the otherwise common allele *Aat*-1_3, but in the fourth (SA4) it was high (0.99). There were similar trends at *Me*-1 for subsp. *apoda* and subsp. *scias*, and at *Gpi*-2 for subsp. *callimastha*.

Between the broad geographic zones of distribution of *E. pellita*, populations in Australia and New Guinea had different common alleles at *Pgd*-1 and *Pgd*-2.

Distribution of Genetic Diversity

Measures of genetic diversity (Table 4*a*, *b*) were all reasonably high for most populations of both species. The mean number of alleles per locus (A) in *E. pellita* was variable, and was especially low in populations NG6 and CYP6. Estimates of the percentage of polymorphic loci (P) in these populations were also low. On a geographic basis the highest levels of both these measures were found in populations from Cape York ($A = 2.5$; $P = 77\%$), followed by north-east Queensland ($A = 2.3$; $P = 72\%$) and New Guinea ($A = 2.1$; $P = 70\%$). Similarly, all populations of *E. scias* had high levels of A and P, with little variation between them. In *E. pellita,* there was a significant positive correlation between latitude and observed heterozygosity (Fig. 2). Correlations between other genetic parameters (A, P, H_e) and latitude were not significant. There were no clear patterns relating to the subspecies of *E. scias*.

Table 3*a*. Allelic frequencies at eight variable loci in populations of *E. pellita*
See Table 1 for populations codes. A full list of allelic frequencies is lodged with the Journal

	Population															
Locus	NQ1	NQ2	NQ3	NQ4	CYP1	CYP2	CYP3	CYP4	CYP5	CYP6	NG1	NG2	NG3	NG4	NG5	NG6
Aat-1																
(*n*)	73	51	75	64	62	65	123	77	50	50	91	65	85	91	86	55
1	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.01	0.00	0.00
2	0.98	1.00	0.98	0.98	1.00	0.92	1.00	0.96	1.00	1.00	1.00	0.89	0.98	1.00	0.99	0.98
3	0.01	0.00	0.01	0.02	0.00	0.07	0.00	0.03	0.00	0.00	0.01	0.04	0.02	0.00	0.01	0.02
4	0.01	0.00	0.01	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Est-1																
(*n*)	73	51	72	64	61	65	123	77	50	50	91	63	84	91	95	55
2	0.14	0.39	0.03	0.15	0.14	0.21	0.02	0.00	0.08	0.00	0.09	0.06	0.20	0.14	0.01	0.01
3	0.65	0.30	0.74	0.52	0.70	0.77	0.84	0.92	0.90	1.00	0.85	0.83	0.79	0.84	0.91	0.99
4	0.21	0.30	0.23	0.33	0.16	0.02	0.14	0.08	0.02	0.00	0.06	0.10	0.01	0.02	0.08	0.00
Gpi-2																
(*n*)	73	51	75	64	62	55	103	77	50	50	91	65	75	91	85	55
2	0.00	0.00	0.02	0.00	0.00	0.01	0.08	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.15	0.00
3	0.03	0.00	0.04	0.00	0.01	0.00	0.07	0.09	0.01	0.00	0.09	0.42	0.19	0.10	0.11	0.43
4	0.38	0.35	0.41	0.40	0.03	0.14	0.11	0.04	0.21	0.38	0.11	0.00	0.11	0.07	0.06	0.11
5	0.38	0.49	0.46	0.31	0.83	0.70	0.67	0.64	0.63	0.53	0.79	0.41	0.68	0.82	0.64	0.26
6	0.12	0.09	0.06	0.07	0.07	0.08	0.05	0.21	0.12	0.09	0.01	0.11	0.01	0.00	0.01	0.15
7	0.09	0.07	0.01	0.23	0.07	0.07	0.03	0.02	0.03	0.00	0.00	0.06	0.01	0.00	0.03	0.06
8	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mdh-1																
(*n*)	73	51	75	64	62	55	103	78	50	50	91	65	75	91	85	55
1	0.00	0.01	0.01	0.00	0.03	0.04	0.01	0.05	0.01	0.00	0.00	0.00	0.00	0.00	0.02	0.00
2	1.00	0.99	0.79	1.00	0.94	0.92	0.98	0.81	0.99	0.99	1.00	0.93	0.99	1.00	0.98	1.00

3	0.00	0.00	0.01	0.00	0.02	0.02	0.01	0.10	0.00	0.01	0.00	0.07	0.01	0.01	0.00	0.00
4	0.00	0.00	0.19	0.00	0.01	0.03	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mdh-2																
(*n*)	73	51	75	64	62	55	103	78	50	50	91	65	75	91	85	55
1	0.03	0.00	0.00	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	0.97	1.00	0.99	0.95	0.99	1.00	0.99	1.00	1.00	1.00	1.00	0.99	1.00	1.00	1.00	1.00
3	0.00	0.00	0.01	0.00	0.01	0.00	0.01	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00
Me-1																
(*n*)	65	48	65	52	54	51	91	52	45	37	54	58	65	71	80	55
1	0.98	0.85	0.74	0.96	0.46	0.64	0.83	0.81	0.81	0.82	0.78	0.88	0.96	0.97	0.95	1.00
2	0.02	0.15	0.26	0.04	0.54	0.36	0.17	0.19	0.19	0.18	0.22	0.12	0.04	0.03	0.05	0.00
Pgd-1																
(*n*)	73	51	75	64	62	55	103	77	50	50	91	65	75	91	85	55
1	0.00	0.01	0.00	0.00	0.03	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	0.66	0.66	0.41	0.65	0.80	0.92	0.67	0.51	0.65	0.60	0.19	0.11	0.21	0.10	0.02	0.00
3	0.02	0.00	0.00	0.01	0.00	0.00	0.00	0.05	0.11	0.00	0.00	0.02	0.00	0.00	0.00	0.00
4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6	0.32	0.33	0.59	0.34	0.17	0.08	0.27	0.40	0.19	0.40	0.81	0.87	0.79	0.90	0.98	1.00
7	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ugp-2																
(*n*)	73	51	59	64	61	55	103	78	50	50	91	65	75	91	85	55
1	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	1.00	1.00	0.79	0.93	0.89	0.98	0.77	0.85	0.98	1.00	1.00	0.75	0.99	0.97	1.00	0.98
3	0.00	0.00	0.20	0.07	0.05	0.02	0.10	0.14	0.02	0.00	0.00	0.25	0.01	0.03	0.00	0.02
4	0.00	0.00	0.00	0.00	0.05	0.00	0.05	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5	0.00	0.00	0.00	0.00	0.01	0.00	0.08	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table 3*b*. Allelic frequencies at eight variable loci in populations of *E. scias*

See Table 1 for population codes. A full list frequencies is lodged with the Journal

Locus	SA1	SA2	SA3	Population SA4	SS1	SS2	SC1	SC2
Aat-1								
(*n*)	50	48	79	80	33	58	51	79
1	0.00	0.01	0.09	0.00	0.02	0.02	0.02	0.00
2	0.40	0.65	0.41	0.01	0.06	0.24	0.42	0.23
3	0.19	0.24	0.23	0.99	0.83	0.73	0.56	0.65
4	0.40	0.10	0.27	0.00	0.09	0.01	0.00	0.11
5	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Est-1								
(*n*)	49	52	81	77	33	58	51	79
1	0.00	0.00	0.00	0.00	0.02	0.01	0.00	0.00
2	0.01	0.03	0.07	0.21	0.55	0.60	0.82	0.48
3	0.21	0.45	0.61	0.70	0.18	0.28	0.04	0.44
4	0.78	0.52	0.33	0.10	0.26	0.11	0.14	0.08
Gpi-2								
(*n*)	50	52	81	80	33	58	51	79
1	0.00	0.08	0.03	0.01	0.02	0.00	0.00	0.22
2	0.04	0.01	0.02	0.21	0.29	0.12	0.02	0.2
3	0.09	0.31	0.18	0.43	0.35	0.32	0.14	0.12
4	0.77	0.06	0.22	0.12	0.03	0.08	0.06	0.10
5	0.00	0.12	0.09	0.11	0.06	0.11	0.72	0.14
6	0.02	0.08	0.14	0.06	0.05	0.03	0.03	0.06
7	0.04	0.15	0.23	0.06	0.21	0.34	0.04	0.02
8	0.04	0.20	0.10	0.00	0.00	0.00	0.00	0.11

Mdh-1								
(*n*)	50	52	81	80	33	59	51	79
1	0.00	0.00	0.01	0.00	0.02	0.00	0.00	0.01
2	0.95	0.99	0.95	0.98	0.97	0.98	0.98	0.94
3	0.05	0.01	0.04	0.00	0.02	0.02	0.02	0.06
4	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00
Mdh-2								
(*n*)	50	52	81	80	33	59	51	79
2	0.94	0.53	0.66	0.98	0.77	0.97	0.97	0.78
3	0.06	0.47	0.34	0.02	0.23	0.03	0.03	0.22
Me-1								
(*n*)	44	51	79	68	30	58	49	79
1	0.41	0.23	0.52	0.93	0.62	0.34	0.37	0.37
2	0.59	0.78	0.48	0.07	0.38	0.66	0.63	0.63
Pgd-1								
(*n*)	49	52	81	80	33	59	51	79
2	0.89	0.81	0.80	0.76	0.74	0.85	0.96	0.84
3	0.02	0.19	0.18	0.22	0.03	0.11	0.00	0.13
4	0.00	0.00	0.01	0.01	0.00	0.02	0.01	0.00
5	0.07	0.00	0.01	0.00	0.00	0.00	0.00	0.00
6	0.02	0.00	0.01	0.01	0.00	0.02	0.03	0.03
7	0.00	0.00	0.00	0.00	0.23	0.01	0.00	0.00
Ugp-2								
(*n*)	50	52	81	60	33	59	51	65
2	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00
3	0.99	0.99	1.00	0.99	0.99	0.94	1.00	0.92
4	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00
5	0.00	0.00	0.00	0.00	0.02	0.06	0.00	0.08

Table 4. Measures of genetic diversity in populations of *E. pellita*
A = mean number of alleles per locus; P = percentage of loci polymorphic (0.99 criterion); F = Wright's fixation index. Standard errors are given in parentheses; *Unbiased estimate (see Nei 1978)

Population	Mean sample size per locus	A	P	Mean heterozygosity: Direct-count H_o	Mean heterozygosity: HdyWbg expected H_e*	F
E. pellita						
NQ1	71.0	2.1	60.0	0.141	0.194	0.230
	(1.0)	(0.3)		(0.044)	(0.064)	
NQ2	50.5	2.1	66.7	0.136	0.221	0.241
	(0.3)	(0.3)		(0.047)	(0.070)	
NQ3	72.9	2.6	80.0	0.161	0.252	0.290
	(1.2)	(0.4)		(0.042)	(0.060)	
NQ4	62.5	2.4	80.0	0.151	0.209	0.326
	(1.0)	(0.3)		(0.050)	(0.066)	
CYP1	61.0	2.7	80.0	0.157	0.218	0.236
	(0.6)	(0.3)		(0.037)	(0.053)	
CYP2	57.6	2.5	80.0	0.119	0.197	0.291
	(1.5)	(0.3)		(0.033)	(0.053)	
CYP3	106.1	2.9	86.7	0.161	0.249	0.325
	(2.4)	(0.4)		(0.042)	(0.061)	
CYP4	73.3	2.8	80.0	0.159	0.269	0.389
	(1.9)	(0.3)		(0.036)	(0.055)	
CYP5	49.6	2.5	86.7	0.137	0.196	0.235
	(0.3)	(0.3)		(0.037)	(0.049)	
CYP6	48.6	1.6	46.7	0.107	0.164	0.266
	(1.0)	(0.2)		(0.045)	(0.062)	
NG1	86.1	1.8	60.0	0.075	0.174	0.508
	(2.6)	(0.2)		(0.031)	(0.046)	
NG2	64.3	2.5	93.3	0.108	0.256	0.540
	(0.5)	(0.2)		(0.027)	(0.045)	
NG3	76.0	2.1	73.3	0.069	0.152	0.498
	(1.3)	(0.3)		(0.025)	(0.045)	
NG4	87.3	2.0	80.0	0.056	0.112	0.380
	(2.0)	(0.2)		(0.019)	(0.029)	
NG5	83.4	2.3	73.3	0.065	0.197	0.712
	(1.9)	(0.3)		(0.029)	(0.059)	
NG6	53.3	1.7	40.0	0.055	0.105	0.291
	(1.7)	(0.3)		(0.041)	(0.057)	
E. scias						
SA1	49.5	2.5	86.7	0.171	0.192	0.049
	(0.4)	(0.3)		(0.052)	(0.054)	
SA2	50.3	2.5	80.0	0.217	0.256	0.147
	(1.3)	(0.4)		(0.066)	(0.067)	
SA3	80.6	3.1	93.3	0.198	0.291	0.265
	(0.2)	(0.4)		(0.054)	(0.072)	
SA4	77.7	2.6	86.7	0.166	0.193	0.074
	(1.5)	(0.4)		(0.050)	(0.058)	
SS1	32.4	2.9	80.0	0.221	0.317	0.243
	(0.4)	(0.4)		(0.053)	(0.067)	
SS2	58.7	2.9	93.3	0.174	0.251	0.209
	(0.1)	(0.4)		(0.050)	(0.062)	
SC1	50.8	2.5	73.3	0.125	0.172	0.070
	(0.1)	(0.4)		(0.039)	(0.049)	
SC2	77.1	2.7	80.0	0.250	0.294	0.106
	(1.3)	(0.5)		(0.058)	(0.067)	

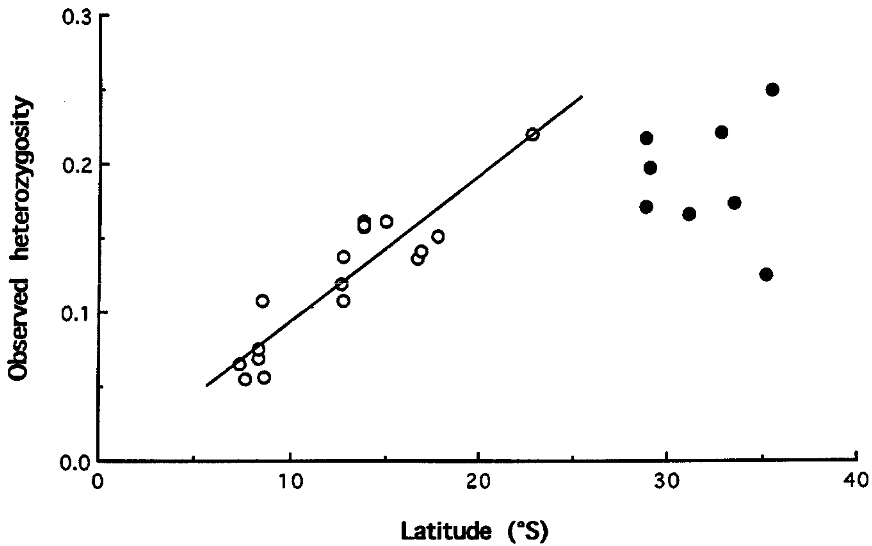

Fig. 2. Relationship between latitiude and observed heterozygosity in *E. pellita* ○, and *E. scias* ●. A regression line fitted through the *E. pellita* points gives $r = 0.91$, $P < 0.001$ $n = 17$.

Table 5. Mean measures of genetic diversity in three red mahoganies (*E. pellita*, *E. scias*, and the closely related *E. urophylla*) and five other widespread eucalypts
H_T = total genetic diversity; H_S = mean genetic diversity within populations; G_{PS} = mean proportion of diversity (%) between populations; A = mean no. alleles per locus and P = mean proportion (%) of polymorphic loci. [A]House and Bell (1994); [B]*E. cloeziana* (Turnbull 1980); *E. grandis*, *E. saligna* (Moran and Bell 1983); *E. delegatensis* (Moran, unpub. data); *E. nitens* (Moran 1992)

	E. pellita	*E. scias*	*E. urophylla*[A]	five other *Eucalyptus* spp.[B]
H_T	0.299	0.343	0.195	0.231
H_S	0.249	0.295	0.172	0.198
G_{PS}	20.4	15.0	11.8	14.7
A	2.3	2.7	2.4	2.2
P	72.9	84.2	77.5	69.9

Table 6. Within-species analysis of genetic diversity in *E. pellita* and *E. scias*
H_S = within-species diversity; H_P = mean diversity within populations; D_{ZS} = mean differentiation between zones or subspecies; D_{PZ} = mean differentiation between populations within a zone and subspecies

	E. pellita		*E. scias*	
		as % of H_S		as % of H_S
H_S	0.24858		0.29472	
H_P	0.19780	79.6	0.24579	83.4
D_{ZS}	0.02153	8.7	0.00246	0.8
D_{PZ}	0.02925	11.7	0.04647	15.8

Population means of the fixation index 'F' were all positive in both species, indicating a deficiency of heterozygotes compared to expectations assuming panmixia. This is a common finding in plants with predominant outcrossing (Brown 1979).

Total genetic diversity (taking all populations of both species) was 0.326, 65.6% of which can be found within any population; 16.7% was the amount of differentiation between the two species; 3.7% was the amount of differentiation between zones and subspecies; and 14.0% was the differentiation between populations within a zone or subspecies. Total genetic diversities in the species individually (*E. pellita* $H_T = 0.299$; *E. scias* $H_T = 0.343$) were high. These figures are compared to the mean values of measures of genetic diversity in five species of *Eucalyptus* with widespread distributions in Table 5.

In common with other species in the genus, the bulk of genetic diversity in both *E. pellita* and *E. scias* can be found within any population (Table 6). Population divergence as measured by G_{ST} accounted for 20.4% of total genetic diversity in *E. pellita* and 15.0% in *E. scias*. The mean value of G_{ST} for 17 species of *Eucalyptus* is 18.1% (Moran 1992). The geographic zones of *E. pellita* (8.7% of total diversity is accounted for between zones) and the subspecies of *E. scias* (0.8% of total diversity accounted for between subspecies) were poorly differentiated isozymically.

Genetic Distance and Phylogeny

Populations of *E. pellita* group on a geographic basis into those from New Guinea and Australia, with a further subdivision of Australian populations into those from Cape York Peninsula and north-east Queensland. The population from Hopevale (NQ3), geographically intermediate between Cape York and north-east Queensland, groups with CYP populations. A cluster diagram based on Nei's genetic distance and using the UPGMA algorithm (Fig. 3) shows a clear separation of most populations of *E. pellita* from *E. scias* at a relatively large genetic distance (0.271). Within *E. scias*, three populations of subsp. *apoda* cluster together, but otherwise genetic distances between populations are greater than between populations of *E. pellita*.

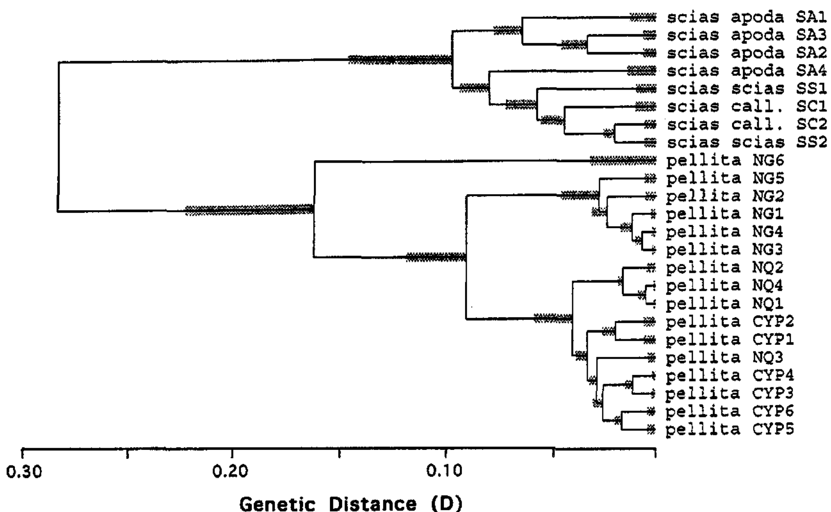

Fig. 3. Cluster diagram of *E. pellita* and *E. scias* using Nei's (1978) unbiased genetic distance and the UPGMA algorithm, and using the GD program (Ritland 1989) to fit error bars. Clusters are significant where the error bar is less than half the branch length. See Table 1 for population codes.

Table 7. Matrix of mean measures of Nei's genetic distance (*D*) between major groups of populations of *E. scias* and *E. pellita*

(*a*) Each subspecies of *E. scias* and *E. pellita* from Australia and New Guinea; and (*b*) *E. pellita* from North Queensland, Cape York Peninsula and New Guinea. [A]mean genetic distance between populations within subspecies

(*a*)

	scias scias	*scias apoda*	*scias callimastha*	Aust. *pellita*	NG *pellita*
scias scias	0.042[A]	0.085	0.042	0.241	0.369
scias apoda		0.085[A]	0.085	0.212	0.347
scias callimastha			0.048[A]	0.208	0.355
Aust. *pellita*				–	0.088
NG *pellita*					–

(*b*)

	NQ *pellita*	CYP *pellita*	NG *pellita*
NQ *pellita*	–	0.039	0.094
CYP *pellita*		–	0.086
NG *pellita*			–

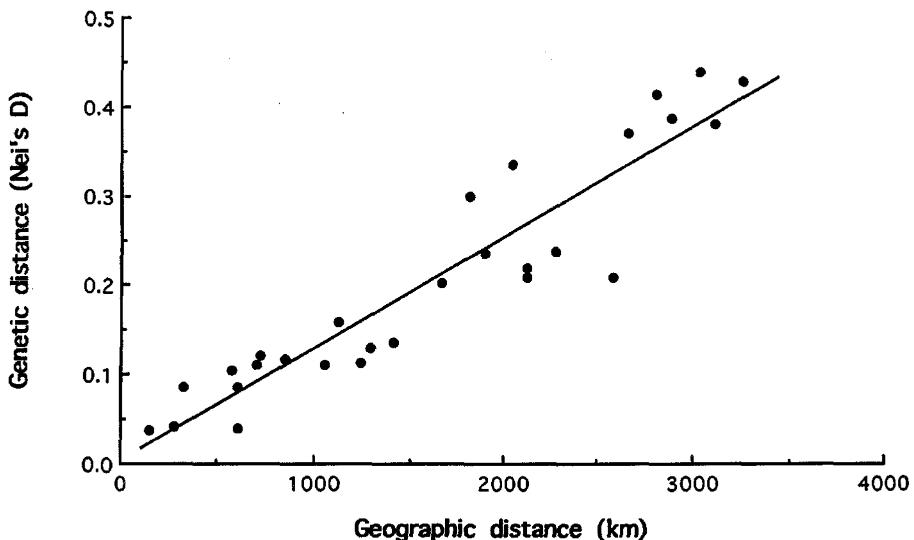

Fig. 4. Relationship between genetic distance (Nei's D) and geographic distance[A] between local centres of distribution in *E. pellita* and *E. scias*. A regression line fitted through the points gives r = 0.95, $P < 0.001$, n = 21. [A]This is defined as pair-wise estimates of the approximate straight line (great circle) distance between the centres of the main areas of species or subspecies occurrences as given in Table 1 (with the exception that populations in New Guinea are split into those from Papua New Guinea (NG1, NG2, NG3, NG4, NG6) and that from Irian Jaya (NG5)).

The mean distance between populations of *E. pellita* is 0.072 and between *E. scias*, 0.076. These figures are slightly higher than a mean for outbreeding tree species (see Gottlieb 1977; Crawford 1983), and is partly due to the combining of three subspecies of *E. scias*. The individual estimates of mean genetic distance for each subspecies are: 0.042 for *E. scias* subsp. *scias*, 0.085 for *E. scias* subsp. *apoda* and 0.048 for *E. scias* subsp. *callimastha*. Based on the cluster diagram, mean genetic distances between *E. pellita* geographic zones and *E. scias* subspecies are shown in Table 7. Genetic distance is positively and significantly correlated with geographic distance (Fig. 4).

The phylogenetic tree based on the distance Wagner procedure and using Rogers' (1972) genetic distance (Fig. 5) confirms that *E. scias* is clearly separated genetically from

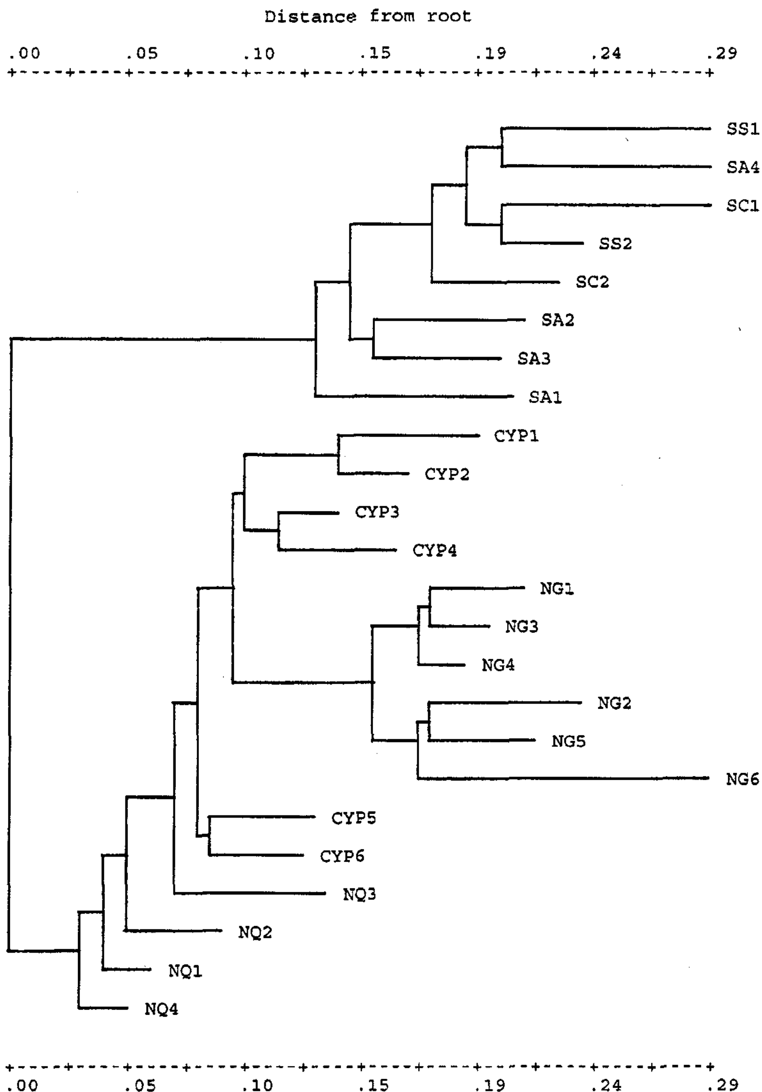

Fig. 5. Phylogenetic tree of *E. pellita* and *E. scias* based on the distance Wagner procedure and using Rogers' (1972) genetic distance. See Table 1 for population codes.

Table 8. Multi-locus estimates (t) of outcrossing in three populations of *E. pellita*

Population	No. of trees	Mean t	Family range
NQ2 NW Kuranda, Qld	10	0.73 ± 0.05	0.60–0.86
CYP4 Lankelly Ck, Qld	10	0.45 ± 0.06	0.17–0.63
NG5 Bupul-Muting, Irian Jaya	7	0.49 ± 0.08	0.42–0.55

E. pellita. Within *E. pellita,* the populations from New Guinea are clearly separated from those in Australia. Both the cluster diagram and the phylogenetic tree separate population SA4 from other subsp. *apoda* populations, which accords with the disjunction in the distribution of SA4 from the others (see Fig. 1).

Mating System in E. pellita

Estimates of outcrossing rates in three populations of *E. pellita* are shown in Table 8. These are based on four variable loci (*Pgd*-1, *Ugp*-2, *Gpi*-2, *Est*-1) for the three populations tested. The outcrossing rates for populations NG5 and CYP4 are low compared to other eucalypts. Eldridge *et al.* (1993) list outcrossing rates of the species of *Eucalyptus* that have been measured to date. In the closely related *E. urophylla,* outcrossing rates are high (0.91) with little variation between trees or populations (House and Bell 1994). Here, low rates are combined with considerable variability in individual tree rates, a feature found in other species of the genus (e.g. *E. obliqua* (Brown *et al.* 1975) and *E. pulverulenta* (Peters *et al.* 1990). The outcrossing rate for population NQ2 is closer to the overall mean for natural populations of the genus of 0.74 (Eldridge *et al.* 1993).

Discussion

Genetic Diversity and Mating System

The estimates of total genetic diversity in both *Eucalyptus pellita* and *E. scias* are within the range for trees generally (Hamrick and Godt 1989), and are comparable to other species in the genus *Eucalyptus* with similar geographic ranges. Genetic diversity is greater within populations than between, another common finding for angiosperm tree species pollinated by animals. Despite the fact that *E. scias* is a regional species in terms of its distribution (i.e. with a range between 150 and 600 km; Moran and Hopper 1987), G_{ST} is relatively low (16.7%): six other species of *Eucalyptus* with regional distributions had a mean G_{ST} of 24.9% (Moran 1992). *E. pellita* and the closely related *E. urophylla* have very similar genetic diversity statistics, except for a lower total genetic diversity (H_T) in *E. urophylla* (House and Bell 1994).

Low levels of outcrossing were noted in two of the three populations tested. In CYP4, trees were sampled over a linear distance of 20 km. The population is ecologically restricted to a narrow (< 2 km) fringe between species-rich rain-forest and open sclerophyll woodland containing other species of *Eucalyptus* and *Acacia.* In population NG5, trees were sampled over a linear distance of 6–7 km from within a large, more or less continuous stand of many thousands of individuals. Possible reasons for low levels of outcrossing in these populations include: (1) the establishment of neighbourhood structures (Loveless and Hamrick 1984); (2) failure of 'normal' eucalypt selection against selfing (Griffin *et al.* 1987); (3) asynchronous flowering within the population; (4) limited pollen movement (local foraging by pollinators) (Loveless and Hamrick 1984); (5) unusual weather conditions at the time of flowering, limiting the movement of pollen between trees. The outcrossing estimate may not therefore accurately reflect the true rate at which pollen is transported between trees (Ennos and Clegg 1982).

All or any of these factors could result in low levels of outcrossing. If these estimates of outcrossing are accurate, they have potentially significant implications for tree breeding and provenance–progeny trials. Trees which receive pollen from closely related individuals or which self-fertilise, are liable to set seeds that give rise to trees with inferior growth for commercial forestry purposes (Sedgley and Griffin 1989).

Systematic Relationships

The results of this study demonstrate some degree of concordance between classifications based on electrophoretically detectable genetic differentiation and those based on morphology. Substantial differences in allele frequencies at a number of loci were found to separate *E. pellita* from *E. scias*, and the data presented lend support to the recent splitting of *E. pellita* and *E. scias* (Johnson and Hill 1990). However, despite considerable within-species genetic diversity, isozyme divergence is lacking between *E. pellita* populations from Cape York–New Guinea and those from north-east Queensland. Genetic differentiation between *E. pellita* and *E. scias* is nevertheless small, and that between the subspecies of *E. scias* is minimal and does not reflect the new taxonomy proposed by Johnson and Hill (1990).

Genetic distances between geographic zones of *E. pellita* are comparable to those between *E. scias* subspecies. In the latter, distances are similar to those between geographic zones (proposed as subspecies) in *E. delegatensis* (Boland 1985; Moran *et al.* 1990). All three subspecies of *E. scias* are closer to Australian populations of *E. pellita* than those in New Guinea. Genetic distances between *E. pellita* and *E. scias* (0.208–0.369) are at the high end of the range for interspecific comparisons in eucalypts (Moran *et al.* 1990).

The cline in observed heterozygosity in *E. pellita*, with highest levels in the south of the species' range, perhaps gives a clue to its origin. Assuming *E. pellita* and *E. scias* share a common ancestor, this could have been centred in the southern coastal parts of New South Wales, from where it has spread and changed through genetic drift and selection. Similar clines in measures of genetic diversity have been found in *Acacia melanoxyon* R.Br. (Playford *et al.* 1993) and *Casuarina cunninghamiana* Miq. (Moran *et al.* 1989), but in the opposite direction to that in *E. pellita*.

Many widespread species of *Eucalyptus* are characterised by large disjunctions in their distributions, and in some (e.g. *E. grandis*, Burgess and Bell 1983) the most geographically separated populations are the most similar isozymically. There is a lack of adequate fossil evidence to properly document the evolutionary spread of species and species groups. Assumptions have been made about refugia (especially for species adapted to moist environments) into which species retreat in times of aridity (e.g. Pedley 1974). Presumably, species such as *E. pellita* and its relatives, have expanded and contracted their ranges to adapt to changing environments over several hundred thousand years (Nanson *et al.* 1992), leaving populations reproductively isolated from each other. Reproductive barriers between the major areas of the species' distributions are currently substantial (especially Torres Strait and the dry Laura gap around Princess Charlotte Bay). At various stages in the evolution of these taxa, these barriers may have been much less significant or perhaps, absent altogether, allowing renewed gene flow. It is during these periods that the 'core' areas of the distribution as we see it now probably became established.

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