



**Breeding Systems and Interspecific Hybridisation  
in the Genus *Eucalyptus* L'Hér.**

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**APPENDIX:** Ellis, M. F., Sedgley, M., and Gardner, J. A. 1991. Interspecific pollen-pistil interaction in *Eucalyptus* L'Hér.(Myrtaceae): The effect of taxonomic distance. *Annals of Botany* **68**, 185-94.

## SUMMARY

This study investigated the reproductive biology of four *Eucalyptus* species of the subgenus *Symphyomyrtus*; *E. spathulata*, *E. cladocalyx* and *E. leptophylla* of the section *Bisectaria*, and *E. leucoxyton* of the section *Adnataria*.

Aspects of the breeding system, floral morphology and pistil cytology were studied in three trees each of *E. spathulata*, *E. cladocalyx* and *E. leptophylla*. *E. spathulata* and *E. leptophylla* were found to be highly self incompatible, setting very low levels of seed from controlled self pollination. *E. cladocalyx* trees ranged from self compatible to self incompatible. Reductions were seen in both the number of capsules and the numbers of seed per capsule, from self pollination. The mechanism of self incompatibility was investigated in the pistil by following the success of cross and self pollinations with fluorescence microscopy. In *E. cladocalyx* and *E. leptophylla* no reduction in ovule penetration was seen from self pollination while in *E. spathulata* a significant reduction was seen in two trees but not the third, indicating post-zygotic mechanisms of self incompatibility operating in all three species, with some pre-zygotic control in *E. spathulata*. Floral architecture differed between the three species in the structure of the inflorescence units, flower morphology, and anther, pollen and ovule numbers per flower. Pistil cytology was similar for all three species but there were differences in the length of the stylar canal, the degree of sclerotinisation of the style, stigma morphology and volume of transmitting tissue.

The breeding system of *E. leucoxyton* was investigated with emphasis on the unusual features of gynodioecy and secondary pollen presentation. Fifty seven percent of trees in the study population were found to be male sterile, with pollen grains aborted during development between tetrad formation and anthesis. Hermaphrodite trees presented 93 percent of pollen grains on the upper style and stigma, with only seven percent of pollen grains remaining on the anthers. In the absence of pollinators hermaphrodite trees set low levels of autogamous seed compared with open pollinated pistils. Counts of pollen tubes in open pollinated pistils of each morph revealed that female trees were pollen limited in the study population. Multilocus estimates of outcrossing rates were determined for female

isozyme electrophoresis. Female trees showed values of  $\hat{t}$  approaching 1.0, indicating complete outcrossing, while hermaphrodite trees showed significant levels of selfed seed in open pollinated seed crops, but still maintained an outcrossing rate higher than that reported for most eucalypts.

The pollen-pistil interaction was investigated in three intraspecific, 57 interspecific and six intergeneric crosses using the three species *Eucalyptus spathulata*, *E. cladoclayx* and *E. leptophylla* as female parents. Interspecific prefertilisation isolation was found to occur in the pistil and manifested as a number of pollen tube abnormalities in the style and ovary associated with a lowered probability of ovule penetration. The major selection points in the pistil were the upper style and the ovary. The severity of abnormalities and the probability of pollen tube arrest in the pistil was proportional to the taxonomic distance between parent species. Ovule penetrations were seen mainly in crosses within the section *Bisectaria* or between the sections *Bisectaria* and *Adnataria*. Pollen storage, style length and mean maximum temperature during the flowering period of the male parent had no significant effect on pollen tube growth in the crosses used.

Intra and interspecific combinations which showed ovule penetration were repeated the following flowering season and monitored for seed set. Most combinations produced capsules although only combinations within the section *Bisectaria* and between the sections *Bisectaria* and *Adnataria* set viable seed. There was considerable variation between years in cross compatibility with some female trees which showed no ovule penetration in the first year setting viable seed in the same combinations the following year. Many close combinations set levels of seed approaching intraspecific cross pollinations, while interspecific crosses outperformed intraspecific self pollinations in both capsule set and the number of seeds per capsule. Some crosses between the sections *Bisectaria* and *Adnataria* showed hybrid breakdown at the early seedling stage. Hybrid parentage was confirmed through intermediate seedling morphology.

The findings of this study have implications for the ecology and conservation of *Eucalyptus* species, for breeding strategies in tree improvement programmes and for the taxonomic relationships of the species.