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Inbreeding Depression and Changes in Variation After Selfing in *Eucalyptus globulus* ssp. *globulus*

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(Received 20th October 1994)

Summary

The effect of self pollination (selfing), open-pollination (OP) and controlled cross pollination (outcrossing) on progeny of 11 *Eucalyptus globulus* ssp. *globulus* seed parents was examined from seed set to 43 months after planting. Selfing severely depressed seed set and field growth relative to outcrossing. No effect was found on germination percent and rate, nursery and field survival, nor the proportion of plants possessing adult foliage or flower buds. Inbreeding depression for height first occurred between germination and 8 months after planting but occurred later for diameter. There was a trend for inbreeding depression to increase with age. The performance of OP families was intermediate between self and outcross treatments but was only significantly different from outcrossing for volume at 43 months after planting. Selfing also appeared to increase variation between and within families relative to outcrossing. The results are discussed in terms of the biology and genetics of the species and the relevance to tree improvement programs.

Key words: inbreeding depression, selfing, genetic variances, open-pollination, progeny testing, tree improvement, *Eucalyptus globulus*, breeding.

FDC: 165.41; 165.53; 232.11; 176.1 *Eucalyptus globulus*.

Introduction

A knowledge of the effects of inbreeding can be important in tree improvement programs. On the one hand, potential genetic gains may be compromised by inbreeding depression (MESKIMEN, 1983; GRIFFIN, 1989); on the other hand, employing inbreeding offers benefits not otherwise available (BARKER and LIBBY, 1974; GULLBERG and KANG, 1985; LINDGREN, 1975; KANG and NIENSTAEDT, 1987; GRIFFIN and COTTERILL, 1988; NAMKOONG *et al.*, 1988). Theory also predicts that inbreeding may change the distribution of variation within a population (ROBERTSON, 1952; WEIR and COCKERHAM, 1977), yet this effect has received little attention in forest trees (*e. g.* SORENSON and WHITE, 1988).

The reproductive system of eucalypts offers ample opportunity for self pollination (GRIFFIN and HAND, 1979; ELDRIDGE *et al.*, 1993). The effects of selfing in eucalypts have been reported to include reduced seed set (HODGSON, 1976a; PRYOR, 1976; GRIFFIN *et al.*, 1987; POTTS and SAVVA, 1988a; SEDGLEY and SMITH, 1989; SEDGLEY *et al.*, 1989; TIBBITS, 1989), decreased germination percent (ELDRIDGE, 1978; ELDRIDGE and GRIFFIN, 1983), increased frequency of abnormal phenotypes (HODGSON, 1976b; POTTS *et al.*, 1987), depressed field growth and vigour

(HODGSON, 1976b; VAN WYK, 1981; ELDRIDGE and GRIFFIN, 1983; POTTS *et al.*, 1987; GRIFFIN and COTTERILL, 1988) and decreased nursery and field survival (ELDRIDGE and GRIFFIN, 1983; POTTS *et al.*, 1987).

In this paper, the effects of inbreeding in *E. globulus* LABILL. ssp. *globulus* are examined from seed set up to 43 months after planting in the field. The species is widespread in the lowland forests of Tasmania (JORDAN *et al.*, 1993) and is important in breeding programs, both in Australia and internationally (VOLKER *et al.*, 1990). However, with the exception of seed set (POTTS and SAVVA, 1988b), the consequences of inbreeding on the performance and genetic variance in this species have not been reported.

Materials and Methods

Crossing design and techniques

In late 1986, 4 pollination treatments (assisted outcrossing, assisted self pollination, unassisted self pollination and unassisted open-pollination), were undertaken on 11, mainly ornamental, trees of *E. globulus* ssp. *globulus* growing near Hobart. The ornamentals were of unknown origin and most were growing in a linear roadside planting at the Hobart Airport. There were at least 4 to 5 ssp. *globulus* trees within 100 m of each female allowing the opportunity for natural outcrossing, although this may have been less than that expected in natural stands. The assisted outcross and the assisted self pollination treatments were undertaken by emasculating anthers at the stage of operculum lift prior to the stigma becoming receptive and then isolating with a Terylene bag (see TIBBITS, 1989). Six to 8 days after emasculation, when the stigmas were receptive, bags were briefly opened and pollen applied to the stigmas with a match stick. Pollen was collected at anthesis and stored at -18°C in a freezer until required. The pollen used for assisted outcrossing was a poly-mix from 5 parents growing in a natural population near Proctors Road, Hobart, and was unlikely to be related to the female parents. Unassisted self pollination involved bagging unopened flowers. Flowers for the unassisted open-pollination (OP) treatment were tagged at the same time as the other pollination treatments.

Experimental design and measurement

Seed set

The number of flowers for each pollination treatment was counted immediately after applying the treatment. Mature capsules were harvested 12 months after pollination and the numbers of viable (filled) and inviable (collapsed) seeds per capsule were counted. The number of viable seeds per pollinated flower, the number of viable seeds per capsule, the total number of seeds (viable+inviable) per capsule and the ratio of viable seeds to total seeds (seed viability) were calculated.

Germination and nursery survival

Controlled germination tests were undertaken at 22°C with seeds that were classified on appearance as viable. Seeds from the 2 self pollination treatments (assisted and unassisted) were pooled into a single cross type, selfing. Each day the number of newly germinated seeds was counted. From this information, the daily cumulative number of germinated seeds was calculated. Germination capacity was calculated as the percentage of viable seed that had germinated by 25 days after the start of the germination tests. The rate of germination was quantified as the time taken to reach 10%, 50% and 90% of germination capacity by linear interpolation. Germinants were planted into pots in a greenhouse and survival within each family was

recorded at 4 months after germination when plants were transferred outdoors for hardening.

Field Trial

A field trial was established in 1988 with healthy 7 month old plants from the nursery. There were five blocks. Within each block, OP and outcross progenies were planted in a different sub-block to the selfs so as to eliminate competition effects between progenies from markedly different cross types. Within each sub-block, families were allocated randomly to plots of three trees. Trees were planted at 3 m x 3 m spacing. Survival of each plant was recorded at 2, 8, 19 and 43 months after planting, and plot survival was calculated as the proportion of planted individuals alive per plot. Diameter (D) was measured on surviving plants at 0.1 metres above ground at 8 and 19 months and at 1.3 m at 43 months. Total tree height (H) was also measured at these ages. Conic volume ($1/12 \cdot \pi \cdot D^2 \cdot H$) at 43 months was calculated for each tree. The presence of adult foliage and the presence of flower buds were also assessed for each tree at 43 months and expressed as a proportion of the individuals alive per plot.

Survival up to 43 months after planting

Cumulative survival was examined at seed set, germination, 4 months after germination and at 43 months after planting by calculating the number of individuals alive per 100 flowers crossed. If a family was not present at any of these stages, then the survival ratio for that stage was set to zero. The number of families present at each stage was also calculated for each cross type.

Statistical Analysis

Several models were used to test the effect of inbreeding on the trait means (Table 1). The 4 levels for pollination type (PT) were assisted outcrossing, assisted self pollination, unassisted self pollination and unassisted OP. For cross type (CT), there were 3 genetically different treatments; outcrossing, OP and selfing (the progenies from assisted and unassisted self pollination being combined into 1 cross type). If an entire level of a fixed effect (pollination type or cross type) was missing for a female for a trait, all families for that female were excluded for the analysis of that trait. An analysis of variance was undertaken with the GLM procedure in the statistical package SAS (SAS, 1988) to test the assumption that the residuals were normally distributed, check the assumption of homogeneity of resid-

Table 1. – Models used in the analyses of inbreeding effects in *Eucalyptus globulus* ssp. *globulus*. (PT = pollination type [fixed], Female = female parent [random], Rep = replication, block [random], CT = cross type [fixed]).

Trait	Model
Seed set	(1) $Y = \mu + \text{PT} + \text{Female} + e$
Germination, Field diameter, height, volume	(2) $Y = \mu + \text{Rep} + \text{CT} + \text{Female} + \text{Rep} \cdot \text{CT} + \text{Rep} \cdot \text{Female} + \text{CT} \cdot \text{Female} + \text{Rep} \cdot \text{CT} \cdot \text{Female} + e$
Nursery survival, Cumulative survival	(3) $Y = \mu + \text{CT} + \text{Female} + e$
Field Survival, Buds, Adult foliage (43 months)	(4) $Y = \mu + \text{Rep} + \text{CT} + \text{Female} + \text{Rep} \cdot \text{CT} + \text{Rep} \cdot \text{Female} + \text{CT} \cdot \text{Female} + e$
Variation within each cross type	(5) $Y = \mu + \text{Rep} + \text{Female} + \text{Rep} \cdot \text{Female} + e$

ual variance and test the significance of the factors for each model. The response variable was transformed to an optimal scale if the residual variance was not normally distributed or exhibited a trend against the fitted values. The transformations examined were logarithmic, square root, square and, for proportions, arcsin. For unbalanced designs with missing factor combinations, it was not possible to test contrasts with the GLM procedure. Therefore, to specifically test the difference between assisted self pollination and assisted outcrossing for seed set, and between selfing and outcrossing for the other traits, a GLM analysis was undertaken with the data restricted to the respective pollination treatments or cross types.

Best linear unbiased estimates of pollination treatment or cross type means and standard errors of the difference between estimates were calculated using Restricted Maximum Likelihood (REML) in Genstat (GENSTAT, 1991) and a test for the least significant ($p < 0.05$) difference between effects was constructed. Relative inbreeding depression due to self pollination, for seed set, and selfing for the other traits, was calculated as:

$$ID = [(OutX - Self)/OutX] * 100, \quad (1)$$

where OutX and Self were the mean estimates of assisted outcrossing and assisted self pollination or selfing and outcrossing respectively (GRIFFIN and COTTERILL, 1988). Inbreeding depression due to open-pollination was calculated by replacing the mean of self pollination (or selfing) with the OP mean.

The effect of inbreeding on the variation within and between families was examined for diameter, height and volume at 43 months using model 5 (Table 1) within each cross type. The significance of each factor was tested with GLM as detailed above. Variance components and their respective standard errors were estimated and the effect of each female parent were predicted (BLUP) using REML. Total phenotypic variation was obtained by summing the variance components for female, female x block interaction and the residual.

Results and Discussion

Inbreeding depression

Seed set, germination and nursery survival

Of the 44 possible mating combinations only 1 assisted self pollination and 1 assisted outcrossing pollination treatment, from different females, failed to produce any seed. All seed set traits were significantly affected by pollination type (Table 2). The contrast of assisted self pollination against assisted outcrossing was highly significant ($p < 0.01$) for all traits except the number of capsules per flower and total seeds per capsule (Table 2 and 3). Assisted self pollination resulted in significantly less viable seeds per flower and per capsule ($p < 0.001$, ID = 74%), less total seeds per capsule ($p < 0.05$, ID = 67%) and

Table 2. – Probabilities for the significance of the effect of pollination treatment and female parent on 5 seed set traits, and the effect of pollination treatment when analyses were restricted to assisted self pollination and assisted outcrossing (Self_{as} v OutX_{as}). (Transformations are shown in parentheses).

Seed Set Traits	transformation	Source of Variation		
		Pollination Treatment	Female	(Self _{as} v OutX _{as})
Capsules/flower	(none)	0.01	0.01	0.05
Viable seeds/flower	(ln[x+1])	0.00	0.00	0.00
Viable seeds/capsule	(ln[x+1])	0.00	0.00	0.00
Total seeds/capsule	(ln[x+1])	0.00	0.05	0.02
Viable seeds/total seeds	(x ²)	0.00	0.00	0.00

Table 3. – Back transformed pollination treatment means and inbreeding depression due to assisted selfing for 5 seed traits. (Self_{as} = assisted self pollination, OutX_{as} = assisted outcrossing, Self_{un} = unassisted self pollination, OP_{un} = unassisted open pollination, lines connect means which were not significantly different at the $p < 0.05$ level, * = $p < 0.05$, ns = not significant, and ID = relative inbreeding depression due to assisted self pollination).

Pollination Type	transformation	Self _{un}	Self _{as}	OutX _{as}	OP _{un}	ID
Capsules/flower	none	0.4	0.4	0.5	0.7	33 % ns
Viable seeds/flower	(ln[x+1])	1.1	1.2	4.5	4.7	74 %*
Viable seeds/capsule	(ln[x+1])	1.4	2.1	8.4	7.0	74 %*
Total seeds/capsule	(ln[x+1])	1.8	3.2	9.8	9.9	67 %*
Viable seeds/total seeds	(x ²)	0.6	0.6	0.8	0.9	25 %*

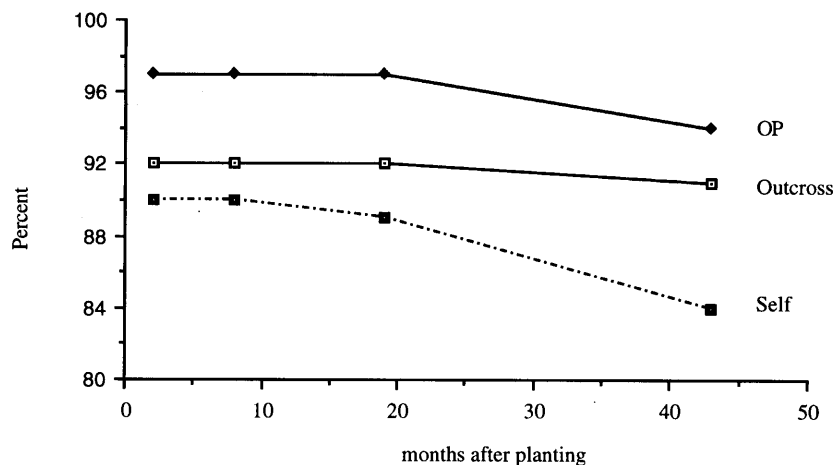


Figure 1. – Survival (percent) of planted seedlings at 2, 8, 19 and 43 months after planting in the field for outcross (at planting, $n = 68$), open-pollination (OP) ($n = 86$) and self ($n = 64$) cross types.

lower seed viability ($p < 0.001$, $ID = 25\%$). The severe effect of selfing on the number of viable seeds per flower and per capsule is comparable to the selfing depression for the same traits in *E. gunnii* (73%, POTTS and SAVVA, 1988a), *E. nitens* (73%, TIBBITS, 1989) and *E. ovata* (76%, POTTS and SAVVA, 1988a), but less than that for *E. morrisbyi* (90%, POTTS and SAVVA, 1988a), *E. grandis* (87%, HODGSON, 1976b) and *E. woodwardii* (95%, SEDGLEY, 1989), and greater than that reported for *E. urnigera* (57%, POTTS and SAVVA, 1988a), *E. regnans* (7% to 47%, ELDRIDGE, 1970; GRIFFIN *et al.*, 1987; SEDGLEY *et al.*, 1989) and *E. urophylla* (11%, ELDRIDGE, 1978). The total number of (viable+inviable) seeds per capsule most likely reflects the number of fertilised ovules. Thus selfing in *ssp. globulus* appears to result in both fewer fertilised ovules (total seeds per capsule) and an increase in the rate of abortion of fertilised ovules (ratio of viable seeds to total seeds).

No significant differences were observed between the effect of assisted outcrossing and unassisted open-pollination on seed set (Table 3). However, this comparison may confound the genetic effect of different pollen type (outcross versus a mixture of outcross and inbred) with (i) mechanical damage to the flower due to the emasculating and bagging procedure in the assisted outcrossing treatment (GRIFFIN *et al.*, 1987; TIBBITS, 1989) and (ii) differences in pollen load. Between 10% and 30% of the seeds in OP families of eucalypts are expected to originate from self-pollination (GRIFFIN *et al.*, 1987; MORAN *et al.*, 1989). However, because OP seed yield per capsule was high relative to outcrosses, OP seed yield seems to be more influenced by the absence of emasculating or bagging damage than by the genetic constitution of the pollen. The influence of emasculating damage on seed yield may be reduced as controlled pollination techniques are improved. The seed yield from assisted and unassisted self-pollination also did not differ significantly for any seed set trait (Table 3). Although this suggests that differences due to mechanical damage may be negligible, this result could also be due to an increased pollen load counteracting emasculating damage in assisted self-pollination.

In contrast to the strong effect of selfing on seed set, there was no significant effect of cross type on germination percent, number of days taken to reach 10%, 50% or 90% of total germination, nor on the proportion of germinants alive 4 months after germination.

Survival and growth in the field trial

Survival was high for all cross types (Figure 1) and there was no significant effect of cross type on survival to 43 months after planting. However, there was a clear trend with time for the difference in survival between the OPs and outcrosses to decrease, and the difference between the outcrosses and selfs to increase. ELDRIDGE and GRIFFIN (1983) found that in *E. regnans*, significant inbreeding depression for mortality did not arise until 5 years after planting. In *E. gunnii*, POTTS *et al.* (1987) reported significantly lower survival of progenies from selfing relative to those from outcrossing in the nursery and after one year in a field trial.

There was significant ($p < 0.05$) inbreeding depression in height and diameter by 19 months (Table 4). For height, there was a marginal difference between self and outcross progenies by 8 months after planting but inbreeding depression occurred later (19 months) for diameter (Figure 2). For both

Table 4. – Probabilities for the significance of the cross type and female parent effects and their interaction on diameter, height and volume at 8, 19 and 43 months. Also shown is the probability for the significance of the effect of cross type when the analyses were restricted to selfing and outcrossing (Self v OutX).

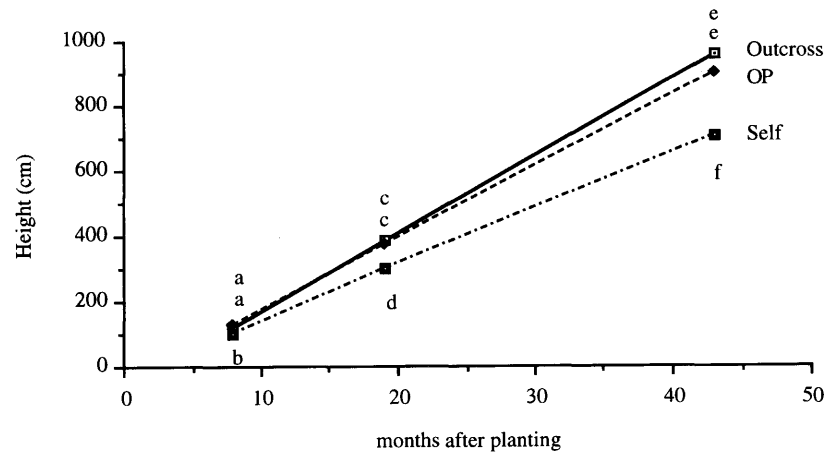
Traits	Source of Variation			
	Cross Type	Female	Cross Type X Female	(Self v OutX)
Height (cm)				
8 months	0.06	0.06	0.38	0.10
19 months	0.03	0.09	0.64	0.04
43 months	0.01	0.21	0.36	0.02
Diameter (cm)				
8 months	0.14	0.15	0.64	0.33
19 months	0.04	0.09	0.52	0.04
43 months	0.02	0.19	0.49	0.04
Volume (m³)				
43 months	0.01	0.11	0.48	0.02

traits, inbreeding depression tended to increase with age. At 43 months, inbreeding depression due to selfing was 26% for height and 24% for diameter (Figure 2). At 43 months, the effect of cross type on conic volume was significant ($p < 0.05$) and inbreeding depression due to selfing was 48% (Figure 3). OP progenies were intermediate between the self and outcross progenies for all traits at 19 and 43 months, but only differed significantly ($p < 0.05$) from outcrosses for conic volume at 43 months. Assuming that the outcross pollen in the open-pollination treatment was genetically comparable to the pollen used in the controlled outcrosses, then the relative depression in OPs is estimated to be 23%. For all traits, no significant effect was detected for female parent nor the interaction between cross type and female parent by the GLM analysis of variance. In addition, the significance of the female effect tended to decrease with time for height (Table 4). The exact significance of the ANOVA tests for cross type effects needs to be qualified,

however, as heterogeneity of residual and higher order variances between cross types was found (Table 5 and later discussion). There was no significant effect of cross type on the proportion of plants possessing adult foliage or flower buds at 43 months.

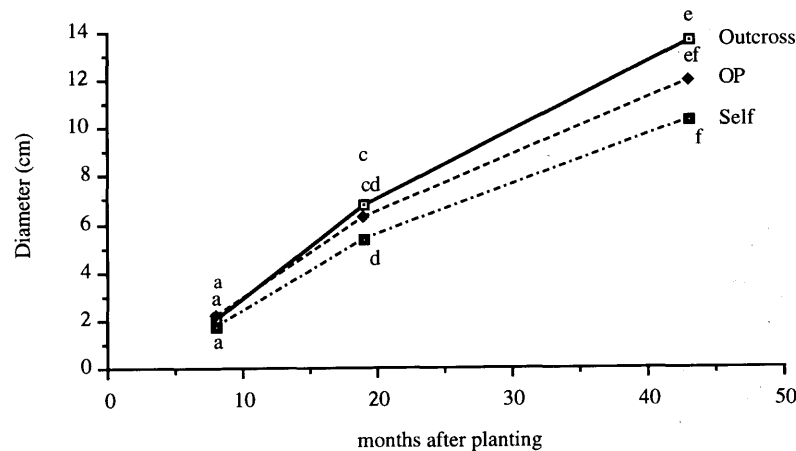
The failure to detect a consistent significant effect of inbreeding due either to selfing or open-pollination until the second year after planting (19 months) accords with studies of *E. regnans* (20 months after planting; GRIFFIN and COTTERILL, 1988), *E. grandis* (1 year after planting, HODGSON, 1976a; VAN WYK 1981), and *E. gunnii* (1 year after planting; POTTS *et al.*, 1987). The levels of selfing depression observed for diameter, height and conic volume at 43 months in *E. globulus* ssp. *globulus* were substantially greater than those reported for *E. regnans* at a similar age (11%, 18% and 37% respectively; GRIFFIN and COTTERILL, 1988). This is despite our estimates being in the absence of competition between cross types whereas cross types

(a)



selfing	17 % *	22 % *	26 % *
OP	-2 % ns	3 % ns	6 % ns

(b)



selfing	19 % ns	21 % *	24 % *
OP	-4 % ns	8 % ns	13 % ns

Figure 2. - Estimates of cross type means for height (a) and diameter (b) at 8, 19 and 43 months for outcross, open pollination (OP) and self progenies. Relative inbreeding depression due to selfing and level of significance are indicated below each graph. (Common letters connect means which were not significantly different at the $p < 0.05$ level, * = $p < 0.05$, ns = not significant).

were mixed in the *E. regnans* trials. Assuming a linear relationship between inbreeding depression and inbreeding coefficient (F) (e.g. SEDGLEY and GRIFFIN, 1989), our results suggest that, for *E. globulus* ssp. *globulus*, every 0.1 increase in the level of inbreeding would be associated with about a 5% reduction in height and diameter and a 10% reduction in conic volume at 43 months. This compares with a 5% decrease in height for *Pinus radiata* at 36 months (SEDGLEY and GRIFFIN, 1989) and a general figure of a 10% reduction in growth for the same change in F for *E. grandis* (REDDY *et al.*, 1983).

The intermediate performance of OP progeny suggests that there was a considerable proportion of selfs or other forms of inbred progeny in OP families. The intermediacy of an OP family between self and outcross progeny from the same parent

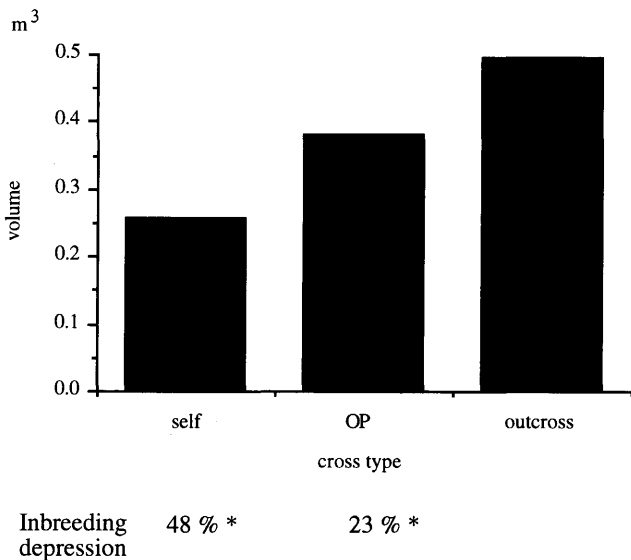


Figure 3. – Estimates of cross type means for volume at 43 months for outcross, open-pollination (OP) and self progenies and relative inbreeding depression due to selfing and open-pollination. (All cross types differed at the $p < 0.05$ level, * = $p < 0.05$).

has been used by some authors to estimate the outcrossing rate of an individual (HODGSON, 1976b; GRIFFIN *et al.*, 1987; GRIFFIN and COTTERILL, 1988). This approach, however, compares the additive effects of a female parent when outcrossed with several males to the mean of the self family which may include both additive and non-additive effects. Nevertheless, this method may give reasonable estimates if the effect of inbreeding depression is large relative to additive effects. Population estimates based on cross type means may also be reasonable if the outcross, self and OP populations are represented by a large number of female parents. Our results for volume suggest that the average outcrossing rate in the OP families was 56%. This compares to 48% and 78% calculated using the means for height and diameter respectively. The presence of a considerable proportion of self progeny is not unexpected. Restricted movements of the insect pollen vectors would favour high frequencies of self pollination as well as matings with possibly related neighbours. However, the frequencies of selfs in the OP families of this study are probably higher than would be obtained from OP families collected from native forests as many of our females were relatively isolated being ornamentals. In addition, our estimate of the average selfing rate may be inflated as the OP seedlings were growing in direct competition with the outcrosses, whereas the selfs were in a less competitive environment.

Survival from seed set to 43 months after planting

The main deleterious impact of selfing occurred at the seed set stage (ID = 72%), although the difference was not significant in this instance, probably due to pooling the two self pollination treatments into the single cross type, selfing. The accumulation of inbreeding effects resulted in a significant depression in survival of the self progenies by the nursery stage of 80% (Figure 4). While never significant, the mean number of individuals per 100 outcrossed flowers was less than for OP at all stages, effectively due to greater flower abortion in the controlled outcrosses (see earlier discussion and Table 3). At 43 months after planting, 82% of self, 91% of outcross and 100% of OP families were surviving. In addition to the loss of an outcross and a self family at seed set (see earlier discussion), a second self family was lost 4 months after germination.

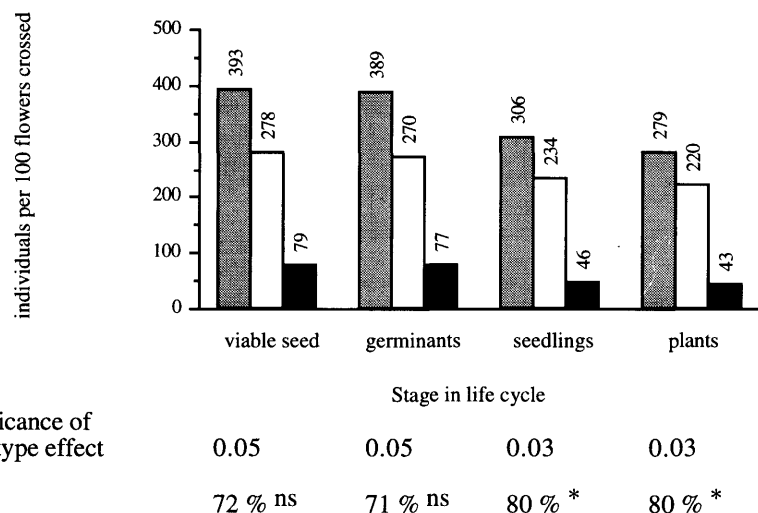


Figure 4. – Back transformed means for the number of individual per 100 flowers from seed set to 43 months after planting for *Eucalyptus globulus* ssp. *globulus* for open-pollination (▒), outcross (□) and self (■) cross types. The probability for the significance of the cross type effect and relative inbreeding depression due to selfing (ID) on the back transformed scale are also shown. (seedlings = 4 months after germination, plants = 43 months after planting; * = $p < 0.05$, ns = not significant).

This study confirms the view that, although self pollination is relatively frequent (GRIFFIN and HAND, 1979; GRIFFIN *et al.*, 1987) and seed dispersal is restricted (POTTS, 1990), the breeding system of eucalypts is generally outcrossing and is maintained by severe selection against inbreds at seed set and in the field (PHILLIPS and BROWN, 1977; POTTS *et al.*, 1987; GRIFFIN *et al.*, 1987). Similar to that reported for conifers (KOSKI, 1973; PARK and FOWLER, 1984), in *E. globulus* ssp. *globulus* there is a severe impact of inbreeding at seed set, but the effect of genetic load on germination and early survival appears to be masked and only becomes evident again after field planting. During early field growth, genetic effects may be obscured by maternal and nursery effects. However, at later ages inbreeding effects may be re-expressed as these effects diminish, stresses (induced by a harsher field environment and competition) on individual plants develop, and ontogenetic differences accumulate. The decline with age of the effect of female parent for height may reflect the decline in the importance of maternal effects with age. Outcross and self progenies were segregated in this study specifically to avoid competition. Thus, the deleterious effect of selfing on survival and growth is underestimated compared to situations in which self and outcross progenies are randomly mixed. Under the latter condition (*e.g.* ELDRIDGE and GRIFFIN 1983; GRIFFIN and COTTERILL 1988), suppression of inbred individuals by the more vigorous outcross progeny would lead to greater differences between cross types. For the same reason, the estimate of selfing depression reported in this study will underestimate the differential performance of inbred and outcross plants within the same OP family that would occur in a regenerating natural forest.

Table 5. – The effect of female parent and the interaction between female parent and block on diameter (cm), height (m) and conic volume (m³) at 43 months after planting, for the same 6 female parents in three different cross types. Shown are the significance of the effects (Pr[F], variance component estimates [σ^2 (standard error)] and phenotypic variation. (degrees of freedom: self, Female = 5, Female.Block = 16, Residual = 31; outcross, Female = 5, Female.Block = 16, Residual = 37; Open-pollination, Female = 5, Female.Block = 20, Residual = 52).

Trait Source	Self		Outcross		Open-pollination	
	σ^2 (s.e.)	Pr[F]	σ^2 (s.e.)	Pr[F]	σ^2 (s.e.)	Pr[F]
Diameter						
Female	1.51 (1.95)	0.04	0 (0.79)	0.81	1.29 (1.28)	0.03
Female.Block	0 (2.40)	0.68	3.08 (1.74)	0.01	0 (1.26)	0.49
Residual	12.78 (3.12)		4.06 (0.94)		9.38 (1.83)	
Phenotypic	14.29		7.14		10.67	
Height						
Female	0.53 (0.59)	0.04	0 (0.20)	0.58	0 (0.21)	0.45
Female.Block	0.61 (0.61)	0.28	0.82 (0.43)	0.00	0.83 (0.47)	0.01
Residual	2.84 (0.70)		0.87 (0.20)		1.66 (0.32)	
Phenotypic	3.98		1.69		2.49	
Volume						
Female	0.06 (0.05)	0.01	0 (0.00)	0.82	0.05 (0.04)	0.01
Female.Block	0 (0.01)	0.40	0.14 (0.07)	0.00	0 (0.01)	0.52
Residual	0.15 (0.04)		0.11 (0.03)		0.22 (0.04)	
Phenotypic	0.21		0.24		0.26	

The effect of inbreeding on the variation between and within families

Partitioning of the variance within each cross type suggested that the size and distribution of variation between and within families was affected by the degree of inbreeding (Table 5). There was no significant variation between females when outcrossed and the estimate of the variance component was near zero, whereas the variation between self families was significant ($p < 0.05$) for all traits. This differential response appears to be important despite the failure to detect a significant female parent x cross type interaction in the previous analyses where error variances were pooled. In addition, the average within plot variation (residual) was approximately 3 times greater within self families than within outcross families for diameter and height at 43 months. Although the standard errors on the variance component estimates are large, the observed trend accords with results from other studies. A similar trend was found in *E. regnans* for height and diameter at 45 months after planting (GRIFFIN and COTTERILL, 1988). WILCOX (1983 – Table 5) reported that the variation was higher among self than among outcross families for all traits studied in *Pinus radiata*. For *Pinus sylvestris*, there was also a greater percentage of phenotypic variation between and within self than outcross families (LUNDKVIST *et al.*, 1987).

The effect of female parent in the OP treatment, however, was not as consistent across traits (Table 5). For diameter and volume, the female parent effect was significant ($p < 0.05$ and $p < 0.01$ respectively) and the variance component estimate was only slightly less than that for selfing. This further suggests that a large proportion of OP progeny have arisen from inbreeding. However, the female parent effect was not significant and the variance component estimate was near zero for height. In *E. regnans*, GRIFFIN and COTTERILL (1988) reported a highly significant ($p < 0.01$) effect of female parent on diameter, height and volume for OP progeny.

For height, female parent means predicted from outcrossing were better correlated to the female parent means predicted using self progenies ($r^2 = 0.57$) than from OP families ($r^2 = 0.04$) and, while not marked, the correlations for volume ($r^2 = 0.25$) and diameter ($r^2 = 0.14$) were also better than those observed for OP families ($r^2 = 0.02$ and $r^2 = 0.04$ respectively). Despite the low number of families ($df = 4$), the poor discrimination between females following outcrossing, and no female parent x cross type interaction for any of the traits (Table 4), these observations are of interest as they are consistent with those of GRIFFIN and COTTERILL (1988) who also reported that predicted parent effects for volume at 45 months from outcrossing were better correlated with self families ($r^2 = 0.72$) than with OP families ($r^2 = 0.58$). BARKER and LIBBY (1974) suggest that the correlation between self performance and outcross performance may be improved by culling the lower 50% of each self family to reduce the bias that may occur due to the segregation of rare, recessive, deleterious alleles. However, such an improvement was not found by GRIFFIN and COTTERILL (1988) nor this study.

Without a knowledge of gene frequencies and gene action it is not possible to accurately predict how genetic variation is partitioned between and within self families and the magnitude of additive and non-additive components (WEIR and COCKERHAM, 1977; HALLAUER and MIRANDA, 1981). Several non-epistatic models have been used in an attempt to predict the variation between self and outcross families (Table 6), although these may be simplistic approaches as inbreeding induces correlations between alleles similar to that arising from linkage disequilibrium (Namkoong *et al.*, 1988, p. 16). The assumption

of only additive gene effects for height, diameter and volume (as used by GRIFFIN and COTTERILL, 1988, to predict variance components from self families in *E. regnans*) would appear to be invalid as the traits in this study exhibited inbreeding depression (implicating dominance in the gene action). In addition, this additive model (Model B – Table 6) predicts that inbreeding will decrease variation within self families relative to poly-mix outcrossing which is contrary to the results of this study. The observed increase in variation within self families is also inconsistent with the dominance model which assumes equal gene frequencies (Model C – Table 6, used by WILCOX, 1983, to predict variance components in *Pinus radiata*) as this model also predicts a decrease in the within self family variation relative to outcrossing. It is important to note, however, that differences in the residual variation may have been caused by non-genetic factors. On the other hand, using a model where a trait was influenced by a single rare ($1-p \approx 0$) recessive ($a = d$) allele, ROBERTSON (1952) predicted that after 1 generation of selfing ($F = 0.5$) there would be an increase in total genetic variation, the genetic variation between families, the genetic variation within families and the additive component of the genetic variation within families. This model seems to fit with the results of this study and suggests that the increase in variation between and within self families may be due to the expression of rare independent deleterious alleles. Non-additive effects appear to be an important factor in the genetic variation of growth traits for *E. globulus* ssp. *globulus* and the expression of deleterious alleles may be a major component of these effects. The implication of deleterious recessive genes is consistent with LEDIG'S (1986) argument that most inbreeding depression in forest trees is due to the expression of deleterious recessive gene effects rather than over-dominance.

Table 6. – Summary of non-epistatic models used to predict the additive (σ^2_A) and dominance (σ^2_D) variation of a randomly outcrossing population that occurs between and within polymix and self families.

source of variation	genetic component	reference
A) polymix outcross		
between families	$1/4 \sigma^2_A$	BECKER (1984)
within families	$3/4 \sigma^2_A + \sigma^2_D$	
B) selfing — additive model		
between families	σ^2_A	FALCONER (1989)
within families	$1/2 \sigma^2_A$	
C) selfing — dominance model, equal gene frequencies ($p = q = 0.5$)		
between families	$\sigma^2_A + 1/4 \sigma^2_D$	HALLAUER and MIRANDA (1981)
within families	$1/2 \sigma^2_A + 1/2 \sigma^2_D$	

Implications for tree improvement programs

The apparent presence of inbred progenies, particularly selfs, in OP families has several implications for the management of tree improvement programs. Firstly, if seeds from OP seed orchards are used to establish new plantations, potentially achievable rates of gain may be compromised. The results from this study suggest that natural selection from seed set to 43 months after planting is not sufficient to remove all inbred progeny from OP families. Culling selfs in the nursery (e.g.

EKBERG and ERIKSSON, 1985) is not an option in *E. globulus*, as inbreeding effects are not detectable for germination or nursery survival, and only become significant sometime between germination and 8 months after planting in the field. MORAN *et al.* (1989) estimated that approximately 10% of seed from a OP seed orchard of *E. regnans* was from selfing. If this were the case for *E. globulus*, the 48% selfing depression in volume (43 months) would translate into an OP depression in a field plantation of around 5%. In comparison, the expected rates of gain from breeding of *E. globulus* ssp. *globulus* in Tasmania are 7% to 17% (VOLKER *et al.*, 1990). Secondly, the use of OP progeny tests may bias selection and parameter estimates. Additive genetic variance components estimated from OP families may be inflated due to the presence of selfs (NAMKOONG, 1966). Further bias will arise if there is variation in the proportion of selfs within each OP family due to variation in self fertility between parents. In collections from natural stands, the level of outcrossing may also vary between families due to differing population sizes and the amount of crossing with related neighbours (ELDRIDGE, *et al.*, 1993). Provenance tests using OP families may be biased for the same reasons.

Controlled selfing, however, may be a useful method for progeny testing and for the management of breeding populations. Selfing by simply bagging unopened flowers and relying on unassisted pollination will be cheaper than controlled outcrossing and, as indicated in this study, gives similar seed yield to selfing by assisted pollination. The results of this study and those of GRIFFIN and COTTERILL (1988) suggest that selfing may be superior to OP progeny tests for the identification of superior parents. Increase in variation between and within self families may also help improve selection efficiency. Simulation work by DE RESENDE and VENCovsky (1992) suggested that progeny testing based on selfing gave higher rates of gain than half-sib testing.

The management of breeding populations has tended to avoid inbreeding because of its deleterious effects. However, there is a conflict between intense selection within a finite population and the avoidance of inbreeding (ROBERTSON, 1961). This conflict may be resolved if breeding populations can be managed as a set of small populations where inbreeding is allowed, or even enforced. Relative to a single large population, by using a strategy that employs selfing, it may be possible to breed more efficiently for negatively correlated traits, purge deleterious alleles early, maintain flexibility to enable a rapid response to changing breeding objectives, and better conserve alleles (KANG and NIENSTAEDT, 1987; NAMKOONG, *et al.*, 1988). The ability to utilise inbreeding in the management of breeding populations will be compromised, however, if inbreeding depression causes a substantial loss of genetic material (KANG, *et al.*, 1992). The results presented here suggest that, for *E. globulus* ssp. *globulus*, the loss of genetic material from selfing may not be great. A high proportion of families were represented from seed set to 43 months after planting, although inbreeding did reduce the absolute number of individuals, mainly at the stage of seed set. Most inbred lines may thus survive if large numbers of self crosses are made and the progeny are grown in moderate environments under low competition intensity.

Acknowledgments

The authors thank Drs. NUNO BORRALHO, ROLAND BURDON, GARY HODGE and TONY SHELBOURNE for their comments on the manuscript; Dr. PAUL COTTERILL for advice on trial design; PETER GORE for technical assistance; and North Forest Products for financial support and for the provision and maintenance of the trial site. Establishment of this trial was also financially supported by the Tasmanian Forestry Commission

and APM, and undertaken under an Australian Government National Research Fellowship (BMP). Latter measurements and data collation were supported by the Tasmanian Forest Research Council.

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