

Acknowledgments

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Stand Density Influences Outcrossing Rate and Growth of Open-Pollinated Families of *Eucalyptus globulus*

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Summary

Using isozymes markers, we test BORRALHO and POTTS' (1996) hypothesis that poorer growth of open-pollinated (OP) families of *Eucalyptus globulus* grown from seed collected from less dense stands is a consequence of lower outcrossing rates. A significant relationship between stand density class and outcrossing rate was found, with outcrossing rates near one for families from closed forests. In support of BORRALHO and POTTS' (1996) hypothesis, lower parental breeding values were generally associated with lower outcrossing rates. This is no doubt due to a higher proportion of less vigorous selfs within an OP family depressing mean growth relative to families where inbred progeny were virtually absent. It is suggested that the precision of predicting breeding values with OP progenies may be improved by adjusting family performance for individual outcrossing rate.

Key words: Inbreeding depression, breeding values, selfing rate, mating system.

FDC: 232.11; 165.41; 176.1 *Eucalyptus globulus*.

Introduction

In a recent paper, BORRALHO and POTTS (1996) suggested that the growth of native forest open-pollinated (OP) families of

Eucalyptus globulus was significantly affected by the density of the stand from which the seed was collected. They argued that differences in stand density would influence outcrossing rates and that accounting for this would improve heritability estimates and the prediction of breeding values. The density of the stand surrounding each parent was classified on a four point scale with 1 representing isolated parent trees (i.e. no observable neighbours) to 4 being for parent sampled from closed continuous forests. Classification was based on photographs taken at the time of the original seed collection because it was impractical to relocate all 596 parent trees used in the analysis. Families from parents classified as isolated were on average significantly smaller at 4 years than families of parents from closed forests. BORRALHO and POTTS (1996) suggested this trend could result from selection for faster growth in closed forests, but was more likely due to inbreeding depression caused by reduced outcrossing rates at lower stand densities.

Eucalypts are pollinated by a variety of generalist insects and animals (GRIFFIN, 1980). There is ample opportunity for self pollination and at least some seed is set following controlled self pollination (GRIFFIN *et al.*, 1987; HARDNER and POTTS, 1995). Estimates of outcrossing rates for individuals trees vary

between 0.26 and 1.00 (GRIFFIN *et al.*, 1987; MORAN *et al.*, 1989; BURGESS *et al.*, 1996). A relationship between outcrossing rate and stand density has been suggested by several isozyme studies. Lower estimates have been obtained from isolated parents or very small populations compared to larger stands of the same species (SAMPSON *et al.*, 1989, 1995; PETERS *et al.*, 1990).

Inbreeding depression is severe in *E. globulus* with selfs being 25% smaller in diameter at 4 years than controlled cross progenies from the same parents (HARDNER and POTTS, 1995). Variation in inbreeding depression, due to the variation in outcrossing rate, may therefore obscure differences in breeding values between parents when estimated with OP families (BURGESS *et al.*, 1996). This may partly explain inflated OP heritability estimates and the poor correlation between breeding values predicted from controlled outcross and native stand OP families (HODGE *et al.*, 1996). In this paper, we test BORRALHO and POTTS' (1996) hypothesis that differences in the growth of their *E. globulus* OP families between stand density classes reflect differences in outcrossing rate.

Materials and Methods

The genetic material for this study was a subset of the 596 *E. globulus* OP families studied by BORRALHO and POTTS (1996). Two families were chosen from each of 4 localities (West Cape Barren Island, King Island, Central Flinders Island, and Moogara), one where the parent had been classified as being isolated with no observable neighbours (stand density class 1) and one where the parent had been classified as coming from a closed continuous forest (stand density class 4, except for King Island where the forest was open and continuous, stand density class 3). Progenies from the 596 families had been planted in 5 trials across Northern Tasmania in 1989. Spacing between plants was 4 m x 2.5 m. Diameters were measured at 4 years after planting and the mean of each parent was predicted across sites as discussed in BORRALHO and POTTS (1996) with their full data set and without their term for stand density in the genetic model.

In each of the 8 families selected for study, between 34 and 73 seed from stored samples of the original collection were assayed for allozyme polymorphisms using starch gel electrophoresis (WENDEL and WEEDEN, 1989). Six to 12 day old germinates were ground in 0.1 M Tris-HCl, pH 8.0, containing 10% glycerol, 10% PVP-40T, 0.1% mercaptoethanol and 0.2% Triton X100. A lithium borate-Tris citrate, pH 8.1 system was used to assay the enzyme systems GPI, ACP, EST, AAT and LAP; and a histidine-citric acid pH 6.5 system to assay for SKD and PGD (VAILLANCOURT *et al.*, 1993). Enzyme specific staining followed WENDEL and WEEDEN (1989).

One zone of activity was noted for SKD and LAP, 2 zones for GPI, ACP, and PGD and 3 zones for AAT. There were multiple zones for the EST, however, only the most anodal was considered. For ACP, only the most cathodal zone was considered. These zones of activity were scored as different loci. GPI-1 and PGD-2 were monomorphic. Two allozymes were scored for AAT-3, AAT-2 and ACP-2; 3 allozymes for AAT-1 and LAP; 4 allozymes for PGD-1; and 5 allozymes for SKD, GPI-2, and EST-1. Outcrossing rate for each parent was estimated by expectation maximisation with an updated version of RITLAND's (1990) MLT program.

Results and Discussion

Individual tree outcrossing rate estimates for native forest *E. globulus* OP families varied between 0.48 and 1.00 (Table 1) and are within the range of other estimates for native forest

OP families (GRIFFIN *et al.* 1987; BURGESS *et al.*, 1996) and seed orchard families (MORAN *et al.*, 1989). Across localities, parents classified as isolated (stand density class 1) had significantly lower outcrossing rates than parents from open or closed forests (class 3 and 4) (paired t test, $t_3 = 6.25$, $P < 0.01$; Table 1). Inbred progeny were virtually absent from all closed forests families (class 4). Although BORRALHO and POTTS (1996) classified stand density from photographs, they were generally successful in differentiating between stand densities. Lower outcrossing rates in less dense stands is no doubt due to reduced inter-tree movements by pollinators (KARRON *et al.*, 1995), however, even families from our isolated parents contained a considerable proportion of outcross progeny.

There was considerable variation in outcrossing rate within stand density classes (Table 1). In particular, the 2 trees from King Island had much lower outcrossing rates than trees from comparable classes in other localities. This is probably due to difficulties in specifying stand density from photographs. After relocating some of the trees, the King Island parents were found to be more isolated than comparable parents in other localities. The class 1 tree was approximately 100 m from any other *E. globulus*, while the open forest tree (class 3) was in a patch of 4 trees within 50 m of each other but isolated by about 300 m from any other *E. globulus*.

Table 1. – Multilocus outcrossing rate estimates (t) and bootstrap standard errors (s.e., estimated from 50 bootstraps) of individual trees from 4 localities and different stand density classes (after BORRALHO and POTTS, 1996). Also shown are the number of polymorphic loci (from the 11 assayed) and the average number of progeny per loci used for the estimation of t.

Locality	Stand density class	No. loci	Ave no. progeny	t	s.e.
Moogara	M1 - Isolated	6	40	0.81	0.17
	M4 - Closed forest	7	40	1.00	0.00
Central Flinders Island	F1 - Isolated	5	42	0.68	0.15
	F4 - Closed forest	8	43	0.98	0.03
West Cape Barren Island	W1 - Isolated	7	25	0.81	0.17
	W4 - Closed forest	6	35	0.99	0.00
King Island	K1 - Isolated	5	69	0.48	0.13
	K3 - Open forest	4	46	0.63	0.16

The association between outcrossing rate and stand density may be improved if the stand is described at the time of seed collection (BORRALHO and POTTS, 1996), particularly a record made of the distance to neighbouring trees and their number. However, lower outcrossing rates may also be a consequence of temporal isolation due to variation in phenology (FRIPP *et al.*, 1987), lower self fertility (GRIFFIN *et al.*, 1987) or lower pollinator density (MONCUR *et al.*, 1995). Further research is needed to quantify how these factors may influence outcrossing rate within and between trees, and between seasons.

This study supports the BORRALHO and POTTS (1996) hypothesis that the poorer growth of OP families from less dense stands is a consequence of lower outcrossing rates. OP families with a smaller mean diameter were consistently associated with lower outcrossing rates and lower stand density in the Central Flinders Island, Moogara and West Cape Barren localities (Figure 1), no doubt due to a high proportion of less vigorous selfs within an OP family depressing mean growth

relative to families where inbred progeny were virtually absent. The similarity in mean diameter of the 2 families from King Island, despite a difference in outcrossing rates (Figure 1), may be due to the class 3 parent having a lower breeding value or a higher genetic load than the class 1 parent. It is possible that outcrossing rates determined at seed set may not be a good predictor of mean growth of OP families at larger ages in some species it selfs die through competition with the more vigorous outcrosses (e.g. *E. regnans*; HARDNER and POTTS, *in press*). However, selection against selfs may not occur in other species grown for short rotation at wide spacing. Regardless, the preferred age for early selection in *E. globulus* in 4 years (BORRALHO *et al.*, 1992) and clearly breeding values at this age are biased by variations in outcrossing rate.

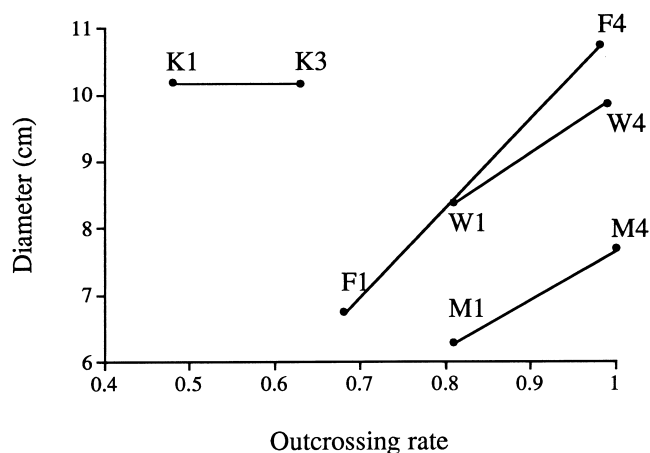


Figure 1. — The relationship between mean diameter at 4 years and individual tree outcrossing rate for *E. globulus* OP families of parents from different stand density classes (after BORRALHO and POTTS, 1996) and 4 localities (abbreviations as described in Table 1) when stand density class was not included in the genetic model. Lines connect parents from the same locality.

This study suggests that parental breeding values should be relatively accurately predicted from closed forest OP families, as selfs are virtually absent (Figure 1). However, outcrossing rates below 0.8 may markedly depress growth in *E. globulus* (Figure 1). In *E. grandis*, growth to 4 years appears to be strongly affected at rates below 0.6 (BURGESS *et al.*, 1996). Outcrossing rates in seed orchards also vary between 0.42 and 1.00 (MORAN *et al.*, 1989), suggesting the prediction of breeding values using seed orchard OP families may be less precise than expected (cf. HODGE *et al.*, 1996). Further work is needed in eucalypt and other insect or animal pollinated species to: (i) determine the nature of the relationship between inbreeding depression and individual outcrossing rate; and (ii) quantify the effect of variation in outcrossing rates of seed orchard OP families on breeding value prediction.

While one option for breeding is to only use OP families collected from closed forest, this will reduce selection intensity and genetic diversity, particularly if the population is fragmented such as in *E. globulus* (POTTS and JORDAN, 1994). BORRALHO and POTTS (1996) suggested the mean performance of OP families from lower stand density classes could be adjusted upwards to remove the effect of variation in in-

breeding depression between stand density classes. This approach, however, does not account for the variation within a stand density class, which is evident from this study. Adjustment using individual outcrossing rate may be a more precise method for the prediction of breeding values from OP progenies, particularly if estimates of outcrossing rates are improved by using more markers or progeny. The benefits of this approach will depend on whether the gain from more accurate breeding value prediction offsets the cost of determining accurate outcrossing rates on a large scale.

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