Australian Flora Foundation Final Report

Reproductive ecology and population genetics of *Grevillea barklyana:* a model for the conservation of small populations of endangered plants.

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Background

Extensive fragmentation of plant populations due to land clearance has occurred in Australia during the last 100 years. An important concern in conservation biology is the predicted reduction in viability and persistence of small, isolated populations. Population genetic theory suggests that inbreeding will increase in small populations, leading to increased expression of deleterious alleles (inbreeding depression). However, there have been few attempts to quantify these parameters, or to integrate this theory with a knowledge of plant breeding systems and interactions with pollinators.

Funding from Australian Flora Foundation during 1993 has allowed examination of the effects of small population size on the rare plant species, *Grevillea barklyana*, using an integrated ecological and genetic approach Specifically, the aim of this study was to examine pollinators, seed set, mating patterns, and inbreeding depression in *G. barklyana* populations that differ in size (numbers of plants).

Study sites

Observations and experimental work were conducted in five populations of *Grevillea barklyana* spp. *maccleayana* near Jervis Bay on the NSW south coast. Populations varied in size from approximately 50 to 500 plants.

Summary of Results

(1) Self-fertility and inbreeding depression

Hand-pollination experiments in several populations indicated that G. *barklyana* plants are fully self-fertile. Plants are also able to automatically self-pollinate, thereby ensuring reproduction in the absence of pollinators. Selfed and outcrossed seeds are viable and produce vigorous seedlings. Self-fertility and the apparent high fitness of selfed progeny relative to outcrossed progeny

suggest that inbreeding depression is not occurring in *G. barklyana* populations, although further examination especially under field conditions is necessary. Lack of inbreeding depression may be the result of recessive lethal alleles being purged from populations by repeated setting over a long time period.

(2) Mating system analyses

Electrophoretic examination of mating patterns in several populations indicated that the majority of seeds produced were the products of self-fertilization. In one population, where the genotypes of individual plants were determined by electrophoresis, there was virtually no exchange of genes between immediately adjacent plants. The implications of high levels of selfing include increased homozygosity, decreased genetic diversity and, in the long term, increased nsk cf extinction as a result of reduced ability' to adapt to changing environmental conditions, interestingly, some variation in mating patterns was evident, with relatively high levels of outcro-ssing being detected in one large population. In general, mating system analyses were hampered by low levels of electrophoretic variability. More powerful molecular techniques ore new required to further investigate the mating system and genetic variation within and betw een populations.

Although high selfing -ares are not uncommon in self-fertile plants, the high selfing rates in *G. barklyana* were inconsistent with the abundance and foraging behaviour of bird-pollinators in the study populations. Bird pollinators were abundant in all populations examined and birds moved frequently between plants during their foraging bouts, potentially transferring cross pollen. The high levels of selfing, despite the abundance of cross-pollinating floral visitors, may be explained by the presence of introduced honeybees *(Apis mellifera)* which visit newly-opened flowers to collect pollen. Foraging behaviours of pollen-collecting honeybees at newly opened flowers results in self-pollination. Self pollen deposited by bees would pre-empt ovules, thereby rendering later visits by cross-pollinating birds ineffective. Pollen-collecting honeybees may also -educe crcss-pollination by birds because less pollen is available for transfer between plants.

Self-pollination by honeybees has importance consequences for *G. barklyana* because low outcrossing rates will occur regardless of the abundance of birds. In conservation biology there is concern about the effects of introduced honeybees on native plants, although at present there is a dearth of experimental data. Most work to date has focused on the fact that honeybees often remove floral rewards but do not pollinate flowers. The present results suggest that introduced honeybees can also have more subtle effects, that nevertheless, have far-ranging implications for plant populations.

(3) Selective abortion

Although plants rely on external agents for pollen transfer, they can nevertheless exhibit considerable control over paternity and consequently the genetic quality of their progeny. Mechanisms promoting non-random mating is an active area of research and has profound implications for both the basic and applied plant sciences. In *G. barklyana*, many more fruits are initiated than mature. I investigated whether plants were able to selectively abort selfed fruits in favour of outcrossed fruits. Experiments in two populations in which some flowers on inflorescences were cross-pollinated and others were self-pollinated indicated that *G. barklyana* lacks the ability to selectively abort selfed fruits. This result is particularly interesting because selective abortion of selfed fruits has been demonstrated in related species (e.g. Vaughton and Carthew 1993, Biol. J. Linn. Soc. 50: 35-46). The inability of plants to discriminate between self and crossed progeny would contribute to the observed low outcrossing rates in *G. barklyana.*

(4) Fruit and seed production

Seed production in five populations was found to be consistently low (about 0.1%). Seed set was was primarily limited by the availability of resources for fruit production and seed filling; most fertilized ovules do not initiate fruits and many juvenile fruits abort during development. Reproductive output was not limited by either the quantity or quality of pollen received. Hand cross-pollinations using pollen from the same and different populations failed to increase seed set over natural levels.

Caging experiments indicated that pre-dispersal predation of immature fruits by parrots contributed to the low number of mature seeds produced per plant. Moreover, not all seeds produced by plants are available for recruitment. About 90% of mature seeds are eaten shortly after release from the parent plant by nocturnal mammal predators, probably *Ratttus fuscipes.* This is important because *Grevillea barklyana* is killed by fire and thus relies on its soil-stored seed bank for recovery. Estimation of seed bank size in five populations suggested that although most stored seeds were viable, small seed bank size may potentially limit recruitment after fire. This finding has implications for management, particularly the frequency of controlled burns.