

# F<sub>1</sub> hybrid inviability in *Eucalyptus*: the case of *E. ovata* × *E. globulus*

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The impact of inbreeding and hybridization on fitness was compared in the two co-occurring forest tree species, *Eucalyptus ovata* and *E. globulus*, aimed at explaining the rarity of their hybrids in nature. The success of selfing, open-pollination and outcrossing of both species and interspecific hybridization was monitored from seed-set to 10-year's growth in a field trial. There was a unilateral barrier to hybridization with seed-set obtained only with *E. ovata* females. The F<sub>1</sub> hybrids exhibited reduced viability compared to intraspecific cross-types at virtually all stages of the life cycle and are clearly at a selective disadvantage compared with their open-pollinated *E. ovata* half-sibs with which they would directly compete in nature. *Eucalyptus ovata* and *E. globulus* overlap in their flowering time but the F<sub>1</sub> hybrids flowered later with virtually no overlap with either species. The asynchronous flowering and reduced reproductive fitness of F<sub>1</sub> hybrids would markedly limit the opportunity for advanced generation hybridization. Inbreeding similarly had a deleterious effect on the fitness of both species, and the F<sub>1</sub> hybrids were most competitive with the *E. ovata* selfs. It is argued that changes in inbreeding levels of parental populations may be a key factor affecting the relative fitness of hybrids and their potential to impact on the pure species gene pool. Reduced fitness of the pure species through inbreeding may result in hybridization having its greatest evolutionary impact in small founder or relict populations.

**Keywords:** *Eucalyptus globulus*, *Eucalyptus ovata*, hybridization, hybrid fitness, inbreeding, reproductive isolation.

## Introduction

Eucalypt species are well known for their weak reproductive barriers (Potts & Wiltshire, 1997). However, the number of natural hybrid combinations recorded is relatively low given the opportunities for hybridization between sympatric taxa in nature (Griffin *et al.*, 1988). The extent of natural hybridization varies depending upon numerous factors, including the degree of taxonomic and spatial separation, flowering synchrony, flower size and hybrid fitness (Griffin *et al.*, 1988; Gore *et al.*, 1990; Ellis *et al.*, 1991). Although there are many studies of natural hybridization in the genus (reviewed in Griffin *et al.*, 1988; Potts & Wiltshire, 1997) only a few address hybrid fitness (e.g. Drake, 1981; Potts, 1986). However, these fitness studies suffer from a poor knowledge of hybrid pedigree as identification is morphologically based (Arnold, 1992). Studying the fitness

of artificial hybrids with common environment trials is one solution, yet with tree taxa there is a paucity of detailed, long-term studies.

The relative fitness of hybrids and their parental taxa may be habitat dependent or determined by endogenous factors such as genomic incompatibilities which result in hybrids being less fit than parent taxa in all environments (Levin, 1978; Arnold, 1997; Emms & Arnold, 1997). A key endogenous factor that has received little attention is the inbreeding level of parental species. This is particularly important for plants with mixed mating systems (e.g. *Eucalyptus*; Hardner & Potts, 1995) and in small populations, where natural interspecific hybrids may compete with inbred pure species progenies. The present study examines pre- and postzygotic barriers to hybridization between two species of *Eucalyptus*, *E. ovata* Labill and *E. globulus* Labill ssp. *globulus*, aimed at explaining the rarity of their hybrids in nature. We report the success of selfing, open-pollination and intra- and interspecific cross-pollination of both

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species and compare the relative effects of interspecific hybridization and inbreeding on fitness from seed-set to 10-year's growth in a field trial.

*Eucalyptus globulus* ssp. *globulus* (*E. globulus* hereafter) and *E. ovata* are forest trees from different series (subgenus *Symphyomyrtus*; section *Maidenaria*; series *Viminales* and *Ovatae*, respectively; Pryor & Johnson, 1971) which differ markedly in flower morphology and size (Gore *et al.*, 1990). Like most eucalypts, *E. globulus* has a mixed mating system, is pollinated by a variety of insect and bird taxa (Hingston & Potts, 1998) and exhibits extreme inbreeding depression following selfing (Hardner & Potts, 1995). There is no information on the breeding system or effect of inbreeding on *E. ovata*.

Natural hybridization between *E. globulus* and *E. ovata* would be expected because they exhibit extensive overlap in geographical ranges and flowering time on the island of Tasmania (Williams & Potts, 1996). On a local scale they occupy different habitats but grow in close proximity in ecotones where their crowns may contact, yet hybridization is rare (Williams & Potts, 1996). Putative F<sub>1</sub> hybrid seedlings have not been observed in open-pollinated progenies of *E. globulus* but occur at a rate of ≈1% in open-pollinated progenies of *E. ovata* sampled within ≈500 m of *E. globulus* (unpubl. data). Natural hybrids in mature stands are restricted to rare, isolated trees or small, localized patches of hybrids in ecotones (e.g. McAulay, 1937).

## Materials and methods

### Crossing design

Three types of intraspecific controlled pollinations (assisted outcrossing, assisted self-pollination, unassis-

ted self-pollination), interspecific F<sub>1</sub> crosses, as well as open-pollinated (OP) controls were undertaken using 23 (13 used as females for controlled crossing) *E. globulus* and 12 (five were used as females for controlled crossing) *E. ovata* trees as parents. The numbers of crosses and families produced for each cross type are given in Table 1. The pollen used for intraspecific outcrossing or interspecific F<sub>1</sub> hybridization was derived from: (i) a mixture of pollen from five *E. globulus* trees (polymix); (ii) a mixture of pollen from five *E. ovata* trees (polymix); or (iii) single pollen collections. In each case, the pollen was collected from trees that were unrelated to the female parents. Crossing methodology, seed traits, germination and nursery procedures are detailed in Hardner & Potts (1995).

### Seed-set, germination and nursery growth

Cross success was assessed based on the number of viable seeds per capsule, the number of viable seeds per pollinated flower and seed viability (the ratio of viable seeds to total seeds). Controlled germination tests were undertaken at 22°C with seeds that were classified on appearance as viable. Each seed lot (family) was partitioned into two separate Petri dishes containing a maximum of 20 seeds which were allocated to two randomized blocks within the germination chamber. Selfed seed from unassisted and assisted self-pollination treatments were pooled into a single seed lot at this stage. The proportion of seed germinated and the mean time to germination of each seed lot was calculated. After 25 days, healthy germinants were planted into individual pots and maintained in family plots in a greenhouse. After 4 months, plants were transferred outdoors for hardening and at age 6 months, just prior

**Table 1** Number of parents involved in the mating design, crosses carried out and number of families and seedlings planted in the nursery and field trial of *Eucalyptus ovata*, *E. globulus* and their F<sub>1</sub> hybrids

	Parents	Crosses	Nursery		Field trial	
			Families	Seedling	Families	Seedling
<i>E. ovata</i>						
Self	6	6	6	96	6	35
OP	5	5	12*	415	12	206
Outcross	9	14	14	647	14	216
F <sub>1</sub>	10	10	10	452	10	111
<i>E. globulus</i>						
Self	13	13	12	207	11	116
OP	20	20	20	656	20	284
Outcross	23	31	27	628	26	282
F <sub>1</sub>	18	28	0	0	0	0
Total	104	127	104	3101	99	1250

\* Includes additional open-pollinated families from pollen parents.

to setting up the field trial, the number of runts (dwarfs and plants with extremely poor vigour), unhealthy and healthy plants were assessed. Family arrangement within the greenhouse and nursery was randomized with respect to cross type.

### Field trial

A field trial was established in 1988 near Ridgley in north-western Tasmania (latitude 41°10'S longitude 145°46'E) with healthy 7-month-old plants from the nursery. There were five replicates, containing five sub-blocks: (i) OP (GLop) and outcrosses (GL × GL) of *E. globulus*; (ii) *E. globulus* selfs (GLself); (iii) OP (OVop) and outcrosses (OV × OV) of *E. ovata*; (iv) *E. ovata* selfs (OVself); and (v) *E. ovata* × *globulus* F<sub>1</sub> hybrids (OV × GL). This sub-blocking was chosen to minimize 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 × 3 m spacing. The trial included a total of 1250 initial trees without buffers. Stem diameter (D) was measured on surviving plants at 0.1 m above ground level at 1 and 2 years (8 and 19 months) and at 1.3 m above ground level at 4 and 10 years of age (43 and 118 months). These data were used to calculate stem basal area ( $D^2 \times \pi/4$ ) for each tree and the proportion of planted individuals alive per sub-block. All surviving trees were assessed for flower buds and capsules in October 1991 (age 3 years), May 1992 (4 years) and April 1993 (5 years) and the percentage of reproductive trees in each sub-block calculated for each cross type. At age 4 years, all trees with sufficient numbers of reproductive buds were monitored for flowering time at monthly intervals from April 1992 to May 1993. At each assessment the percentage of the current season's bud crop which was flowering was visually estimated.

### Statistical analysis

The five types of pollination undertaken on each *E. ovata* female tree were compared for seed-set (viable seed per capsule and per flower) in a one-way analysis of variance based on the logged transformed values for each tree. Seed-set results for *E. globulus* were compiled from Hardner & Potts (1995). All other one-way analyses of seed or nursery traits compared the seven cross types based on individual tree averages for each female. Proportional data were arcsine transformed prior to analysis. As cross types were arranged in sub-blocks within replicates in the field trial; they were compared in one-way analyses of variance based on cross type means or proportions for each replicate. In all analyses of cross type differences, contrasts were undertaken specifically to

compare: (i) the parental taxa under outcrossing; (ii) the selfs and outcrosses within each taxon; (iii) the F<sub>1</sub> hybrid against the mid-parent value derived from the outcross treatment for each parental taxon; and (iv) the F<sub>1</sub> hybrids against the open-pollinated progeny of *E. ovata*. The level of inbreeding depression (ID%) resulting from either selfing or open-pollination was calculated as:

$$ID\% = 100(X_{\text{out}} - X_{\text{inbred}})/X_{\text{out}}$$

where  $X_{\text{out}}$  is the mean for the controlled outcrosses and  $X_{\text{inbred}}$  is the mean for inbred progenies (self- or open-pollinated). Outbreeding depression was measured in a similar manner as deviation below the average of the *E. ovata* and *E. globulus* outcrosses. Where traits were transformed prior to analysis, the depression was calculated using back-transformed means. All analyses were undertaken with the GLM procedure of SAS (SAS version 6.12) and the Tukey test ( $P < 0.05$ ) used for *a posteriori* comparisons of cross-type means.

## Results

### Seed-set

Interspecific F<sub>1</sub> crosses between *E. ovata* and *E. globulus* were only successful when *E. ovata* was used as the female. In this case the number of viable seed per flower pollinated was not significantly different from controlled intraspecific outcrossing (means of 1.0 and 0.9 viable seed/flower for OV × GL and OV × OV, respectively). As expected from the flower and capsule size, *E. globulus* produced more viable seeds per flower than *E. ovata* following open-pollination (GLop 4.7 and OVop 0.9) and outcrossing (GL × GL 4.5 and OV × OV 0.9). Self-pollination significantly reduced seed-set in both species (*E. globulus*  $P < 0.01$ , *E. ovata*  $P < 0.001$ ), and no significant difference was found between assisted and unassisted self-pollination means (1.2–1.1 for *E. globulus* and 0.1–0.1 for *E. ovata*, respectively). Similar results were obtained from the analysis of the number of viable seeds per capsule (GL × GL 8.4, GLop 7.0, OV × OV 4.8, OVop 2.1). Assisted and unassisted self-pollination means did not differ significantly (*E. globulus* 2.1 and 1.4, *E. ovata* 1.2 and 1.9, respectively) and the number of seed obtained in capsules from the interspecific crosses (OV × GL 5.4) was not significantly different from intraspecific outcrosses on the *E. ovata* females (OV × OV 4.8).

Seed viability differed significantly between cross types ( $P < 0.001$ ). The outcross (Table 2) and open-pollinated seed of *E. ovata* was more viable than the corresponding seed of *E. globulus*, with the F<sub>1</sub> hybrid seed intermediate (OV × GL 93%), but significantly ( $P < 0.001$ ) reduced compared to the intraspecific outcrosses from the same *E. ovata* females. Inbreeding

**Table 2** The effects of F<sub>1</sub> interspecific hybridization and inbreeding over the life cycle in *Eucalyptus globulus* and *E. ovata*. For each trait, the table shows the percentage and significance of the difference between *E. globulus* and *E. ovata* for outcrosses (GL × GL vs. OV × OV); inbreeding depression following selfing in *E. globulus* (GL × GL vs. GLself) and *E. ovata* (OV × OV vs. OVself); outbreeding depression estimated as the deviation of the F<sub>1</sub> hybrid performance from the mid-parent value (F<sub>1</sub> vs. mp.); and between the F<sub>1</sub> hybrid and open-pollinated progenies of *E. ovata* (with which the hybrids would be competing in nature; F<sub>1</sub> vs. OVop)

Trait	Percentage of depression and significance									
	GL × GL vs. OV × OV		GL × GL vs. GLself		OV × OV vs. OVself		F <sub>1</sub> vs. mp.		F <sub>1</sub> vs. OVop	
Germination										
Proportion of seeds										
viable/total	-6	***	8	***	17	***	2	*	2	NS
germinated/sown	-1	NS	-1	NS	1	NS	7	*	8	*
Time to germination (days)†	19	*	10	NS	-3	NS	-38	***	-51	***
Nursery										
Proportion of seedlings										
planted/germinated	6	*	2	NS	-7	*	-2	NS	0	NS
healthy at 1 month/planted	33	**	28	NS	-27	NS	79	***	81	***
alive at 6 months/planted	7	NS	9	NS	30	*	46	***	44	***
healthy/alive at 6 months	16	*	12	NS	10	NS	15	NS	17	NS
Field trail										
Diameter										
1 year	33	***	26	***	40	***	10	*	-27	*
2 years	22	***	25	***	42	***	20	***	-4	NS
4 years	18	**	27	***	46	***	47	***	35	***
10 years	13	NS	15	NS	40	NS	4	NS	-20	NS
Survival										
1 year	2	NS	0	NS	1	NS	6	NS	-4	NS
2 years	4	NS	0	NS	6	NS	7	NS	-2	NS
4 years	2	NS	2	NS	17	*	26	***	19	*
10 years	7	NS	30	**	43	***	78	***	74	***
Basal area										
1 year	51	***	40	***	57	***	28	**	-56	**
2 years	47	***	41	***	65	***	34	***	-29	NS
4 years	33	***	46	***	70	***	70	***	53	**
10 years	25	***	48	***	79	***	78	***	62	***
Proportion reproductive‡										
3 years	81	**	-28	NS	100	*	90	**	28	NS
4 years	48	***	20	NS	100	***	67	***	11	NS
5 years	17	NS	19	NS	86	***	70	***	51	NS

NS, not significant.

\*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ .

† Negative values for time to germinate means that more time is required to germinate.

‡ Selfs did not reach reproductive maturity until the fifth year.

significantly reduced seed viability in both species (OV × OV 97%, OVop 94%, OVself 80%, GL × GL 92%, GLop 87%, GLself 85%; Table 2).

### Germination and nursery survival

The F<sub>1</sub> hybrid seed had the lowest percentage germination (92% compared with the OVop 100%) and was

slower to germinate than all other cross types (Table 2). No significant differences between the F<sub>1</sub> and parental cross types occurred in the very early stages of growth following germination as measured by the proportion of germinants transplanted. However, the proportion of planted germinants that produced healthy seedlings after 1 month was significantly ( $P < 0.001$ ) less in the F<sub>1</sub> hybrids than the pure species outcrosses. By

6 months, the mortality of the F<sub>1</sub> hybrids was nearly three times that of the pure species outcrosses (survival F<sub>1</sub> 44%, GL × GL 86%, GLop 85%, GLself 78%, OV × OV 80%, OVop 80%, OVself 78%) (Table 2). The proportion of F<sub>1</sub> plants classified as unhealthy (including runts or plants with abnormal morphology) at this stage did not differ from the parental species (Table 2).

### Growth and survival in the field trial

There was no significant difference in the survival of the different cross types in the first 2 years of the trial, but there was for growth (Table 2). *Eucalyptus globulus* grew significantly faster (as measured by stem diameter) than comparable cross types of *E. ovata* (Table 2). The F<sub>1</sub> hybrids were intermediate in growth rate in the first year, but by the end of the second year their growth started to lag behind outcrosses of both species, with their average stem diameter significantly ( $P < 0.001$ ) below the mid-parent value of both species (Table 2). This poorer performance of the hybrids was reflected in significantly lower survival from age 4 years onwards and by year 10, only 19% of the F<sub>1</sub> hybrids planted were still alive compared with 88% and 82% of the outcrosses and 77% and 71% of the open-pollinated progenies of *E. globulus* and *E. ovata*, respectively.

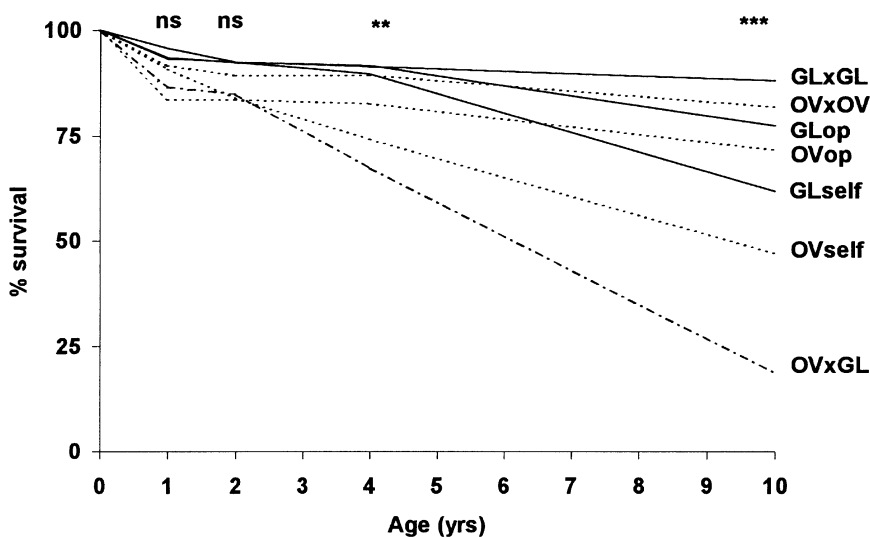
The selfed progeny of both species exhibited poorer growth and survival than comparable outcrosses, with the open-pollinated progenies intermediate (Table 2; Fig. 1). Inbreeding depression for the diameter of surviving selfed plants was 40–46% in *E. ovata* and relatively stable with age (Table 2). In *E. globulus* the depression was 25–27% over the first 4 years but dropped to 15% by age 10 years, suggesting that the selfs that do survive to age 10 years are relatively

vigorous. When survival is taken into account by calculating the average basal area from each tree planted, the level of inbreeding depression from selfing was markedly higher, ranging from 57 to 79% in *E. ovata* and 40–48% in *E. globulus*, and increased with age (Table 2). This inbreeding depression compares with the 28–78% outbreeding depression for basal area in the F<sub>1</sub> hybrids (Table 2). The F<sub>1</sub> hybrids grew better than the *E. ovata* selfs in the first 2 years, but their mortality was higher in subsequent years (Fig. 2). By age 10 years, the average basal area of the F<sub>1</sub> hybrids was equivalent to the *E. ovata* selfs but significantly ( $P < 0.05$ ) less than the other cross types (data not shown), including the *E. ovata* open-pollinated progenies (Table 2).

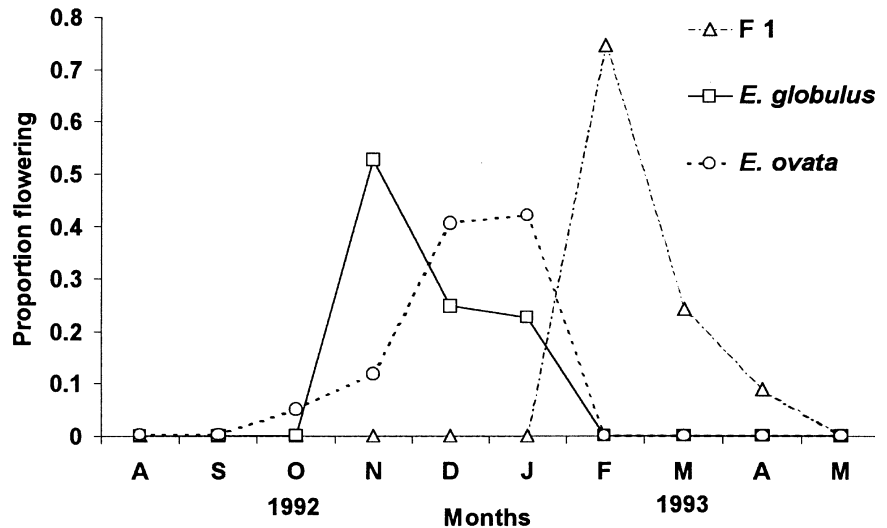
### Flowering

There were highly significant ( $P < 0.001$ ) differences between cross types in the percentage of reproductively mature trees at ages 3–5 years (Table 2). *Eucalyptus globulus* was more precocious than *E. ovata*, but by the fifth year there was little difference between the two species (Table 2). However, at this stage, the proportion of reproductive trees declined with increasing inbreeding in both species (only significant in *E. ovata*), and the reproductive success of the F<sub>1</sub> hybrids was markedly less than outcrosses of the parental species (Table 2). By 5 years of age only 10% of the hybrids were reproductively mature compared with 38% and 32% of the outcrosses, 41% and 22% of the open-pollinated progenies and 31% and 5% of the selfs of *E. globulus* and *E. ovata*, respectively.

Monitoring of flowering time of reproductive trees at age 4 years indicated that the flowering time of the F<sub>1</sub> hybrids in the trial was asynchronous with that of the



**Fig. 1** Percentage survival at 1, 2, 4 and 10 years after planting in the field of *Eucalyptus globulus* (GL) and *E. ovata* (OV) progenies derived from self-pollination (self), natural open-pollination (op), intra-specific outcrossing (GL × GL or OV × OV) and inter-specific hybridization of *E. ovata* × *E. globulus* (OV × GL). Proportions were arcsine transformed for analysis and means have been back-transformed for presentation (\*\* =  $P < 0.01$ ; \*\*\* =  $P < 0.001$ ; n.s. = not significant).



**Fig. 2** The proportion of reproductively mature trees of *E. globulus* ( $n=26$ ), *E. ovata* ( $n=15$ ) and their interspecific F<sub>1</sub> hybrids ( $n=7$ ) which were flowering each month in the field trial. Observations were recorded from April 1992 to May 1993, when trees were from 4 to 5 years old. No flowering occurred between April and August 1992.

pure species (Fig. 2). Flowering commenced in October in *E. globulus* and in September in *E. ovata*, and both completed flowering by February. There was extensive overlap in their flowering, although *E. globulus* peaked 1–2 months earlier than *E. ovata*. In contrast, there was virtually no overlap in the flowering of either species with the F<sub>1</sub> hybrids which did not commence flowering until February.

## Discussion

### Prezygotic barriers to hybridization

There is a strong, unilateral barrier to artificial hybridization between the large-flowered *E. globulus* and the small-flowered *E. ovata*. The cross fails when *E. globulus* is used as the female, consistent with the absence of F<sub>1</sub> hybrids in its open-pollinated progenies. This prezygotic barrier is probably equivalent to that reported between *E. globulus* and the small-flowered *E. nitens*, where the barrier is structural and caused by the pollen tubes of the small-flowered species being unable to grow the full length of the *E. globulus* style (Gore *et al.*, 1990). However, artificial hybridization using *E. ovata* as the female was just as successful as outcrossing with unrelated *E. ovata* pollen, suggesting that there is no barrier through physiological incongruity to successful seed-set once *E. globulus* pollen is applied to the *E. ovata* stigma (e.g. Ellis *et al.*, 1991). The low level of putative F<sub>1</sub> hybrids observed in open-pollinated seed lots of *E. ovata* therefore must reflect pre-mating barriers, such as localized spatial separation arising from fine-scale habitat differences, localized differences in flowering time, and/or differing pollen vectors.

### Postzygotic barriers to hybridization

Postzygotic barriers to F<sub>1</sub> hybridization between *E. ovata* and *E. globulus* were severe and were manifest early in the life cycle. F<sub>1</sub> hybrids had reduced seed viability, delayed germination and higher mortality after 6 months growth in the nursery compared with outcrosses of either parental taxon. Such early expression of F<sub>1</sub> hybrid inviability has been reported in other F<sub>1</sub> hybrid combinations of *Eucalyptus*, where early stages in the life cycle have been monitored (Tibbits, 1988; Potts *et al.*, 1992; Oddie, 1996). The inviability of the *E. ovata* × *globulus* interseries F<sub>1</sub> hybrids appears greater than that reported for intraseries hybrids involving *E. globulus* (e.g. *E. nitens* × *globulus*, Potts *et al.*, 1992; *E. gunnii* × *globulus*, Potts unpubl. data). However, in the one case of intersection crossing studied in detail (*E. camaldulensis* × *globulus*), extremely high rates of F<sub>1</sub> hybrid dysfunction were evident over 22 months of nursery growth (72% cf. <1% in pure species controls; Oddie, 1996). These results are consistent with general observations for increasing hybrid inviability with increasing taxonomic distance between parental taxa (Potts *et al.*, 1987; Griffin *et al.*, 1988).

There are few studies in forest trees where hybrid fitness has been monitored over such a long period. Many studies of *Eucalyptus* F<sub>1</sub> hybrids are based only on nursery growth or 1–2 years field performance (Tibbits, 1988; Potts *et al.*, 1987; Potts *et al.*, 1992). The present study clearly shows how early field growth can give a misleading indication of long-term success when forest trees are concerned. The first indication of the inviability of the F<sub>1</sub>s after field planting was at the 4-year assessment. By 10 years however, hybrid survival was significantly reduced even compared with the inbred

progenies of either species (Fig. 1). This inviability occurred despite the  $F_1$  hybrids growing in separate sub-blocks to the parental controls and not competing directly with the more vigorous parental types. Indeed with increasing mortality with time, the hybrids were clearly not experiencing the same level of intra-tree competition as the other cross types and were growing in an increasingly open, less competitive environment. The present results are therefore an underestimate of the inviability of these  $F_1$  hybrids and the intense post-dispersal selection that would occur against them in nature.

### Genetic causes of $F_1$ inviability

Lethality or semi-lethality of  $F_1$  hybrids after germination has been reported in many genera of plants, with hybrid weakness and dwarfs a common feature (Levin, 1978). The main genetic causes of inviability of the  $F_1$  hybrid sporophyte include: (i) genome disharmony and incompatible development cues; (ii) the deleterious, complementary action of one or a few genes; or (iii) cytoplasmic effects (Levin, 1978).

Incompatible development cues are a possible explanation for the high later age inviability of the *E. ovata* × *globulus*  $F_1$ s because the species differ considerably in ontogenetic development. *Eucalyptus globulus* is markedly heteroblastic retaining a distinctive juvenile foliage type (opposite, sessile and highly glaucous) for 2–3 years before producing the more typical ‘adult’ foliage (petiolate, alternate, isobilateral and green). In contrast, the ontogenetic differentiation in *E. ovata* is less marked and the transition to the petiolate, opposite ‘adult’ leaf type occurs in the first few months of growth (after ≈4–8 nodes).

Deleterious genetic interactions have also been implicated in many cases of inviability of  $F_1$  hybrids (Levin, 1978). Such genes have no deleterious effects within a species and probably accumulate as a by-product of divergence, but may cause inviability or sterility in combination with genes from another species. There is increasing evidence that such deleterious interactions involving dominant complementary genes cause  $F_1$  hybrid inviability in both plants (e.g. Levin, 1978; Christie & Macnair, 1987) and animals (e.g. Palopoli & Wu, 1994; Orr, 1995). Deleterious epistatic effects involving recessive genes would not affect the  $F_1$  sporophyte generation. Deleterious interactions between alleles from the same locus have been implicated in  $F_1$  hybrid inviability and sterility in several cases (Abbo & Ladizinsky, 1994). This mechanism would require the species to have different, derived alleles that are incompatible with each other in the heterozygous state.

$F_1$  hybrid inviability resulting from the deleterious interactions of nuclear and cytoplasmic genes has been reported in several plant genera (e.g. *Iris*, Cruzan & Arnold, 1999), and is often detected by reciprocal differences in the frequency of dwarf or abnormal plants (Levin, 1978). Such reciprocity could not be tested in the present case because of the unilateral barrier to the formation of hybrid seed. The chloroplast DNA is highly polymorphic within both *E. globulus* (Jackson *et al.*, 1999) and *E. ovata* (McKinnon, unpubl. data) and some coadaptation between the chloroplast and nuclear genes is possible. However, there is extensive sharing of chloroplast haplotypes across species (Jackson *et al.*, 1999), and the effect of combining either chloroplast type with a hybrid nuclear genome should be no more serious than when intraspecific crossing occurs between the different chloroplast types. Such interaction is thus unlikely to be a cause of the observed  $F_1$  hybrid inviability.

### Reproductive potential and isolation of $F_1$ hybrids

Despite survival or even vigorous growth of  $F_1$  hybrids, sterility or reduced reproductive output may result in a major barrier to gene flow between species (Levin, 1978). Several studies of *Eucalyptus* have shown that  $F_1$ -type hybrids may survive to reproductive maturity in natural populations but exhibit significantly reduced reproductive output compared to competing parental taxa (e.g. Drake, 1981; Potts, 1986). In the present case, reproductive output was not quantified on an individual plant basis yet the *E. ovata* × *globulus*  $F_1$  hybrids were observed to flower (Table 2), and viable pollen and seed has been collected (Potts, unpubl. data). Nevertheless, even when  $F_1$  hybrids do survive, there would appear to be a major barrier to introgression arising from (i) a reduced proportion of the surviving  $F_1$ s reaching reproductive maturity (no doubt because of their poor growth) and (ii) the  $F_1$  hybrids flowering out of synchrony with either parental taxon (Fig. 2).

Flowering time has been reported to be under strong genetic control and inherited in a more-or-less intermediate manner in other eucalypt  $F_1$  hybrids (Gore & Potts, 1995), suggesting that once established,  $F_1$ s would act as a ‘bridge’ for further gene exchange. Several hypotheses could explain the displacement of flowering time in the present case including: (i) an epistatic effect of genes directly affecting flowering time *per se*; (ii) delayed flower bud development as a pleiotropic effect of genes (or their interactions) which cause the poor growth of the  $F_1$  hybrids; and (iii) a predominantly additive effect of genes controlling bud development time. The last mechanism appears to be operating in the present case, because despite their smaller size, *E. ovata*

flower buds take nearly two seasons to develop from initiation to flowering, whereas this development is complete in one season in *E. globulus* (Lopez, unpubl. data).

### Effects of inbreeding

Severe inbreeding depression at all stages in the life cycle has been reported previously in *E. globulus* (Hardner & Potts, 1995), but this is the first report for *E. ovata*. Inbreeding and hybridization clearly result in a severe reduction in fitness, although the genetic causes are likely to be different (Potts *et al.*, 1992). In nature, the unilateral crossing barrier, coupled with limited seed dispersal from the female, would result in most *E. ovata* × *globulus* F<sub>1</sub> hybrids being more likely to compete with open-pollinated progeny of *E. ovata* than *E. globulus*. The present study clearly shows that the F<sub>1</sub> hybrids are significantly less fit than open-pollinated progeny of either parental taxon and would rarely survive to reproductive age in nature under direct competition with even mildly inbred parental types. Nevertheless, although the F<sub>1</sub>s were significantly less fit than selfed progeny of *E. globulus* in the test environment, they were vegetatively and reproductively competitive with selfs of *E. ovata*. Thus, despite the potentially high level of endogenous inviability, the *E. ovata* × *globulus* F<sub>1</sub> hybrids would appear to be competitive with highly inbred progeny of the maternal taxon.

### Evolutionary implications

Arnold *et al.* (1999) argue that hybridization may have significant evolutionary consequences despite low hybrid fitness in early generations. The present study suggests that changes in inbreeding levels of parental populations might be a key factor affecting the relative fitness of hybrids and their potential to impact on the pure species gene pool. As with most forest tree taxa, eucalypts exhibit severe inbreeding depression for fitness traits (Potts & Wiltshire, 1997), which would result in population fitness being markedly lowered during population bottlenecks arising from founder events or range contraction. Such bottlenecks would have been an integral part of the response of the Tasmanian eucalypt species to the Quaternary glacial cycles that would have imposed cycles of contraction and expansion of eucalypt forest. There is increasing evidence from extensive sharing of distinctive chloroplast haplotypes that the Tasmanian eucalypt species, including *E. globulus* and *E. ovata*, have historically been involved in extensive reticulate evolution (Jackson *et al.*, 1999). It has been argued that the patterns of chloroplast variation and capture are consistent with extensive hybridization and

expansion from populations confined to glacial refugia (Jackson *et al.*, 1999).

Pollen swamping from more common congeners (Ellstrand & Elam, 1993; Potts & Wiltshire, 1997), coupled with reduced fitness of the pure species through inbreeding, may result in hybridization having its greatest evolutionary impact in small founder or relict populations. Subsequent expansion of such populations could certainly explain cases of introgression despite low hybrid fitness in early generations (Arnold, 1997; Arnold *et al.*, 1999). It is therefore important to account for varying levels of parental inbreeding if the genetic impact of hybridization is to be fully modelled.

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