

Unilateral Cross-incompatibility in *Eucalyptus*: the Case of Hybridisation between *E. globulus* and *E. nitens*

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Abstract

The growth of *E. globulus* and *E. nitens* pollen tubes in styles of *E. globulus* was examined in order to elucidate the site of the unilateral barrier to hybridisation. Pollen tubes of *E. nitens* failed to grow the full length of the larger *E. globulus* style. *E. globulus* pollen tubes grew an average of 1.4 mm per day for the first 4 days, compared with 0.8 mm per day for pollen tubes of *E. nitens*. From days 4 to 14, the growth of *E. nitens* pollen tubes slowed to an average of 0.2 mm per day and virtually no growth occurred after day 14. In contrast, *E. globulus* pollen tubes grew through the style and into the ovary between days 5 and 14. By day 28, at about the time of style abscission, *E. nitens* tubes had grown only 6 mm, well short of the full length of the *E. globulus* style (9-10 mm). A similar difference in growth was obtained *in vitro* where *E. nitens* pollen tubes were significantly shorter than those of *E. globulus*. A comparison also including *E. ovata*, *E. urnigera* and *E. gunnii* indicated a significant correlation between style length and *in vitro* pollen tube length. It is argued that the unilateral cross-incompatibility between *E. globulus* and *E. nitens* is due to a structural barrier arising from an inherent limit to pollen tube growth which is associated with pistil size.

Introduction

Post-mating barriers to interspecific hybridisation within plant genera are often unilateral (Lewis and Crowe 1958; Abdalla and Hermsem 1972; Hogenboom 1975, 1984; de Nettancourt 1977, 1984; Levin 1978a; Williams and Rouse 1988) and *Eucalyptus* is no exception. While hybridisation between species within *Eucalyptus* subgenera is relatively common (Griffin *et al.* 1988), the unilateral failure of artificial hybridisation within subgenera has been reported for *E. caesia* x *E. pulverulenta* (Pryor 1956), *E. caesia* x *E. leucoxyton* (Beardsell *et al.* 1979), *E. globulus* x *E. viminalis* (Pryor 1956), *E. globulus* x *E. nitens* (Tibbits 1986), *E. globulus* x *E. ovata*, *E. globulus* x *E. gunnii* and *E. globulus* x *E. morrisbyi* (Potts and Savva 1988). In each combination reported, the species differ markedly in floral morphology and the barrier to hybridisation is manifest only when the longer-styled species is used as the female. The inability of the pollen tubes of the small-styled species to grow the full length of the larger style has been suggested as the simplest explanation for the unilateral failure of hybridisation (Pryor 1956; Tibbits 1986; Potts and Savva 1988). This certainly appears to be the case for crosses involving species with comparable disparities in style length in other plant genera such as *Datura* (Gardella 1950), *Phlox* (Levin 1978a) and *Rhododendron* (Williams and Rouse 1988). Nevertheless, unilateral failure of hybridisation may arise through S-locus incompatibility where self-incompatible (SI) species are crossed with self-compatible (SC) species (Lewis and Crowe 1958; de Nettancourt 1977) or incongruity (Hogenboom 1975, 1984) operative at either a pre-zygotic (e.g. stigma, style or micropyle) or post-zygotic stage (Levin 1978a; Knox 1984).

The specific site of expression and cause of the unilateral barrier to hybridisation has not been determined in *Eucalyptus*. The present study thus investigates the site and mechanism of the unilateral failure of hybridisation between two commercially important species, *E. globulus* and *E. nitens*. *E. globulus* in particular is widely grown for pulpwood production (Volker and Orme 1988). However, while it is relatively fast growing there is considerable interest in improving characteristics such as frost resistance through interspecific hybridisation with *E. nitens* (Tibbits 1986, 1989) and other smaller flowered species e.g. *E. gunnii* (Potts *et al.* 1987). The styles of *E. globulus* are about four times the length of those of *E. nitens* and hybridisation is successful only when *E. nitens* is used as the maternal parent (Tibbits 1986, 1989). Nevertheless, the use of *E. globulus* as the maternal parent for the production of the *E. globulus* x *E. nitens* hybrids is desirable to tree breeders as the flower is relatively large, is easy to emasculate and pollinate, and has the potential to produce larger and greater numbers of seeds than a *E. nitens* flower. There are thus considerable benefits in breaking this unilateral barrier to hybridisation and, as a prerequisite, the present study was initiated to elucidate the site of incompatibility. The growth of *E. globulus* and *E. nitens* pollen tubes following pollination of *E. globulus* styles is detailed and the evolution of such unilateral barriers to hybridisation is addressed. The *in vitro* pollen tube growth of both species was also compared with three other species which exhibited complete (*E. gunnii* and *E. ovata*) or partial (*E. urnigera*) unilateral cross-incompatibility with *E. globulus* (Potts and Savva 1988).

Materials and Methods

In vivo Pollen Tube Development

Pollen from four *E. nitens* and three *E. globulus* trees was pooled to create separate 'polymixes' of each species. Pollen was extracted as outlined by Potts and Marsden-Smedley (1989) and placed in gelatine capsules which were stored in air-tight phials containing silica gel at -20°C . Prior to flowers being pollinated, the viability of the pollen was tested *in vitro*, using the method of Griffin *et al.* (1982). Pollinations were performed on two ornamental trees of *E. globulus*. Flowers were emasculated by cutting away the staminal ring just prior to anthesis and then enclosed in Terylene bags to prevent natural pollination. Six days after emasculation, at peak receptivity (Tibbits 1986), flowers were pollinated by applying pollen with a matchstick. Flowers were rebagged and collected on days 1, 2, 3, 4, 5, 7, 14 and 28 after pollination. Flowers from the second tree were collected after 8 days, rather than 7 (treated as day 7 in the GLM analysis). Five flowers were collected, from each tree, on days 1 and 2, and four flowers from each tree on the other sampling days.

Preparation of pistils for microscopic examination of pollen tubes was based on the methods of Griffin *et al.* (1982). Pollen tubes were stained with aniline blue and observed using a Nikon Optiphot microscope with an episcopic-fluorescence attachment (UV-excitation). An ocular micrometer was used to measure the five longest pollen tubes in each style which were then averaged for each flower. Pollen tubes that had grown the full length of the style were recorded as 9 mm long, the approximate length of the *E. globulus* styles. The number of tubes per style was estimated by counting visible tubes. Pollen tube lengths were not recorded in cases where there were fewer than 10 tubes per style, as they appeared to be unrepresentative of both cross types. The significance of mother tree, pollen species, time and interaction effects on pollen tube length and number was tested using the generalised linear models procedure (GLM) of SAS (SAS 1987), and the least squares means and their standard errors were obtained from the same procedure. To examine the style anatomy and to determine the path of *E. globulus* pollen tubes through the style, transverse sections of six *E. globulus* styles were studied. Styles were frozen with carbon dioxide and transverse sections cut at $30\ \mu\text{m}$ using a Reichert sliding microtome. Sections were stained and observed as previously described.

In vitro Pollen Tube Growth

The growth of pollen from five trees each of *E. globulus*, *E. urnigera*, *E. gunnii* and *E. ovata*, and four trees of *E. nitens* was compared *in vitro*. Pollen was collected, extracted and stored as outlined previously. Pollen from each tree was kept separate and germinated on a modified form of Cauvin's

(1984) medium (0.5% agar, 20% sucrose and 150 ppm boric acid), plated into cells in 5 × 5 replidishes. Pollen from each of the 24 trees was replicated once in each of three replidishes. The pollen was incubated at 25°C and after 24 h the mean pollen tube length in each cell was estimated from the length of the longest pollen tube in 10 randomised fields of view of a compound microscope (100x magnification). Pollen was scored in replidish blocks and variation between and within species analysed by a hierarchical analysis of variance within the block stratum using GENSTAT. The difference between specific means was tested for significance using the l.s.d. procedure.

Floral and Pollen Dimensions

The diameters of 10 pollen grains from 8–10 trees each of *E. nitens*, *E. globulus*, *E. gunnii*, *E. urnigera* and *E. ovata* were measured using a graduated ocular microscope (400x magnification). To differentiate between inviable and viable pollen grains the pollen samples were rehydrated at 5°C on an agar medium (20% sucrose, 200 ppm boric acid and 0.5% agar) for approximately 24 h prior to measurement (see Heslop-Harrison and Heslop-Harrison 1985, p. 139). Only large, swollen pollen grains were measured. Style length and ovary depth were measured at the stage of stigma receptivity from three fresh flowers per tree.

Results

The Pollen Tube Path and Anatomy of the E. globulus Style

The style of *E. globulus* contains a lobed canal for approximately two-thirds of its length, similar to that described for *E. woodwardii* (Sedgley and Smith 1989). The canal comprises 4 to 5 lobes (Fig. 1), which corresponded to the number of locules in the specimens examined. Large oil bodies occur throughout the length of the style in the outer region of the cortex (Fig. 1). Vascular bundles occur throughout the length of the style (Fig. 1b), while sclerenchyma was only found in the lower half of the style. Pollen tubes germinate on the stigma, grow through the stigmatic tissue into the style where for the first 1 to 6 mm they are confined to the transmitting tissue surrounding the stylar canal (Fig. 1a). At approximately 6 mm, the transmitting tissue narrows inwards and the tubes converge into the center of the style (Fig. 1b). This arrangement continues to the base of the style. At approximately 1 mm below the base of the style the tubes radiate outwards and grow down the ovary in, or on the surface of, the tissue surrounding the locules (Fig. 1c). The confinement of *E. globulus* pollen tubes to the transmitting tissue and the absence of growth in the stylar canal is similar to pollen tube growth patterns reported in *E. regnans* (Sedgley *et al.* 1989) and *E. woodwardii* (Sedgley and Smith 1989).

Comparison of in vivo Pollen Tube Development

The estimated number of pollen tubes per *E. globulus* style only differed significantly ($P < 0.05$) between pollen species with nearly twice as many tubes of *E. globulus* (102) than *E. nitens* (63) counted per style. However, this is unlikely to reflect a differential response of pollen to germination on the *E. globulus* stigma as this ratio was consistent with species differences in percentage germination *in vitro* (i.e. *E. globulus* 33.3% and *E. nitens* 13.5%). In contrast, the length of pollen tubes differed significantly ($P < 0.001$) between pollen species, female trees and sampling times and the tree × time interaction was also significant ($P < 0.05$) for the transformed data. The pollen tubes of *E. globulus* were significantly longer than those of *E. nitens* at most time intervals, with the difference tending to increase with time (Fig. 2). On average, *E. globulus* pollen tubes grew at a rate of 1.4 mm per day for the first 4 days (Fig. 2), compared with 0.8 mm per day for pollen tubes of *E. nitens* (Fig. 2). From day 4 to day 14, the average rate of growth for *E. nitens* tubes decreased to 0.2 mm per day and virtually no growth occurred after day 14. The decrease in growth rate of *E. nitens* pollen tubes coincided with a change in growth form, the pollen tubes tending to exhibit a winding growth

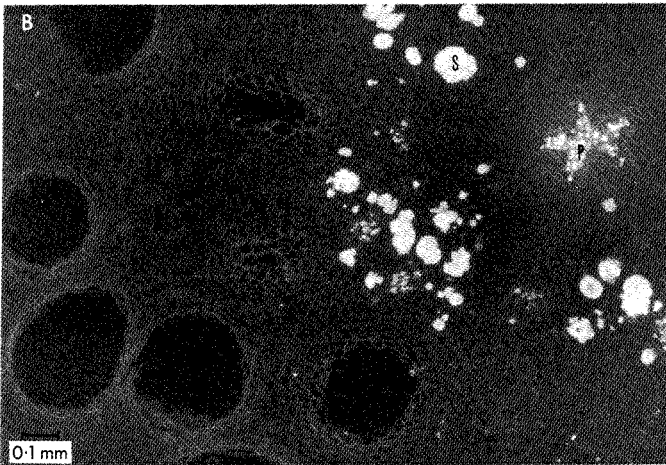
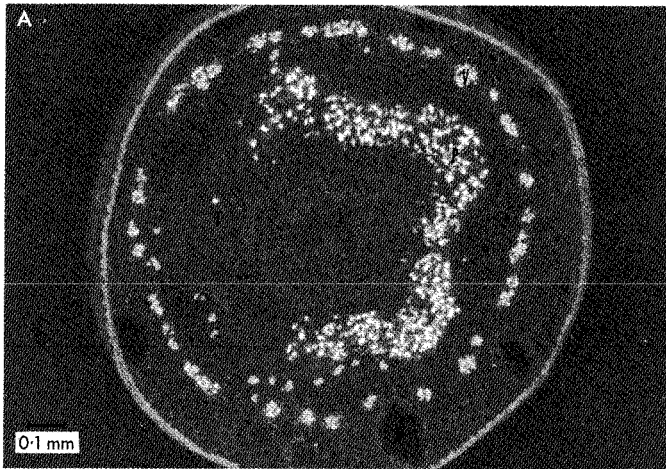


Fig. 1. The growth of *E. globulus* pollen tubes through the *E. globulus* style. (a) Transverse section 2 mm down the style of *E. globulus*, showing the lobed canal (L), pollen tubes (P), transmitting tissue (T), vascular bundles (V) and oil bodies (O). (b) Transverse section through the base of the style showing pollen tubes (P), sclerenchyma (S) and oil bodies (O). (c) Transverse section 1.5 mm below the base of the style showing pollen tubes (P) and locules (LO).

pattern after day 4. *E. globulus* pollen tubes had grown the full length of the style and entered the ovary 5–14 days after pollination (Fig. 2). However, after 28 days, when style abscission was commencing, *E. nitens* pollen tubes had grown no more than 6 mm (Fig. 2), well short of the full length of the *E. globulus* style (c. 9–10 mm, Table 1). Pollen tubes of *E. nitens* reached maximum length about 14 days after pollination (Fig. 2) and near the limit of growth the tube tips often appeared distorted (Fig. 3), sometimes with irregular callose deposits and/or swelling.

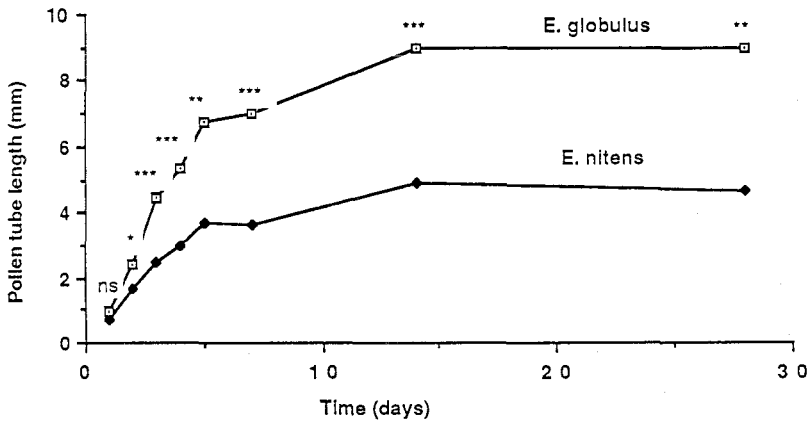


Fig. 2. Average growth rate *in vivo* of *E. globulus* and *E. nitens* pollen tubes. Data have been pooled from the 2 trees and means have been back-transformed from \log_{10} values and the level of significance of the difference in means between *E. globulus* and *E. nitens* at each time interval are indicated (ns, not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$).

Table 1. Style length (mm), ovary depth (mm), pollen grain diameter (μm) and the number of seed obtained per flower following cross pollination of *E. nitens* females

Crossing data are derived from Tibbits (1989, table 3)

	Style length			Ovary depth		Pollen grain diameter			No. of seed / <i>E. nitens</i> flower	
	<i>n</i>	Mean	s.e.	Mean	s.e.	<i>n</i>	Mean	s.e.	Mean	s.e.
<i>E. globulus</i>	9	9.9	0.43	8.2	0.31	10	27	0.3	2.3	1.70
<i>E. nitens</i>	7	2.3	0.10	3.5	0.13	8	19	0.3	3.6	0.62
<i>E. gunnii</i>	10	3.5	0.15	2.5	0.72	8	25	0.7	2.9	0.71
<i>E. urnigera</i>	166	5.2	0.06	4.1	0.04	9	26	0.5		
<i>E. ovata</i>	8	4.0	0.10	2.2	0.08	8	25	0.5		

There was no significant female \times pollen species interaction or three-way interaction indicating that the relative difference in pollen tube length between species was similar between trees. However, the initial rate of growth of pollen tubes of both species varied ($P < 0.01$) between females with pollen tubes growing much faster on Tree 2 than Tree 1. While this difference in growth rate could reflect a developmental or physiological difference between females, it is more likely to be directly related to differences in temperature during pollen tube growth. The two trees were pollinated 6 days apart and the initial period of pollen tube growth in Tree 1 coincided with 1 week of lower daily temperatures commencing on day 3.

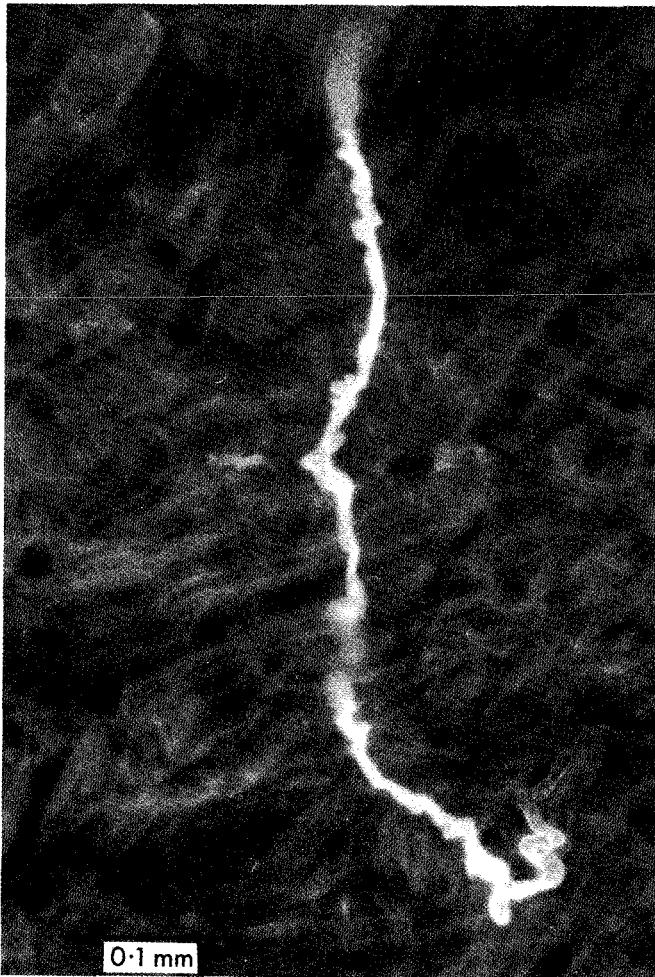


Fig. 3. Distorted pollen tube tip of *E. nitens* after 14 days.

Table 2. Analysis of variance of *in vitro* pollen tube lengths (n.s., not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$)

Source of variation	d.f.	<i>F</i>	<i>P</i>
Pollen tube lengths (\log_{10} transformed)			
Block stratum	3		
Within block stratum			
Species	4	26.1	***
Trees within species	19	3.6	***
Within species stratum			
<i>E. globulus</i>	4	3.2	*
<i>E. nitens</i>	3	2.3	n.s.
<i>E. gunnii</i>	4	6.2	***
<i>E. ovata</i>	4	0.4	n.s.
<i>E. urnigera</i>	4	5.4	***

In vitro Pollen Tube Growth

After 24 h growth *in vitro*, significant differences in pollen tube length occurred both amongst the five species as well as between trees within species (Table 2). The latter variation resulted from significant differences in pollen tube lengths amongst trees of *E. urnigera* ($P < 0.001$), *E. gunnii* ($P < 0.001$) and *E. globulus* ($P < 0.05$) (Table 2). The pollen tubes of *E. globulus* grew significantly longer than those of the other four species (Fig. 4). Under these specific experimental conditions, virtually all *in vitro* pollen tube growth was completed within 24 h (unpublished data), and thus the pollen tube lengths measured are close to maximum length obtainable *in vitro* for each species. *E. nitens* pollen tubes were the shortest (Fig. 4) and *in vitro* grew only one-third of the length of *E. globulus* pollen tubes which is consistent with the *in vivo* results. There was a significant ($P < 0.05$) association between *in vitro* pollen tube length and style (Table 1) or pistil (style + ovary depth) length amongst these five species and, at least for *E. globulus* and *E. nitens*, the ratios of the pollen tube length *in vitro* and distance from the stigma to the base of the ovary were identical (3:2:1).

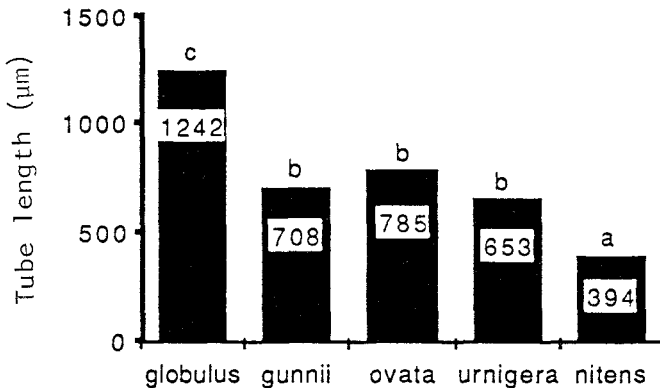


Fig. 4. Mean *in vitro* length (μm) of *E. globulus*, *E. gunnii*, *E. ovata*, *E. urnigera* and *E. nitens* pollen tubes. Common letters indicate means which are not significantly different ($P > 0.05$).

Discussion

The *in vivo* study indicates that the unilateral failure of crosses between *E. globulus* and *E. nitens* occurs prior to fertilisation and is directly due to the failure of the pollen tubes of *E. nitens* to grow much beyond half the length of the *E. globulus* style. Fewer *E. nitens* pollen tubes were also counted in the *E. globulus* style. However, this was consistent with the lower *in vitro* germination of the *E. nitens* pollen utilised and there is no evidence of stigmatic inhibition of pollen germination or initial penetration of the style (Knox *et al.* 1976; Stettler *et al.* 1980).

Two distinct, although not mutually exclusive, groups of mechanisms (de Nettancourt 1984) have been proposed to account for interspecific incompatibility (*sensu* de Nettancourt 1977, p. 141). A number of authors, Lewis and Crowe (1958), Abdalla and Hermsen (1972) and de Nettancourt (1977), consider that interspecific incompatibility depends on the self-incompatibility system governed by the S-supergene. Interspecific incompatibility is frequently unilateral and usually prevents self-incompatible species from being successfully crossed with pollen from self-compatible species (i.e. SI \times SC relationships: Lewis and Crowe 1958; Abdalla and Hermsen 1972; de Nettancourt 1977, 1984). A dual function of the S-alleles (self-rejection and rejection

of SC pollen, e.g. Lewis and Crowe 1958; Pandey 1969) or the selection of specific unilateral incompatibility (UI) genes to protect self-incompatible species from contamination by self-compatibility genes (Abdalla and Hermsen 1972) have been proposed as explanations for this unilateral relationship. Alternatively, Hogenboom (1975, 1984) has argued that interspecific incompatibility may be completely distinct from the self-incompatibility reaction and may be due to incongruity. According to Hogenboom (1975, 1984), incongruity results from a lack of co-adaptation between species, such that one species lacks genetic information about some relevant character of the other. In general, cross-compatibility declines as the level of taxonomic divergence between population systems increases (Levin 1978a) and Hogenboom (1975, 1984) considers that incongruity arises as a by-product of evolutionary divergence. It can explain interspecific incompatibility and may result from incompleteness of the relationship at the physiological, biochemical or structural level (Hogenboom 1975, p. 366).

The unilateral incompatibility between *E. globulus* and *E. nitens* is unlikely to be associated, even indirectly, with an S-allele system since the operation of an S-allele system has not been demonstrated in *Eucalyptus*. Full self-incompatibility is rare (Potts and Savva 1990) and most species including *E. nitens* and *E. globulus* appear to be partially self-incompatible (Potts and Savva 1988, 1990; Tibbitts 1989). In addition, where the S-locus is implicated in cross-incompatibility in other genera, the site of the incompatibility often corresponds to the site of expression of self-incompatibility (de Nettancourt 1977, p. 145). However, the site of expression of the unilateral barrier to hybridisation between *E. globulus* and *E. nitens* does not correspond to the site of the barrier to self-compatibility in *Eucalyptus* which, when present, appears to operate at the time of ovule penetration (Sedgely 1989) or by post-zygotic mechanisms (Griffin *et al.* 1987; Sedgely *et al.* 1989).

While physiological differences between species in *Eucalyptus* have been demonstrated at the male gametophytic level (Potts and Marsden-Smedley 1989), several lines of evidence suggest that physiological incongruity is unlikely to be important and that structural incongruity (or incompatibility), arising from disparity in style and pollen tube lengths, is the major factor limiting the growth of the *E. nitens* pollen through the *E. globulus* style. Firstly, although *E. globulus* and *E. nitens* differ markedly in flower morphology, they are relatively closely related being grouped in the same subseries (Subseries *Globulinae*, Series *Viminalae*, Section *Maidenaria*, after Pryor and Johnson 1971). When flower sizes are comparable, no barriers to seed-set in wider crosses within or even between series within the section *Maidenaria* have been reported (e.g. Potts *et al.* 1987; Tibbitts 1989) which suggests that strong endogeneous, physiological barriers to fertilisation are rare. If physiological incongruity arising from genetic divergence was important, it may also be expected in the reciprocal cross using *E. nitens* as the female which is not the case. Furthermore, of the 15 different types of interspecific crosses undertaken on *E. globulus* females by Potts and Savva (1988), only three crosses produced viable seed. The most successful was with *E. urnigera* which is more distantly related to *E. globulus* than *E. nitens* but has the longest style of all the pollen species examined. Secondly, neither physiological incongruity nor a stylar incompatibility mechanism explains the marked *in vitro* difference in pollen tube growth between *E. globulus* and *E. nitens*. *In vitro*, *E. nitens* pollen tubes grew to less than one-third the length of those of *E. globulus*. The pollen tubes of neither species achieved the lengths *in vitro* that were observed *in vivo* which is a common phenomenon (Brewbaker and Kwack 1964). Nevertheless, the relative pollen tube lengths are comparable, suggesting that the response is relatively independent of the stylar environment. Finally, the *in vitro* pollen tube length of the five species tested was correlated with style length ($P < 0.05$; Table 3) and the maximum length that the *E. nitens* pollen tubes grew in the *E. globulus* style was virtually identical to the distance from the stigma to the base of the ovary in *E. nitens* flowers (Table 1).

A close co-adaptation between style and pollen tube length is essential for successful fertilisation and differences in the length of reproductive structures have been reported as major barriers to interspecific hybridisation in numerous plant genera (Levin 1978a; Plitmann and Levin 1983). As in the present case, this barrier is frequently asymmetrical resulting in the unilateral failure of interspecific hybridisation when small flowered species are used as pollen parents e.g. *Prunus* (Perez and Moore 1985) and *Rhododendron* (Williams and Rouse 1988). In *Rhododendron*, the success of interspecific pollinations was dependant on the male/female style length ratio, and where the style length ratio was < 0.2 , pollen tubes of the shorter styled species were unable to reach the ovary. Williams and Rouse (1988) consider that in such cases pollen tube growth might be due to limited nutritional reserves within the pollen grain, combined with limited access to stylar reserves or alternatively, pre-programming of the pollen grain for finite growth correlated with pistil size, hypotheses which are difficult to differentiate in the present case.

Successful seed-set has been obtained following both intra- and interspecific pollinations of artificially shortened styles of *Eucalyptus gunnii* (Potts and Cauvin 1988). Nevertheless, to date attempts to break the unilateral barrier to hybridisation between *E. globulus* and *E. nitens* by artificially shortening the *E. globulus* styles have not been successful with neither the *E. globulus* control nor the *E. nitens* pollinations setting seed (Badcock and Volker, unpublished data). Reduced cross success was also reported in

Table 3. Correlations amongst floral characters for five *Eucalyptus* species

(d.f., 3; n.s., not significant; * $P < 0.05$)

	Style length	Ovary depth	Pollen grain diameter
Style length	1.00		
Ovary depth	0.92*	1.00	
Pollen grain diameter	0.71 n.s.	0.38 n.s.	1.00
Pollen tube length (<i>in vitro</i>)	0.93*	0.75 n.s.	0.79 n.s.

Rhododendron when some large-flowered species were used to pollinate small-flowered species, possibly due to mis-matched timing of male and female maturity (Williams and Rouse 1988) or overgrowth of pollen tubes (Williams *et al.* 1986). Such an effect has not as yet been reported in *Eucalyptus* where small-flowered species have been successfully pollinated by quite large-flowered species (e.g. Pryor 1956; Potts and Cauvin 1988; Tibbits 1989). Nevertheless, when *E. nitens* was used as the female, there was a trend for crosses with *E. globulus* to be less successful than both intra-specific crosses and crosses with *E. gunnii* (which has a style length more comparable to *E. nitens*, Table 1), a trend which clearly warrants detailed investigation.

Evolution of Unilateral Cross-incompatibility

There is considerable variability in flower size within *Eucalyptus* (Griffin 1982) and closely related taxa frequently form morphological series varying markedly in flower and fruit size (Kirkpatrick 1975; Bramwells and Whiffin 1984; Potts and Jackson 1986). The structural barrier arising from disparities in pistil and pollen tube length is thus likely to be a major post-mating barrier to interspecific hybridisation and gene flow within subgenera. This barrier is clearly asymmetrical and the evolution of such barriers is of fundamental importance to speciation theories.

While Hogenboom (1975, 1984) only treats incongruity as arising as a by-product of evolutionary divergence (presumably including chance, hitchhiking and some character

displacement processes such as competition for pollinators, Levin 1978*a*, p. 276), the same reproductive barriers (e.g. disparities in flower size) may also result from direct selection for reproductive isolation *per se* from co-generic or co-specific populations, i.e. Wallace Effect (Grant 1971; Butlin 1989). Thus, whether the structural mis-matching of *E. nitens* and *E. globulus* is classified as structural incongruity in the strict sense would appear to rest on the origin of the structural disparity which is difficult to determine. Moreover, while the evolution of unilateral S-allele related incompatibility (Abdalla and Hermsen 1972) and incongruity (Hogenboom 1975, 1984) has been addressed, little attempt has been made to integrate this unilateral phenomenon with the Wallace Effect. Nevertheless, it is easy to envisage situations where disparities in population size (King 1985) or shape (Levin 1978*b*), flowering time or intensity could result in asymmetrical production of maladapted F₁ hybrids and thus asymmetrical selection for reproductive isolation. For example, the actual levels of F₁ hybridisation may not only be greater in small populations (Levin 1978*a*), but the effect would be distributed through a greater proportion of the population compared with larger populations.

Thus, the Wallace Effect could also be expected to result in unilateral reproductive isolation and if operative, its effects are most likely to be detected in the interaction between large ancestral and small derived populations (King 1985) or in relict, founder or ecologically restricted populations surrounded by large populations of potentially interbreeding species. In these circumstances, pre-mating (e.g. flowering time) or pre-fertilisation barriers would be expected to limit the wastage of ovules in the smaller population through inter-taxon fertilisations, and the present study indicates that selection for increased flower size in the smaller populations is one possible scenario. While variation in flower size may be associated with ecological factors and directly or indirectly (e.g. due to genetic correlation with increasing seed size or woodiness of the capsule) selected, the role of the Wallace Effect in explaining the considerable variation in flower morphology both within and between eucalypt species requires investigation.

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References

- Abdalla, M. M. F., and Hermsen, J. G. Th. (1972). Unilateral incompatibility: hypotheses, debate and its implications for plant breeding. *Euphytica* **21**, 32-47.
- Beardsell, D. V., Jones, D. L., and Beardsell, C. (1979). Early success with ornamental eucalypt breeding. *Australian Plants* **10**, 70-1.
- Bramwells, H. W., and Whiffin, T. (1984). Patterns of variation in *Eucalyptus siberoxylon* A. Cunn. ex Woolls. 1. Variation in adult morphology. *Australian Journal of Botany* **32**, 263-81.
- Brewbaker, J. L., and Kwack, B. H. (1964). The calcium ion and substances influencing pollen growth. In 'Pollen Physiology and Fertilization'. (Ed. H. F. Linskens.) pp. 143-51. (North Holland: Amsterdam.)
- Butlin, R. (1989). Reinforcement of premating isolation. In 'Speciation and Its Consequences'. (Eds D. Otte and J. A. Endler.) pp. 158-179. (Sinauer Associates, Inc.: Massachusetts.)
- Cauvin, B. (1984). *Eucalyptus* hybridation contrôlée — premiers résultats. *Annales de recherches sylvicoles* **1983**, AFOCEL Paris, 85-117.
- Gardella, C. (1950). Overcoming barriers to crossability due to style length. *American Journal of Botany* **37**, 219-24.
- Grant, V. (1971). 'Plant Speciation.' (Columbia University Press: New York.)

- Griffin, A. R. (1982). Pollination ecology of eucalypts: a frame work for study. In 'Pollination 82'. (Eds E. G. Williams, R. B. Knox, J. H. Gilbert and P. Bernhardt.) (Plant Cell Biology Research Centre: University of Melbourne.)
- Griffin, A. R., Burgess, I. P., and Wolf, L. (1988). Patterns of natural and manipulated hybridisation in the genus *Eucalyptus* L'Hérit.-a review. *Australian Journal of Botany* **36**, 41-66.
- Griffin, A. R., Ching, K. K., Johnson, K. W., Hand, F. C., and Burgess, I. P. (1982). Processing *Eucalyptus* pollen for use in controlled pollination. *Silvae Genetica* **31**, 198-203.
- Griffin, A. R., Moran, G. F., and Frupp, Y. J. (1987). Preferential outcrossing in *Eucalyptus regnans* F. Muell. *Australian Journal of Botany* **35**, 465-75.
- Heslop-Harrison, J., and Heslop-Harrison, Y. (1985). Germination of stress-tolerant *Eucalyptus* pollen. *Journal of Cell Science* **73**, 135-57.
- Hogenboom, N. G. (1975). Incompatibility and incongruity: two different mechanisms for the non-functioning of intimate partner relationships. *Proceedings of the Royal Society of London* **B188**, 361-75.
- Hogenboom, N. G. (1984). Incongruity: non-functioning of intercellular and intracellular partner relationships through non-matching information. In 'Cellular Interactions. (Encyclopedia of Plant Physiology; new ser., V. 17)'. (Eds H. F. Linskens and J. Heslop-Harrison.) pp. 640-654. (Springer-Verlag: Berlin.)
- King, J. L. (1985). Selection for mating barriers between populations of unequal size. *Evolutionary Theory* **7**, 149-51.
- Kirkpatrick, J. B. (1975). Geographical variation in *Eucalyptus globulus*. Forestry and Timber Bureau, Bulletin 47. (Aust. Gov. Publ. Serv.: Canberra.)
- Knox, R. B. (1984). Pollen-pistil interactions. In 'Cellular Interactions. (Encyclopedia of Plant Physiology; new ser., V. 17)'. pp. 508-608. (Springer-Verlag: Berlin.)
- Knox, R. B., Clarke, A. E., Harrison, S. Smith, P., and Marchalonis, J. J. (1976). Cell recognition in plants: determinants of the stigma surface and their pollen interactions. *Proceedings of the National Academy of Sciences of the United States of America* **13**, 2788-92.
- Levin, D. A. (1978a). The origin of isolating mechanism in flowering plants. *Evolution Biology* **11**, 185-317.
- Levin, D. A. (1978b). Some genetic consequences of being a plant. In 'Ecological Genetics: the Interface'. (Ed. P. F. Brussard.) pp. 189-212. (Springer-Verlag: New York.)
- Lewis, D., and Crowe, L. K. (1958). Unilateral interspecific incompatibility in flowering plants. *Heredity* **12**, 233-56.
- Nettancourt, D. de (1977). 'Incompatibility in Angiosperms.' (Springer-Verlag: Berlin.)
- Nettancourt, D. de (1984). Incompatibility. In 'Cellular Interactions. (Encyclopedia of Plant Physiology; new ser., V. 17)'. (Eds H. F. Linskens and J. Heslop-Harrison.) pp. 624-39. (Springer-Verlag: Berlin.)
- Pandey, K. K. (1969). Elements of the S-gene complex. V. Interspecific cross-incompatibility relationships and theory of the evolution of the S complex. *Genetica* **40**, 447-74.
- Perez, S., and Moore, J. N. (1985). Prezygotic endogenous barriers to interspecific hybridization in *Prunus*. *Journal of the American Society for Horticultural Science* **110**(2), 267-73.
- Plitmann, U., and Levin, D. A. (1983). Pollen-pistil relationships in the *Polemoniaceae*. *Evolution* **37**(5), 957-67.
- Potts, B. M., and Cauvin, B. (1988). Inbreeding and interspecific hybridisation in *Eucalyptus*. In 'Proceedings of the International Forestry Conference for the Australian Bicentenary 1988'. (A.F.D.I.: Albury-Wodonga, Australia.)
- Potts, B. M., and Jackson, W. C. (1986). Evolutionary processes in the Tasmanian high altitude eucalypts, pp. 522-527. In 'Flora and Fauna of Alpine Australasia: Ages and Origins'. (Ed. B. Barlow.) (CSIRO: Melbourne.)
- Potts, B. M., and Marsden-Smedley, J. B. (1989). *In vitro* germination of *Eucalyptus* pollen: response to variation in boric acid and sucrose. *Australian Journal of Botany* **37**, 429-41.
- Potts, B. M., Potts, W. C., and Cauvin, B. (1987). Inbreeding and interspecific hybridisation in *Eucalyptus gunnii*. *Silvae Genetica* **36**, 194-9.
- Potts, B. M., and Savva, M. (1990). Self-incompatibility in *Eucalyptus*. In 'Pollination '88'. (Eds R. B. Knox, M. B. Singh and L. Troini.) pp. 165-170. (School of Botany: University of Melbourne.)
- Potts, B. M., and Savva, M. (1988). The crossability of *Eucalyptus globulus*. In 'Proceedings of the IUFRO Meeting on *Eucalyptus* Provenances and Breeding, Thailand, 1988' (in press).
- Pryor, L. D. (1956). An F1 hybrid between *Eucalyptus pulverulenta* and *E. caesia*. *Proceedings of the Linnean Society of New South Wales* **81**, 97-100.

- Pryor, L. D., and Johnson, L. A. S. (1971). 'A Classification of the Eucalypts.' (Aust. Natl. Univ. Press: Canberra.)
- SAS (1987). 'SAS/STAT Guide for Personal Computers.' 6th Edition. (SAS Institute Inc.: Cary, USA.)
- Sedgley, M. (1989). Ovule and seed development in *Eucalyptus woodwardii* Maiden (*Symphyomyrtus*). *Botanical Gazette* **150**, 271-80.
- Sedgley, M., Hand, F. C., Smith, R. M., and Griffin, A. R. (1989). Pollen tube growth and early seed development in *Eucalyptus regnans* F. Muell. (Myrtaceae) in relation to ovule structure and preferential outcrossing. *Australian Journal of Botany* **37**, 397-411.
- Sedgley, M., and Smith, R. M. (1989). Pistil receptivity and pollen tube growth in relation to the breeding system of *Eucalyptus woodwardii* Maiden (*Symphyomyrtus*). *Annals of Botany* **64**, 21-31.
- Stettler, R. F., Koster, R., and Steenackers, V. (1980). Interspecific crossability studies in Poplars. *Theoretical Applied Genetics* **58**, 273-82.
- Tibbits, W. N. (1986). Frost resistance of *Eucalyptus nitens* (Deane & Maiden) Maiden. Ph.D. Thesis. University of Tasmania.
- Tibbits, W. N. (1989). Controlled pollination studies with shining gum (*Eucalyptus nitens* (Deane & Maiden) Maiden). *Forestry* **62**, 111-125.
- Volker, P. W., and Orme, R. K. (1988). Provenance trials of *Eucalyptus globulus* and related species in Tasmania. *Australian Forestry* **51**(4), 257-65.
- Williams, E. G., Kaul, V., Rouse, J. L., and Palser, B. F. (1986). Overgrowth of pollen tubes in embryo sacs of *Rhododendron* following interspecific pollinations. *Australian Journal of Botany* **34**, 413-23.
- Williams, E. G., and Rouse, J. L. (1988). Disparate style lengths contribute to isolation of species in *Rhododendron*. *Australian Journal of Botany* **36**, 183-91.

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