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Variation in Outcrossing Rates and Growth in *Eucalyptus camaldulensis* from the Petford Region, Queensland; Evidence of Outbreeding Depression

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Summary

Comparison of growth rates of *Eucalyptus camaldulensis* Dehnh. in provenance/progeny trials in Thailand has revealed significant differences among families. One possible cause of differential family performance in eucalypt species with mixed mating systems is variation in the level of inbreeding. Outcrossing rates were estimated for ten trees from each of four populations in the Petford region of north-east Queensland using allozymes. They were amongst the highest recorded in eucalypts (mean t_m = 0.95) with relatively little variation among families (t_m = 0.60–1.0). Regression analyses revealed a significant association between family outcrossing rates and growth which varied among populations. A positive association was observed in one population; negative relationships in the other three populations may reflect outbreeding depression associated with hybridisation. Differences in outcrossing rates did not explain a significant level of variation in seedlot viability or survival assessed at two years of age. The high mean outcrossing rates for the four populations of *E. camaldulensis,*

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together with the low proportion of variation in growth attributed to outcrossing rate, suggest that inbreeding is unlikely to be a problem in the first generation of tree improvement using open-pollinated families sourced from natural populations in the Petford region.

Key words: Eucalyptus camaldulensis, mating system, outcrossing rate, inbreeding depression, outbreeding depression

Introduction

Differential family performance in eucalypt open-pollinated provenance/progeny trials has been attributed to possible inbreeding depression resulting from selfing and/or mating between relatives (for example Matheson and Mullin, 1987; Hodge et al., 1996). Direct comparisons of growth rates of self-pollinated and outcrossed eucalypts have demonstrated negative effects of inbreeding on seed viability and growth (for example Hodgson, 1976; Griffin and Cotterill, 1988; Hardner and Potts, 1995). A positive association between outcrossing rate and growth has also been reported in *Eucalyptus grandis*, using families selected on the basis of different levels of prior inbreeding (Burgess et al., 1996). Findings of significant differences in growth and survival among families and populations of *E. camaldulensis* Dehnh. from the Petford region, in provenance/progeny trials in Thailand (Pinyopusarerk et al., 1996), raised the question of whether this variation could be explained by differences in outcrossing rates.

Eucalyptus camaldulensis is amongst the most widely planted eucalypts with southern populations showing superior growth rates in semi-arid climates while northern populations are best suited to sub-tropical climates (Eldridge et al., 1993). Based on the superior growth performance of populations of *E. camaldulensis* occurring between 14°S and 18°S latitude and 143°E and 145°E in north-east Queensland, the Petford region has become one of the most important seed sources for plantations in the wet/dry tropics (Midgley et al., 1989; Doran and Burgess, 1993). Despite extensive plantings of *E. camaldulensis* in Asia, South America, the Middle East and Africa and the establishment of breeding programs (reviewed in CABI, 2000), reports of outcrossing rates in this species have been limited to a single population (Moncur et al., 1995; McDonald et al., 1996). The species is hermaphroditic and insect pollinated with bees, both native (*Trigona* spp.) and honey bees (*Apis mellifera*), the main pollinators (Visuthitepkul and Moncur, 1993). In common with other eucalypts, *E. camaldulensis* has a mixed mating system. Individual flowers are protandrous but geitonogamous pollinations can occur as flowers on the same tree develop at different times (Moncur et al., 1995). The outcrossing rate for seed crops from individual trees is influenced by a variety of genetic and environmental factors including self-incompatibility (Visuthitepkul and Moncur, 1993), flowering phenology relative to neighbouring trees in the stand (Mullin and Pswarayi, 1990), heterogeneity in the pollen pool (Fripp et al., 1987) and ecological factors which influence pollinator activity (Griffin and Cotterill, 1988; Moncur et al., 1995).

Outcrossing rates determine the coefficient of relationship which is used in calculations of heritability and expected genetic gain from open-pollinated progeny trials. Reports of moderate levels of inbreeding in eucalypts (for example Brown et al., 1975) led Eldridge (1976) to suggest that genetic improvement may be less than expected using recurrent selection from open-pollinated seed orchards. Inbreeding would reduce the number of new combinations of genotypes on which future genetic gain by selection depends, whether from seed orchards or plantations. In addition, when populations are partially inbred and exhibit inbreeding depression for fitness components, the quantitative genetic parameters estimated may not reflect the evolutionary potential of these populations (Willis, 1996). Additive genetic variance will be over-estimated if the parental population is structured (leading to heterogeneity in the pollen pool), or there is a substantial degree of inbreeding (Squillace, 1974). This, in turn will bias estimates of heritability and genetic gain.

The objective of this study was to estimate outcrossing rates in four populations of *E. camaldulensis* from the Petford region of north-east Queensland, using allozyme analysis of open-pollinated progeny arrays. The relationship between outcrossing rates in individual families and their performance in progeny trials in Thailand was then examined. The likely impact of variation in outcrossing rates in *E. camaldulensis* breeding programs was also assessed.

Material and Methods

Genetic material

Four populations of *E. camaldulensis* from the Petford region of northern Australia were selected based on differences in volume among families in provenance/progeny trials in Thailand (Pinyopusarerk et al., 1996). Populations were selected which represented the range in volume production recorded from two-year-old plants, from 8.6 x 10^{-3} m^3/tree for Petford to 6.3 x 10^{-3} m^3/tree for Hales Siding. Families within each population were ranked into five volume classes and two families selected from each class based on their consistency of performance across three trial sites. The population names, seedlot number, location and elevation are listed in *Table 1*. Seedlot numbers refer to the Australian Tree Seed Centre (ATSC) database, CSIRO Forestry and Forest Products (http//www.ffp.csiro.au/tigr/atscmain/index.htm). The same seedlots were used to estimate outcrossing rates as were used to establish the provenance trials.

Table 1. – Populations names, CSIRO seedlot number and description of sampling sites. One family was selected from each seedlot except where noted in parentheses.

Population	Seedlot Number	Latitude (South)	Longitude (East)	Elevation (m)
Eureka	14246,14279,14280,14281	17°16’	145°05’	500
	14286,14289	17°12’	145°04’	480
	14297,14305	17°15’	145°05’	520
	16537 (2 families)	17°11’	145°03’	460
Hales Siding	14309,14310,14312,14319,14321, 14326,14328,14331,14332,14336	17°22’	145°12’	780
Montalbion	14365,14366	17°25’	145°11’	700
	14370,14371	17°24’	145°10’	680
	14374,14375,14377,14378,14379	17°24’	145°09’	680
	14382	17°25’	145°06’	560
Petford	14340,14341,14342,14343,14345, 14346,14347	17°21’	144°57’	460
	16720 (3 families)	17°25’	145°01’	600

Allozyme analysis

Seeds were germinated in potting mix (tanbark, sand, vermiculite, perlite mixed in a 3:1:1:1 ratio) and seedlings allowed to develop to the first leaf-pair stage (2–4 weeks). Forty seedlings from each of ten trees were assayed from four populations using starch gel electrophoresis. Seedlings were ground in a 0.06 M borate extraction buffer (pH 9.0) containing 1 mg ml^{-1} dithiothreitol and polyvinylpyrrolidone (PVP, mol. wt = 40000). Electrophoresis was conducted using standard procedures described by MORAN and BELL (1983). Twelve loci from eight enzyme systems were resolved using three electrode buffer systems: 1. histidine, pH 8.0; malic enzyme (ME) E.C.1.1.1.82; 2. lithium borate, pH 8.2; aspartate amino-transferase (AAT) E.C.2.6.1.1 (3 loci); 3. morpholine citrate pH 6.1; glucosephosphate isomerase (GPI) E.C.5.3.1.9 (1 locus); isocitric dehydrogenase (IDH) E.C.1.1.1.4.2 (1 locus); malic dehydrogenase (MDH) E.C.1.1.1.37 (2 loci); phosphoglucose isomerase (PGI) E.C.5.3.1.9 (1 locus); phosphogluconic dehydrogenase (PGD) E.C.1.1.1.44 (1 locus) and uridine diphosphogluconic pyrophosphatase (UGP) E.C.2.7.7.9 (2 loci).

Genetic interpretation of electrophoretic variants was based on segregation patterns in progeny arrays from open-pollinated families. Loci within each enzyme system and alleles within a locus were numbered according to their migration rates, beginning with the fastest migrating locus/allele designated number 1.

Data analysis

Genetic diversity

Allele frequencies calculated for the 12 loci in each population were used to compute the following genetic diversity measures using the BIOSYS–1 package (SWOFFORD and SELANDER, 1989): mean number of alleles per locus (A); observed heterozygosity (H_o); and unbiased expected heterozygosity (H_E) (NEI, 1978).

Mating system

Mating system estimates were based on the segregation of alleles at 11 polymorphic loci. Chi-square goodness-of-fit tests were performed at each locus in each population to determine whether observed heterozygosity differed from expected heterozygosity for the parental genotypes (data not shown). Ugp-3 was the only locus with high chi-square values in all populations and was therefore omitted from the analysis. Single locus (t_s) and multilocus outcrossing rates (t_m) were estimated for each population using the mixed mating model. Maternal genotypes were inferred from progeny arrays following BROWN and ALLARD (1970). Multilocus estimates of outcrossing were compared with the mean of single locus estimates to give the lower bound for selfing due to biparental inbreeding (BROWN, 1990). The correlation of outcross paternity (r_p) was estimated following RITLAND'S (1989) sibling-pair model, in which r_p is the probability that two individuals drawn at random from the same progeny array are full sibs. These parameters, and the average single-locus inbreeding coefficient of maternal parents (F), were calculated using the MLTR computer program (Version 1.1) (RITLAND, 1996). The NEWTON-RAPHSON method was used for maximising the likelihood equation for parameters t, F and r_p. Standard errors for population estimates of t_m, t_s and r_p were based on 100 bootstraps re-sampling progeny arrays, and family estimates of t_m on 500 bootstraps, re-sampling progeny within a family.

Association between growth and outcrossing rate

Relationships between outcrossing rates and height, diameter over bark at 1.3 m and survival, measured 24 months after planting, were examined using data from provenance/progeny trials established at three sites in Thailand. Estimated plot means and variances for height, diameter and survival for the 40 families were obtained from the REML analyses of each of the three sites, as described by PINYOPUSARERK et al. (1996). These were analysed using the model

$$Y_{ij} = s_i + p_k + \beta_k x + f_j + sf_{ij}$$

where Y_{ij} are the estimated means at site i (s_i,i = 1...3) for family j (f_j, j = 1...40); the p_k (k = 1...4) are parameters for the mean values of the populations, the β_k are coefficients for the regression of outcrossing rate (x) on families within each population and the sf_{ij} are parameters for the site by family interaction. This model is an extension of that used by BURGESS et al. (1996); in essence it submodels the family main effects using populations and regressions within populations. In other words, we have a simple linear regression for each population (p + Bx) to allow for the possibility of differential responses to outcrossing rate among populations. If the site by family interaction was significant then this term could also be submodelled.

For the analyses, the numbers of replicates in each trial for which the families were present (maximum 15) were used as weights in the analysis (as discussed by WILLIAMS and MATHESON, 1994, Chapter 8) and so the average residual mean squares from the individual site analyses (see *Table 2* of PINYOPUSARERK et al., 1996) could be directly used for testing.

The relationship between outcrossing rate and seedlot viability was examined using the same model without the terms involving sites. Seedlot viability was from the ATSC database and was estimated following procedures of the International Seed Testing Association (1985).

Results

Genetic diversity

The level of heterozygosity ranged from 0.25 in the Petford population to 0.28 in Hales Siding (*Table 2*). While heterozygosity estimates would be expected to be inflated by selecting polymorphic loci for the analysis of mating systems, these levels were consistent with values recorded in the Lake Albacutya population of *E. camaldulensis* (H_E = 0.25) in southern Australia (MCDONALD et al., 1996). Heterozygosity was considerably lower than the value of 0.42 reported by ARADHYA and PHILLIPS (1995) for two populations of *E. camaldulensis*, estimated using eight allozyme loci. Seed sources for the latter study included collections from an unknown number of parent trees at Cape River, Queensland and a second unidentified Australian provenance.

Table 2. – Estimates of genetic diversity (standard errors in parentheses) for populations of *E. camaldulensis*. A = mean number of alleles per locus; H_o = observed heterozygosity; H_E = mean expected heterozygosity assuming HARDY-WEINBERG equilibrium.

Population	Sample size	A	Mean heterozygosity	
			H_O	H_E
Eureka	396	4.83 (1.9)	0.251 (0.049)	0.277 (0.057)
Hales Siding	398	4.58 (1.3)	0.278 (0.057)	0.280 (0.057)
Montalbion	397	4.83 (1.1)	0.260 (0.055)	0.260 (0.054)
Petford	399	4.75 (0.9)	0.232 (0.048)	0.249 (0.050)

Mating system

The estimated multilocus outcrossing rates for the four populations of *E. camaldulensis* were all high (*Table 3*). The mean outcrossing rate (t_m = 0.95) is the highest yet recorded in the genus but is similar to that reported for another tropical eucalypt, *E. urophylla* (t_m = 0.91) (HOUSE and BELL, 1994). There were differences among populations in the range of family outcrossing rates. All families in Montalbion were completely outcrossing while families in Eureka were more variable, ranging from 0.60 to 1 (*Table* 4). Outcrossing rates in one family in each of Petford and Hales Siding differed significantly from $t_m = 1$.

Comparison of multi-locus and single-locus outcrossing rates in the three populations which had outcrossing rates less than one revealed little evidence of biparental inbreeding (*Table 3*). The maternal inbreeding coefficients (*F*) were low (*Table 3*) and deviation from random mating was statistically significantly only for Eureka. The level of correlated paternity was also highest in the Eureka population (*Table 3*). Only three loci (*Mdh*-1, *Gpi*-2 in Eureka; *Mdh*-1 and *Aat*-1 in Hales Siding) had more than three alleles with frequencies greater than 0.1. These loci contribute a disproportionate amount of information about correlated mating (RITLAND, 1989).

Table 3. – Estimates of multi-locus (t_m) and mean single-locus outcrossing rate (t_s); the average single-locus inbreeding coefficient of maternal parents (F); and correlated paternity (r_p) in four population of *E. camaldulensis* (standard errors in parentheses).

Population	t_m(se)	t_s(se)	$t_m - t_s$ (se)	F(se)	r_p (se)
Eureka	0.912 (0.035)	0.878 (0.040)	0.035 (0.019)	-0.396 (0.074)	0.365 (0.117)
Hales Siding	0.964 (0.022)	0.936 (0.025)	0.027 (0.013)	0.025 (0.195)	0.119 (0.094)
Montalbion	0.995 (0.020)	0.974 (0.040)	0.021 (0.026)	0.031 (0.157)	0.126 (0.064)
Petford	0.940 (0.022)	0.906 (0.037)	0.033 (0.021)	-0.057 (0.134)	0.079 (0.020)

Table 4. – Population names, family number and CSIRO seedlots (number and viability) used to estimate outcrossing rate (t_m) for *E. camaldulensis* (standard errors in parentheses). Family means for height and diameter over bark 1.3 m above base (dbh) are derived from a combined analysis of progeny trials at three sites in Thailand, assessed 2 years after planting (PINYOPUSARERK et al., 1996).

Population	Family	Seedlot	Viability (seeds/g)	Height (m)	dbh (cm)	Survival (%)	t_m(se)
Eureka	10	14246	610	7.29	5.15	100	0.60 (0.12)
	43	14279	2010	6.64	5.00	97.8	1.11 (0.12)
	44	14280	320	7.41	5.11	95.6	0.86 (0.09)
	45	14281	510	8.21	5.66	100	1.55 (0.21)
	50	14286	180	8.01	5.59	97.8	1.03 (0.31)
	53	14289	570	7.46	5.17	100	0.83 (0.08)
	61	14297	1070	7.31	5.15	97.8	0.88 (0.10)
	69	14305	1020	7.83	5.62	97.8	1.55 (0.23)
	168	16537	300	7.67	5.54	97.8	0.86 (0.09)
	170	16537	390	7.70	5.11	97.8	1.04 (0.36)
Hales Siding	73	14309	590	6.98	4.83	100	1.07 (0.15)
	74	14310	810	6.92	4.59	93.3	1.14 (0.13)
	76	14312	1040	7.03	4.94	88.9	0.95 (0.15)
	83	14319	1080	6.98	4.74	100	0.99 (0.19)
	85	14321	1100	7.46	5.14	100	1.08 (0.24)
	90	14326	1780	7.47	5.48	97.8	0.91 (0.09)
	92	14328	1460	7.08	5.00	95.6	1.11 (0.25)
	95	14331	760	7.31	5.14	100	0.78 (0.09)
	96	14332	730	6.74	4.85	100	0.95 (0.33)
	100	14336	910	6.73	4.88	97.8	1.15 (0.18)
Montalbion	127	14365	910	7.10	5.05	100	1.00 (0.24)
	128	14366	630	7.11	5.15	97.8	1.07 (0.09)
	132	14370	730	7.37	4.80	97.8	1.40 (0.18)
	133	14371	540	7.33	5.40	95.6	0.97 (0.10)
	136	14374	690	7.45	5.45	100	1.14 (0.23)
	137	14375	870	6.38	4.43	95.6	1.15 (0.08)
	139	14377	770	6.92	4.97	97.8	1.41 (0.35)
	140	14378	830	7.73	5.51	100	1.07 (0.22)
	141	14379	830	7.44	5.09	100	1.04 (0.18)
	144	14382	1200	7.25	5.33	95.6	1.12 (0.35)
Petford	102	14340	630	7.98	5.65	100	1.05 (0.13)
	103	14341	850	8.02	5.55	97.8	0.90 (0.16)
	104	14342	580	8.00	5.72	97.8	0.79 (0.10)
	105	14343	1040	7.66	5.28	97.8	1.17 (0.19)
	107	14345	720	8.62	6.15	100	0.93 (0.08)
	108	14346	460	7.79	5.77	88.9	0.96 (0.09)
	109	14347	650	7.65	5.43	95.6	0.98 (0.16)
	183	16720	510	8.08	5.71	97.8	1.05 (0.09)
	184	16720	640	7.89	5.57	91.1	0.96 (0.12)
	186	16720	870	8.15	5.60	93.3	1.26 (0.19)

Outcrossing rates and growth performance

The submodel for families showed that the regression of families within populations on outcrossing rate was highly significant for both height and diameter. The site by family interaction was non-significant for both height and diameter, hence there was no need to further model this component. This contrasts with the significant site x family interaction in the PINYOPUSARERK et al. (1996) data set and reflects the fact that, in the current study, families were selected on the basis of consistency of performance across sites. The analyses of variance for height and diameter are presented in *Table 5*. The relationship between outcrossing rate and growth (height and diameter) differed among the four populations with a positive relationship in the Eureka population and negative relationship in the other three populations (*Figure 1*). The estimated

Table 5. – Analysis of variance for mean height and mean diameter per tree of 40 families of *E. camaldulensis* assessed at 24 months using a family submodel (df = degrees of freedom).

		Mean height			Mean diameter		
Source	df	Sum of squares	Mean squares	Variance ratio.	Sum of squares	Mean squares	Variance ratio.
Site	2	3232.34	1616.17	1346.81***	1696.53	848.26	877.21***
Family	39	394.81	10.12	8.44***	221.29	5.67	5.87***
Population	3	210.89	70.30	58.58***	108.59	36.20	37.43***
t_m.population	4	24.02	6.01	5.00***	26.07	6.52	6.74***
Family remainder	32	159.90	5.00	3.31***	86.62	2.71	2.85***
Site.family	78	117.65	1.51	1.26^{ns}	74.11	0.95	0.98^{ns}
Pooled residual#			1.20			0.967	

#Average of residuals from trials at three sites in Thailand, listed in *Table 2,* PINYOPUSARERK et al. (1996).
***statistically significant (P<0.001); ^{ns} not significant

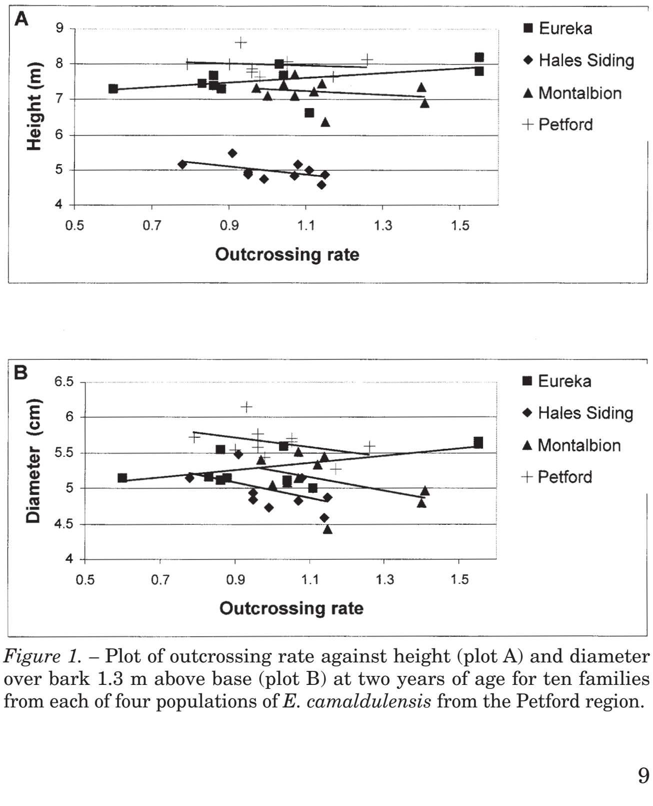

Figure 1. – Plot of outcrossing rate against height (plot A) and diameter over bark 1.3 m above base (plot B) at two years of age for ten families from each of four populations of *E. camaldulensis* from the Petford region.

regression coefficients with t-values are presented in *Table 6*. Survival rates differed among populations and sites ($P < 0.001$), but there was no association with outcrossing rate. Similarly, no association was evident between outcrossing rate and the plot variances of height and diameter or seedlot viability.

Table 6. – Estimated regression coefficients and t-statistics for variation in mean family height and diameter accounted for by outcrossing rates (t_m) in each of four populations of *E. camaldulensis.*

	Mean height		Mean diameter	
	Regression coefficient	t	Regression coefficient	t
t_m.Petford	-0.260	-1.28^{ns}	-0.676	-2.98**
t_m.Eureka	0.685	7.66***	0.510	5.17***
t_m.HalesSiding	-0.923	-3.87***	-1.048	-4.02***
t_m.Montalbion	-0.488	-2.64**	-0.954	-4.65***

***statistically significant ($P< 0.001$); ** ($P < 0.01$); ^{ns} not significant

Discussion

Mating system

The high effective outcrossing rate in *E. camaldulensis* suggests mechanisms are operating to prevent self-pollination or survival of selfed embryos. Self-pollination is avoided at the single flower level as pollen is shed prior to operculum abscission and before the stigma becomes receptive (VISUTHITEPKUL and MONCUR, 1993). However, flowers in an umbel vary in the stage of floral development, allowing vectors to contact a receptive stigma and self-pollination to occur (geitonogamy). Anthers of *E. camaldulensis* are held within a staminal column which may improve pollen dispersal by wind (VISUTHITEPKUL and MONCUR, 1993), thereby increasing the probability of outcrossing. High flower density and its affect on the foraging behaviour of pollinators, high pollen competition, and abortion of selfed embryos may also play a role in maintaining high outcrossing rates (GRIFFIN et al., 1987; SEDGLEY and GRANGER, 1996).

Selfed individuals have been produced from controlled pollinations of three families from the Petford region, which survived to at least twelve months of age (DORAN cited in HARWOOD, 1993). These individuals performed poorly when compared to outcrossed progeny from the same families. However, there were differences among the families in the level of inbreeding depression (calculated as $[1\text{-}(w_s/w_o)]$ where w_s and w_o are the mean fitness of selfed and outcross progeny respectively), ranging from 25% to 41% for height and 24% to 47% for survival at twelve months of age. The fact that viable seed can be produced following controlled self-pollinations, although there is little evidence of selfing in native stands suggests selection is occurring in favour of outcrossed embryos. This is consistent with models of post-zygotic incompatibility and maternal resources allocation proposed by WESTOBY and RICE (1982) and modified by SEAVEY and BAWA (1985), GRIFFIN et al. (1987) and JAMES and KENNINGTON (1993). A survey of mature trees in natural populations of *E. camaldulensis* using RFLP markers reported no evidence of inbreeding (BUTCHER et al., 2002). This indicates selection against selfs may continue during stand development.

The high outcrossing rate in the Petford populations of *E. camaldulensis* compared to other eucalypts may reflect more efficient pollen dispersal in these populations. The lack of evidence of consanguineous mating contrasts with reports of neighbourhood structure in natural populations of many other eucalypts (for example *E. rhodantha* in SAMPSON et al., 1989; *E. marginata* in MILLAR et al., 2000). The outcrossing rate in *E. camaldulensis* was similar to that recorded in a *E. regnans* seed orchard (t_m =0.91) (MORAN et al., 1989) which was significantly higher than in adjacent natural stands (t_m = 0.74). This was attributed to the breakdown of neighbourhood structure in the seed orchard. *Eucalyptus camaldulensis* is typically confined to riverine habitats and neighbourhood structuring within populations may be less likely to develop due to effective seed dispersal along watercourses. Pollinator activity is also important, with a higher concentration of birds and insects expected in habitats where water is readily available. Increasing the density of pollinators has been shown to increase outcrossing rates in a natural population of *E. camaldulensis* from t_m = 0.75 to 0.94 (MONCUR et al., 1995).

The range of values of family outcrossing rates in *E. camaldulensis* was less than reported for most other widespread eucalypts, for example *E. regnans* t_m = 0.23–0.82 (GRIFFIN et al., 1987); *E. pellita* t_m = 0.17–0.86 (HOUSE and BELL, 1996); with the exception of the tropical species *E. urophylla* t_m = 0.83–0.96 (HOUSE and BELL, 1994). The narrow range of values in the *E. camaldulensis* populations is consistent with the proposition of more effective pollen dispersal in this species. The population with the widest range in outcrossing rates, Eureka, was the only population with significant levels of biparental inbreeding and had the highest level of correlated paternity. The lower outcrossing rates in some individuals in this population most likely reflect a limited number, or differential success, of pollen donors.

Outcrossing rates and growth

Differences among families in height and diameter of two-year-old trees from the Petford region in provenance/progeny trials in Thailand (PINYOPUSARERK et al., 1996) were associated with differences in outcrossing rate. However, differences among families were not accounted for by outcrossing rate alone. In the regression analysis after removing the effects of outcrossing rate there remained significant differences among families (*Table 5*). Outcrossing rate accounted for approximately 13% and 23% of variation among families within populations in height and diameter respectively.

A stronger association may have been expected given the reduced fitness of progeny from controlled-self-pollinations in *E. camaldulensis* and other eucalypts; for example depression in height growth of 8–49% in selfed compared with cross-pollinated *E. grandis* seedlings (HODGSON, 1976); 26% in height of 43-month-old *E. globulus* (HARDNER and POTTS, 1995); 12% in height of 30-month-old *E. regnans* (GRIFFIN and COTTERILL, 1988) and 28% in height of two-year-old *E. nitens* (HARDNER and TIBBITS, 1998). A possible explanation for the relatively low proportion of variation explained by outcrossing rate in *E. camaldulensis* is that the number of inbred progeny was too low to affect family means. BURGESS et al. (1996) reported a significant positive correlation between outcrossing and growth in *E. grandis*, but their plot of family mean height vs outcrossing rate indicates lower growth rates were recorded only in families where $t_m < 0.6$. Several comparative studies of inbreeding depression in control-pollinated families with different inbreeding coefficients have found little evidence of inbreeding depression for inbreeding coefficients $f < 0.25$ (GRIFFIN and LINDGREN, 1985; WILLIS, 1993). Using the formula $t = (1–f)/(1+f)$ (WEIR, 1996) this is equivalent to $t < 0.6$. In the current study, only four of the 40 families had outcrossing rates which differed significantly from one and no families had outcrossing rates less than 0.6. The only population with a positive relationship between outcrossing rate and growth parameters, Eureka, had the widest range in outcrossing rates.

While direct relationships between selfing and growth or survival have been demonstrated, attempts to find a relationship between prior inbreeding (*f*) or heterozygosity and growth in individual trees have often been unsuccessful (Aradya and Phillips, 1995; Savolainen and Hedrick, 1995; Aravanopoulos, 2000). Associations between outcrossing rate and family performance are complicated by the fact that the degree of inbreeding depression can vary substantially among families (Hodgson, 1976; Griffin and Cotterill, 1988; Wu et al., 1998). In *E. globulus* lower parental breeding values were generally associated with lower outcrossing rates (Hardner et al., 1996) but a significant correlation between outcrossing and growth was not demonstrated. Similarly, Hardner and Potts (1997) found no correlation between estimated outcrossing rate and genetic load of the parent in *E. regnans.* Morgan and Barrett (1990) and Ivey and Wyatt (1999) suggest the large statistical error in family outcrossing rates means it will be difficult to detect associations with parameters such as growth or floral traits. However, the use of large progeny arrays in the current study reduced the variance of family estimates.

The negative regression coefficients for three of the four populations of *E. camaldulensis* in the Petford region demonstrate that outcrossing does not always enhance fitness. Matings between dissimilar individuals may disrupt favourable gene combinations and lead to outbreeding depression (Falconer, 1989). Outbreeding depression in plants has been documented in crosses between species, varieties and isolated populations (Waser and Price, 1989). However, negative relationships between outcrossing rates and growth have not previously been reported in eucalypts. Outbreeding depression in the Petford, Montalbion and Hales Siding populations may be associated with hybridisation between *E. camaldulensis* and *E. tereticornis*. The distributions of the two species overlap in this region and populations have been reported to contain individuals from both species together with hybrids (Brooker and Kleinig, 1994; Doran and Burgess, 1993). Molecular evidence of hybridisation has also been reported (Butcher et al., 2002). There are no apparent barriers to interbreeding between *E. tereticornis* and *E. camaldulensis*; the two species flower synchronously in eastern Australia and viable seed has been produced following controlled cross-pollination (Doran and Burgess, 1993; Venkatesh and Thapliyal, 1993). The influence of *E. tereticornis* is thought to increase from least in Petford to some influence in Montalbion, with the strongest influence in Hales Siding (Doran, CSIRO Forestry and Forest Products, unpub. data). Similarly, the regression coefficients ranged from the least negative value in Petford to the most negative value in Hales Siding.

Hybridisation would result in a downward bias in estimated outcrossing rates if there is disequilibrium between alleles associated with species differences, and disassortative mating among species. Estimates of outcrossing rate, using the mixed mating model, are based on the assumption that outcrossed individuals in each progeny array are derived independently from a panmictic pollen pool in a population having identical mating systems. With hybridisation, the assumptions of homogeneity of pollen alleles may be violated. However, there was no evidence of a departure from random mating or of subpopulation structure in the three *E. camaldulensis* populations for which a negative association between outcrossing rate and growth was recorded.

Implications for Breeding Strategies

The high mean outcrossing rate for the four populations of *E. camaldulensis* indicate breeding programs based on seed production from open-pollinated seed orchards are unlikely to be adversely affected by inbreeding. While the effect of outcrossing rate on height and diameter was significant, most variation among families remained unexplained. Based on a near two-fold range in outcrossing estimates of open-pollinated *Pinus monticola* trees from a natural stand, El Kassaby et al. (1987) suggested a refined adjustment in family coefficients of relatedness may be necessary if more precise genetic information is to be obtained from open-pollinated progeny tests or from controlled crosses between parents of unknown relatedness. Ranking of parent trees for measurable traits may otherwise reflect ephemeral differences in the proportions of inbred progeny rather than genotypic superiority. Similarly, Hardner et al. (1996) proposed that the precision of predicting breeding values from open-pollinated progeny would be improved by adjusting family performance according to their outcrossing rates. This, however, ignores the reported differences in the level of inbreeding depression among maternal families (for example Griffin and Cotterill, 1988; Wu et al., 1998) and differences in the magnitude of inbreeding depression in different traits (Hardner and Tibbits, 1998). Based on the low level of variation in outcrossing rates in the Petford region, and the relatively low proportion of variation explained by differences in outcrossing rates, such adjustments would appear unnecessary in breeding programs for tropical *E. camaldulensis*. The finding of a negative relationship between growth and outcrossing rates in the Petford region suggests the need for caution when using seed sourced from populations which may include hybrids. Outbreeding depression could well explain the overall poor performance of the Hales Siding population in trials in Thailand (Pinyopusarerk et al., 1996).

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