Pollination in Waratah

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Final Report

<u>Summary</u>

The overall aim of the research project funded by the *Australian Flora Foundation* was to examine the factors limiting seed production by *Telopea speciosissima* (Waratah) in a natural population at Barren Grounds Nature Reserve, near Wollongong, NSW. This project was proposed following a pilot study conducted by Whelan and Goldingay in 1985, in which it was apparent that the low levels of fruit set observed in the field could be increased by experimental outcrossing of flowers using hand pollination. This result contrasted with previous studies on Waratah, in different study sites, by Pyke (1982) and Pyke & Paton (1983).

In order to understand the factors which limit fruit set in nature and to resolve the apparent contradiction between different studies on Waratah, we felt that is was necessary (i) to gain more information about the basic biology of the species: its breeding system and natural pollinators, and (ii) to devise a set of experiments to reveal the factors limiting fruit set and the mechanisms by which they operate.

Three specific questions were addressed:

(i) What is the underlying breeding system of the species? (i.e. is it an obligate out-crosser? If self-compatible, can it set fruit by autogamy - without a pollinator?).

(ii) How do pollinators influence fruit set?

(iii) Is a plant capable of allocating resources to "favoured" pollinations? (i.e. if the plant is self-compatible, does it nevertheless favour the development of fruits on the flowers that receive outcrossing?).

In short, the contributions made by our research to these questions are as follows:

(i) Waratahs are obligate outcrossers; no fruit set by autogamy or self pollination.

(ii) Pollination in the field appears to be less that maximal in some years. In 1985, plants were capable of producing more fruits when inflorescences were hand pollinated with outcross pollen. However, a repeat of this experiment in 1988, as part of the present study, found no such increase in fruit set, indicating that natural levels of pollination were adequate to achive maximum fruit production.

(iii) Lack of self-compatibility removes the significance of this question. However, maturation of fruits on inflorescences appears quite plastic. If 2/3 of the flowers are removed from an inflorecence, it can still produce just as many fruits from the remaining flowers. Hand pollination of a portion of the inflorescence (top 1/3 or botton 1/3, but especially the latter) produces greater initiation of fruits in the treated portion and a lower rate of abortion than for the same portion in control inflorescences.

Taken together, the results of this study reveal that Waratah is an obligate outcrossing species, at least in the Barren Grounds Nature Reserve population. Honeyeaters are abundant in the site and are frequent visitors to Waratah inflorescences (especially New Holland Honeyeaters) - marsupials were also recorded visiting inflorescences. Despite their abundance, the birds carry small pollen loads of Waratah and visit inflorescences in such a way that pick-up and deposition of pollen is minimized. This finding alone may explain the fact that fruit set in the population is sometimes pollen limited. The first flowers to open on an inflorescence (i.e. the bottom flowers) have very low levels of fruit set which can be increased substantially by hand pollination. Hand pollination of flowers either on the top third or bottom third of the inflorescence leads to more fruit initiation in the treated portion and, interestingly, in lower levels of fruit abortion than in equivalent portions of control inflorescences. Active selection for favoured matings, through non-random fruit abortion, appears a real possibility in this species. This is worthy of further investigation.

Results achieved

Detailed descriptions of the experiments conducted and the results achieved will be found in the publications already resulting from this work and from upcoming papers. A summary of findings is presented here.

(i) Breeding system

Eleven inflorescences were bagged to exclude all potential animal pollinators. Ten additional inflorescences were bagged, but bags were opened several times during flowering and all open flowers were hand pollinated with self pollen. These treatments tested for autogamy (automatic self-fertilization) and self-compatibility respectively. None of the autogamy inflorescences produced fruits. Similarly, none of the self-pollinated inflorecences produced fruits. A sample of inflorescences on twenty unmanipulated plants set a mean (± S.E.M.) of 5.95 ± 0.96 fruits per plant. We therefore conclude that this population comprises plants which are obligate outcrossers.

(ii) Tests for pollen limitation of fruit set

The previous study in this Waratah population revealed that natural levels of pollination were insufficient to produce maximum fruit set. Pollen supplementation by hand significantly increased the reproductive output of plants. The proportion of inflorescences with fruits increased from 52% (controls) to 91% (hand-pollinated); the mean number of fruits produced per inflorescence was greater after hand-pollination and the mean number of seeds per fruit was slightly, but significantly, greater after hand-pollination. The sum of these effects for individual plants was that the number of fruits produced per plant after hand-pollination was more than twice that of control plants. Fruit counts per plant translate into a real difference in reproductive output, because, when coupled with the number of seeds produced per fruit, they yield an increase from 133 seeds per plant for controls to 352 for hand-pollinated plants.

(iii) Causes of pollen limitation

There are several possible explanations for the way in which pollen availability can limit fruit set in the field. There may be too few visits by pollinators, pollinators may visit frequently but carry too little pollen, pollination may be inhibited by the presence of self pollen on pollen presenters, and pollinators may be transferring mostly self pollen to flowers on multi-inflorescence plants. We examined several of these possibilities.

Firstly, birds pollinators (predominantly New Holland Honeyeaters) were observed to be abundant in the study site at all times, and were recorded visiting Waratah inflorescences frequently. We also made the surprising discovery that marsupials were not uncommon visitors to this species, formerly viewed to be exclusively bird pollinated. Automated photography recorded the Eastern Pygmy Possum (*Cercartetus nanus*) and spool-and-line tracking of individual Pygmy Possums revealed that they were visiting many inflorescences in a single foraging bout.

Secondly, New Holland Honeyeaters were captured by mist nets in the study area. Pollen loads were assessed by sampling the feathers around the head and identifying pollen grains. Table 1 shows that fewer than 1/2 of the birds captured were carrying any Waratah pollen, and those that were had small amounts relative to the pollen of other plant species.

Table 1: Mean number of pollen grains in standard samples collected from 29 New Holland Honeyeaters

Plant Taxon	No. birds with pollen	Mean no. pollen grains (± s.e.m.)
Waratah	13	1.9 (0.9)
Banksia	16	7.7 (4.1)
Lambertia	13	9.2 (5.8)
Eucalyptus	27	67.4 (15.3)

These data suggest that at least this pollinator species may carry relatively small pollen loads. This was a surprising finding, given the observation that frequencies of bird visits to inflorescences were high. Perhaps these honeyeaters probed flowers in such a way that pollen was not deposited on the feathers. We made close observations of bird visits to Waratah inflorescences and recorded two types of visit: (i) the bird perched on the top of the inflorescence and probed downwards into the flowers, contacting pollen presenters as they did so; or (ii) the bird perched on the side of the inflorescence, or on the stem supporting it, and probed flowers from the side, failing to contact pollen presenters. We collected data at various times during the flowering season on the relative frequencies of the two types of visit (Table 2). These results indicate that the pattern of visits to inflorescences was such as to minimize the amount of pollen deposited on feathers.

Table 2: Pattern of probing exhibited by New Holland Honeyeaters feeding at Waratah inflorescences, for two different "age-classes" of inflorescences.

Stage of inflorescence opening	No. probes observed	Type of probe: Top	Side
<2/3 open	446	16	430
>2/3 open	120	7	113

Thirdly, although ineffective pollination may be sufficient to explain pollen limitation of fruit set, we tested whether the presence of self pollen adhering to the pollen presenters inhibited pollination that would otherwise be effective. Self pollen was removed from newly opened flowers on all inflorescences on a sample of 17 plants. Twenty control plants were tagged simultaneously, but received no manipulations. Fruits were counted on these inflorescences when fruit maturation was complete. This experiment showed that removal of self pollen produced no significant increase in the likelihood of maturing a fruit (Table 3).

Table 3: Effects of removal of self pollen from open flowers on fruit set per plant.

	Pollen removed	Controls
Total no. inflorescences	52	64
Number barren (%)	17 (27)	16 (23)
No. fruits/plant (± s.e.m.)	7.5 (1.1)	5.9 (0.9)

Fourthly, the previous study suggested that hand pollination increased fruit set per plant only on multi-inflorescence plants (see Whelan and Goldingay 1989). One possible explanation for this observation is that visitors to inflorescences on single-inflorescence plants provide mostly outcrossed pollen while visits in multi-inflorescence plants are mostly transferring self pollen. An alternative explanation is that multi-inflorescence plants are larger and therefore have more resources to devote to fruits if they were to receive more pollinations. We attempted to test these possibilities in an experiment in which all but one of the inflorescences were removed from multi-inflorescence plants, but not until after anthesis. Thus pollinations were not affected by the treatment (i.e. many self-pollinations, according to the hypothesis), but removal of inflorescences then allowed all resources to be devoted to the remaining inflorescence. Half of these treatment inflorescences also received hand pollinations, to ensure cross pollination. We are still engaged in the analysis of this experiment.

<u>(iv) Resource allocation</u>

Our previous study of Waratah fruit set revealed that fruit set was not evenly distributed over the rachis. In other words, flowers on an inflorescence did not have an equal probability of setting fruit. The top 1/3 of the inflorescence typically supported most of the fruits. This 1/3 represents the last flowers to open, and the flowers furthest from nutrient supply through the vascular system coming from the stem. Other studies have suggested adaptive roles for this pattern of the first flowers to open on an inflorescence being female-sterile: i.e. they serve a male function only - pollen donation, or they serve to "train" potential pollinators that the inflorescence is about to come "on stream". Alternative hypotheses are: (i) simply that top flowers get more pollinatons; or (ii) there is some maternal choice, through non-random fruit abortion, in which, in this system, the matings received by the flowers on the top of an inflorescence are favoured.

We examined parts of these hypotheses in two experiments. Firstly, 40 single-inflorescence plants were selected. 20 of these were randomly allocated to the experimental group, in which flowers on the the top 2/3 of the inflorescence were removed early in flower development and the remaining flowers were cross pollinated on 5 occasions. Although 2/3 of the flowers had been removed from treatment inflorescences, these initiated and set just as many fruits as the controls (Table 4). These flowers are clearly not sterile.

Table 4: Comparison of fruit set per inflorescence for treatment (in which flowers on the top 2/3 of an inflorescence were removed as buds) and unmanipulated inflorescences.

	Top 2/3 removed	Controls
Total no. inflorescences	20	20
No. setting fruits	17	15
No. fruits/inflorescence (± s.e.m.)	5.6 (1.4)	4.3 (0.9)

In a second experiment, flowers were not removed, but cross pollination was conducted by hand on selected flowers on the inflorescence. This was designed to test whether an inflorescence could selectively mature those flowers which received the best quality pollinations, regardless of their position on an inflorescence. Figure 1 shows that when the bottom flowers on an inflorescence are cross pollinated by hand, they mature most of the fruits for the inflorescence, even though other flowers have not been manipulated. Hand pollinating the top flowers had relatively little effect, because it is these flowers which usually contribute most to fruit set. These results suggest that, in the field, the first flowers to open (i.e. the bottom 1/3 of the inflorescence) typically receive few pollinations. Only 4-6% of the fruits initiated, and only 6-10% of fruits matured, on inflorescences are in this position. Cross pollination of these bottom flowers caused them to produce 45% of the fruits initiated and 52 % of the fruits matured. The proportion of fruit aborted during development was lower for the cross pollinated flowers, whether on the top third or the bottom third, than for fruits in the corresponding position on unmanipulated inflorescences (Figure 2). These results leave some interesting questions unanswered. In particular, they suggest that the hand pollination treatment may be improving pollen quality as well as number of flowers pollinated.

Publications resulting from the study

The pilot study which stimulated the research reported here had been written up as a manuscript at the time of the initial request for *AFF* funding. The first experiments conducted in this study, designed to reveal the breeding system of Waratah, were able to be included in this manuscript before it went to press. In addition, this study has contributed to a "Facts and Figures" article for the International Protea Society, and to a review of factors limiting fruit set in the Proteaceae. We are currently preparing a publication based on the bulk of the work conducted during this project.

Whelan, R.J. and Goldingay, R.L. (1988) Pollination and fruit set in Waratah. *Journal of the International Protea Society* **15**, 11-12.

Whelan, R.J. and Goldingay, R.L. (1989) Factors influencing fruit set in *Telopea speciosissima*: the importance of pollen limitation. *Journal of Ecology* in press.

Ayre, D.J. and Whelan, R.J. (1989) Factors controlling fruit-set in hermaphroditic plants and the contribution of studies with the Australian Proteaceae. *Trends in Ecology and Evolution* in press.

In addition to published papers, results from this study have been presented at a recent conference.

Goldingay, R.L. and Carthew, S.M. (1989) Mammal Pollination: Fact, Fantasy or Fallacy? *Australian Mammal Society Conference*, Alice Springs, April 1989

Expenditure of funds

As anticipated in the original proposal for funding, the bulk of funds ($1600) were expended on salary support for the junior investigator (R.L. Goldingay). The additional $383 was spent on travel and disposable items for field and laboratory work.

Date: 3/7/89

Signed: R.J. Whelan

Chief Investigator