Breeding system and fruit development in *Persoonia juniperina* (Proteaceae)

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Experimental hand pollinations were used to examine factors that influence fruit production in *Persoonia juniperina* (Proteaceae). Assessment of the breeding system indicated no clear pre-zygotic barriers to self-fertilisation. Rates of pollen tube growth and numbers of pollen tubes were similar after selfing and outcrossing. Plants also clearly had the capacity to produce fruit from selfing, although there was some evidence of inhibition in the development of selfed zygotes. More fruit were initiated after cross-pollination, and rates of abortion were greater after selfing, resulting in lower final fruit set. Inhibition of selfed fruit is most probably exhibited post-zygotically since the percentage of ovules penetrated did not differ between treatments. However, at this stage it is difficult to differentiate between late-acting self-incompatibility and genetic load. Supplementary hand pollinations did not increase numbers of pollen tubes or percentage fruit set above natural levels, indicating that plants in the population were not pollen limited. These results contrast with those found in most other species of Proteaceae.

Introduction

The Proteaceae comprises a diverse family of woody perennial plants that are found primarily in Australia and South Africa (Collins & Rebelo 1987). The reproductive biology of members of this family has been the subject of many studies over the past two decades (see Goldingay & Carthew 1998). In Australia there are some 1100 species distributed among more than 46 genera, and the genus *Banksia* has predominated in studies done so far. Twenty one studies on stigma receptivity, breeding systems, selective fruit abortion and mating systems have been conducted on the genus *Banksia*, compared with 16 for all other genera combined (Goldingay & Carthew 1998). As a consequence, many of the generalisations about the family have been based on a limited number of similar species. We will not adequately understand the reproductive biology of this family until species from other genera are studied.

One aspect of the reproductive biology of the family Proteaceae that has been investigated in some detail is the breeding system. Several plant traits indicate that flowers are likely to receive considerable amounts of self (geitonogamous) pollination. Inflorescences are often large and made up of many flowers, a number of which may be open at the same time. It is usual for more than one inflorescence to be open at one time on a plant (Carthew 1993). Consequently, pollinators tend to visit several flowers on an inflorescence and often several inflorescences on a plant (Vaughton 1990, Carthew 1994). Despite these observations, investigations on breeding and mating systems indicate that most members of the family preferentially cross fertilise.

Available allozyme data show extremely high levels of outcrossing (Scott 1980, Carthew et al. 1988, Vaughton & Carthew 1993, Krauss 1994a), and experimental hand pollinations have generally indicated either pre- or post-zygotic selection against self pollen (Whelan & Goldingay 1989, Fuss & Sedgley 1991, although see Harriss & Whelan 1993).

The genus *Persoonia* consists of some 98 species, and members are found in many regions of Australia (Weston 1995). Several attributes of members (e.g. lack of a distinctive inflorescence, lack of pollen presenter, invertebrate pollinators: Bernhardt & Weston 1996) indicate that the reproductive biology of this genus may differ from most other members of the family. However, the reproductive biology of only two species has been reported (Krauss 1994b, Trueman & Wallace 1999). In the present study we examined several factors which influence fruit set in a third species, *Persoonia juniperina*.

Methods

Study system

Persoonia juniperina (prickly geebung) is a small, dense shrub reaching up to 2 m in height. It is a member of the Proteaceae and is commonly found on sandy soils in heathlands in parts of New South Wales, Victoria, South Australia and Tasmania. Flowering occurs during summer mainly between January and March. The flowers are yellow and hermaphroditic. Most plants have only a few flowers open at one time. Unlike most other members of the Proteaceae, flowers do not have a pollen presenter. The perianth is cylindrical, with four segments that curl back when the flower opens. An anther remains attached to each of these segments. The style is thick and straight, and the stigma is located at the tip, projecting beyond the anthers. Styles are 6–9 mm long, and are persistent. Flowers are most likely insect-pollinated, although few insects have been seen visiting flowers. Flowers have a single ovary, and the fruit is a succulent drupe c. 8 mm diameter, with two seeds.

This study was conducted in heathland vegetation at Horsnell Gully Conservation Park, east of Adelaide, South Australia (34°56'S, 138°42'E). Field work was conducted during the flowering seasons of 1993 and 1995.

Pollen tube growth

Hand pollinations were used to determine amounts and rates of pollen tube growth after self- and cross-pollination and to compare these to pollen tube growth for open-pollination and supplementary pollination. In 1993, flowers were allocated to one of four treatments:

i) self-pollination: flowers were bagged with fibreglass mesh and pollinated with their own pollen.

ii) cross-pollination: flowers were bagged, emasculated and then pollinated with pollen from another plant at least 5 m away.

iii) open-pollination: flowers were left open to natural pollination.

iv) supplementary pollination: cross-pollen was added to flowers left open to natural pollination.

For the self- and cross-pollination treatments, each flower was pollinated once, by hand, rubbing a freshly dehisced anther containing many pollen grains across the top of the stigma. Seven different plants were used as recipients for hand pollinations, with between two and seven used for each treatment, depending on the availability of flowers. Flowers were collected either 2 h, 4 h, 8 h, 16 h, or 48 h after pollination and immediately placed into fixative (three parts acetic acid to one part ethanol) to arrest pollen tube growth. Flowers from the open-pollination and supplementary pollination treatments were collected and fixed up to a week after pollination.

Self- and cross-pollination experiments were repeated in 1995, as it was found that 48 hours was not long enough for pollen tubes to reach the ovaries. For this experiment, flowers were collected either three, four or five days after pollination. We tested the ability of flowers to produce pollen tubes in the absence of pollinators via autogamy. For this, flowers were bagged prior to opening, left unmanipulated for one week after they had opened, and were then collected. All styles were prepared for fluorescence microscopy following the methodology of Carthew (1993). Ovaries were removed from styles and were dissected longitudinally before being mounted in a few drops of aniline blue solution and observed under a fluorescence microscope.

Pollen tubes were counted at 50 μm intervals down the length of the style. The distance travelled by the longest pollen tube in each flower was estimated by assuming that a pollen tube present at one 50 μm point, but not at the next ended halfway between. Values were then compared with total style length, and a mean obtained for each time after pollination. Effects of treatment and time on distance grown were analysed using two-way ANOVA of log transformed data.

Fruit set

As for pollen tubes, flowers received one of four treatments: self-pollination, cross-pollination, natural pollination and supplementary pollination. Each manipulated flower was pollinated on two occasions. Fruit development was monitored fortnightly after pollination to determine the extent and timing of fruit abortion.

Results

Pollen tube growth

Some self- and cross-pollen germinated within two hours of being placed on the stigmatic surface, and most flowers contained at least one pollen tube at four hours after pollination. In both treatments, many more pollen tubes germinated and grew down the style than could actually fertilise ovules. The greatest number of pollen tubes observed in any one flower was 35, although there were usually fewer than 20. The maximum number of pollen tubes was recorded in the lower style after 120 hours (Table 1). At this time, ovules of all flowers were penetrated, indicating that potential fruit set was 100%.

Table 1. Percentage of flowers with pollen tubes and mean number (± s.e.) of pollen tubes per flower in the upper and lower styles at different times after pollination. Experiment 1 was conducted in 1993, Experiment 2 in 1995. n is number of flowers.

	Self Pollination			Cross Pollination		
Time after pollination (h)	**% flowers with pollen tubes (n)**	**Mean no. pollen tubes/flower**		**% flowers with pollen tubes**	**Mean no. pollen tubes/flower**	
		Upper style	**Lower style**		**Upper style**	**Lower style**
Experiment 1:						
2	73 (11)	2.27 ± 0.7	0	36 (11)	0.55 ± 0.3	0
4	100 (10)	6.4 ± 0.8	0.2 ± 0.2	80 (10)	3.7 ± 1.2	0
8	70 (10)	5.3 ± 1.8	0.1 ± 0.1	100 (10)	10.4 ± 2.0	0
16	89 (9)	14.56 ± 3.7	0	100 (10)	9.5 ± 0.9	0.1 ±0.1
48	90 (11)	16.73 ± 3.3	0.73 ± 0.3	100 (11)	10.09 ± 2.1	1 ± 0.4
Experiment 2:						
72	100 (10)	6.1 ± 0.9	3.8 ± 0.4	100 (10)	8.0 ± 0.7	6.2 ± 0.8
96	100 (10)	6.5 ± 0.9	4.3 ± 0.5	100 (10)	8.4 ± 1.2	6.8 ± 0.9
120	100 (10)	8.4 ± 0.9	6.3 ± 0.5	100 (10)	8.8 ± 1.8	7.2 ± 1.6

Self- and cross-pollen tubes grew at similar rates for the entire length of the style (Fig. 1, Table 2). Distances grown tended to increase gradually between 2 and 48 hours, and then doubled between 48 and 72 hours (Fig. 1). It generally took between 72 and 96 hours for pollen tubes to reach the ovaries.

The autogamy, open and supplemented pollinations had similar numbers of flowers with pollen tubes (Table 3: $G = 0.55$, $df = 2$, $p > 0.95$). However, the autogamy pollinations had significantly fewer pollen tubes per flower than the other pollinations (Kruskal-Wallis test: $H = 25.67$, $df = 2$, $p < 0.005$) and fewer flowers with their ovules penetrated.

Table 2. Two-way ANOVA for the effects of time and treatment on pollen tube growth after self- and cross-pollination. Data were log-transformed. Flowers from Experiment 1 were collected 2, 4, 8, 16 and 48 h after pollination, and those from Experiment 2 were collected 72, 96 and 120 h after pollination.

	Experiment 1				Experiment 2			
Source	**df**	**MS**	**F**	**p**	**df**	**MS**	**F**	**p**
time	4	0.057	15.235	0.0001	2	0.001	3.184	0.049
treatment	1	0.002	0.565	0.454	1	0.000	0.003	0.959
time*treatment	4	0.006	1.664	0.165	2	0.000	0.003	0.997
error	93	0.004			54	0.000		

Table 3. Pollen tube growth and ovule penetration after different pollination treatments. Pollen tube numbers are means (± s.e.) in the upper style.

Treatment	**Autogamy**	**Open**	**Supplement**	**P**
No. flowers	25	36	26	
% flowers with pollen tubes	88	89	100	NS
Mean no. pollen tubes/flower	3.14 ± 0.31	15.06 ± 2.22	12.81 ± 1.88	< 0.005
% flowers with at least one ovule penetrated	48	72	NA	

Table 4. Fruit production following different pollination treatments. n is the number of plants.

Treatment	**Self**	**Cross**	**Open**	**Supplement**	**P**
No. flowers (n)	50 (3)	50 (3)	70 (3)	24 (5)	
% fruits initiated	50	72	61.4	83.3	< 0.05
% fruits matured	12	30	41.4	37.5	< 0.01
% fruits aborted	76	58	33	55	< 0.01

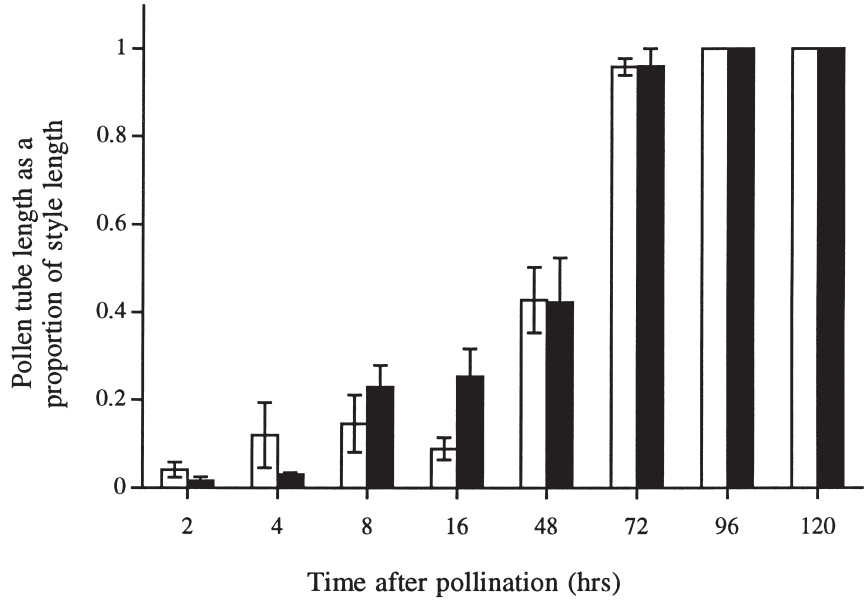

Fig. 1. Distance grown by the longest pollen tubes as a function of style length (mean ± s.e) at various times after self (open bars) and cross (filled bars) pollinations.

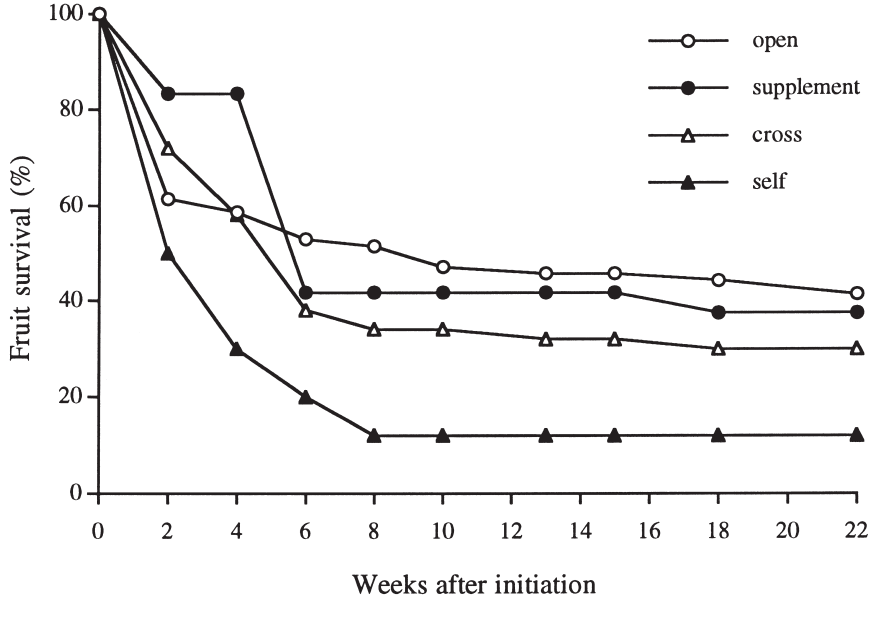

Fig. 2. The percentage of fruit surviving over time for open, supplement, cross and self pollinations. T_o = 3–4 weeks post-pollination.

Fruit set

All pollinations produced some fruit and the percentage of fruits initiated ranged from 50% for selfed flowers to 83% for supplemented flowers (Table 4). The percentage of fruits initiated which subsequently aborted was significantly different among pollinations ($X^2 = 12.93$, df = 3, $p < 0.01$). The highest rate of abortion occurred in self-pollinated flowers (76%) and the lowest in open-pollinated flowers (33%). The percentage of fruits which eventually matured was also significantly different among pollinations ($X^2 = 12.6$, df = 3, $p < 0.01$). Self-pollination produced the fewest fruits, while open pollination produced the most.

Fruit swelling was first detected three to four weeks after pollination. From then, the number of maturing fruits declined steadily over time, until week eight, by which time fruit abortion had slowed considerably (Fig. 2). Self-pollination showed the fastest decline, while the open-pollination was much slower.

Discussion

During the present study, the only visitors observed on flowers were small ants, and these were seen infrequently. Despite the apparent lack of pollinators, most flowers left open to pollination had pollen tubes, and more than 40% matured fruit. This compares to values of 17–34% for open-pollinated *Persoonia mollis* flowers (Krauss 1994b), and 67% for *Persoonia rigida* (Trueman & Wallace 1999). However, it does contrast with most other studies which have mostly documented very low levels of fruit set in this family, usually less than 5% (Ayre & Whelan 1989). In other more commonly studied genera such as *Banksia*, the production of flowers and fruits requires large amounts of energy, as flowers are packaged into large, showy inflorescences which turn into woody infructescences as seeds are matured. It may not be surprising that these plants have high flower:fruit ratios in comparison.

There appears to be considerable variability in breeding systems among species of Proteaceae. Many species have been shown to produce few or no seeds after selfing (e.g. *Banksia prionotes* — Collins & Spice 1986, *Banksia paludosa* — Goldingay & Whelan 1990, *Banksia ericifolia* — Goldingay et al. 1991, *Banksia menziesii* — Ramsey & Vaughton 1991, *Persoonia mollis* — Krauss 1994b, *Telopea speciosissima* — Whelan & Goldingay 1989). Others appear to be capable of relatively high levels of selfing and/or some autogamous seed production (e.g. *Banksia spinulosa* var. *neoanglica* — Vaughton 1988, *Grevillea barklyana* — Harriss & Whelan 1993, *Persoonia rigida* — Trueman & Wallace 1999). Despite this apparent variability, it seems likely that even those species capable of selfing will preferentially outcross when possible (Vaughton & Carthew 1993, Carthew et al. 1996).

In this study, *Persoonia juniperina* exhibited particularly high levels of selfing, as evidenced by both pollen tube growth and fruit production after experimental hand pollinations. This may ensure successful fruit production when pollinators are scarce. It is interesting that no differences in pollen tube growth rates were observed in selfed and outcrossed flowers during this study. This contrasts with many other studies of

self-compatible species, where self pollen tubes grow considerably more slowly than cross pollen tubes (e.g. Hessing 1989, Aizen et al. 1990, Harriss & Whelan 1993). Interestingly, although only two other species of *Persoonia* have been studied to date, there are obvious differences in their breeding systems. A single population of *Persoonia mollis* studied was found to be highly outcrossed, with almost no seed produced from selfing, and no pollen tubes evident in the ovules (Krauss 1994b). In contrast, *Persoonia rigida* showed no evidence of self-incompatibility, in either pollen tubes or seed set (Trueman & Wallace 1999). The population of *Persoonia juniperina* studies here appears to lie somewhere between these two extremes.

Although *Persoonia juniperina* can clearly produce seeds from self pollination, there does appear to be some late-acting selection against selfing, which probably occurs post-zygotically. The lack of differences between self and cross pollination in amounts and rates of pollen tube growth and proportions of ovules that were penetrated indicate no pre-zygotic discrimination. However, selfed flowers were less likely to initiate fruit than other pollinations, and fruit abortion was higher, resulting in significantly lower final fruit set. There are two main reasons for selection against self pollen (Seavey & Carter 1994). There may be allelic self-incompatibility, which is commonly expressed on the stigma or in the style, but may occur at the level of the ovule and perhaps even post-zygotically. However, rejection or acceptance of incompatible compared to compatible pollen should be consistent between individuals. An alternative model implicates genetic load, whereby combinations of recessive deleterious or lethal genes cause embryo abortion. This is likely to be a very common phenomenon in plants (Burbidge & James 1991). In the genetic load model, many genes are implicated, and thus expression should vary between individuals (Waser & Price 1991).

In *Persoonia juniperina* the existence of a pre-zygotic incompatibility reaction cannot be entirely ruled out at this stage, because it is difficult to observe fertilisation events. However, embryo abortion obviously plays a much more important role. It is not clear whether selection against selfing occurs through allelic self- incompatibility or because of a system of recessive lethals. The sample size was too small to determine whether there was variability between plants in their capacity to set selfed seed. Research is now needed to assess in more detail whether individual plants can and do differ in the degree to which they can self.

Several lines of evidence indicate that despite a lack of observed pollinator activity, plants in this population were not pollen limited during the year of the study. First, hand supplementation failed to increase levels of pollen tube growth or initial fruit set over that for natural pollination. Second, the percentage of fruits that were matured was similar for the two treatments. Third, most flowers from all treatments contained pollen tubes and there were usually many more pollen tubes than ovules, even at the base of styles, indicating a surfeit of pollination. This is in contrast to results from other species of Proteaceae studied so far, most of which have shown considerable pollen limitation (*Banksia paludosa* — Goldingay & Whelan 1990, *Telopea speciosissima* — Whelan & Goldingay 1989). However, most taxa studied have been outcrossing species, and may have been limited by quality of pollen rather than quantity. It is clear

that further investigations are required to elucidate the reasons for these differences. Studies on the mating system are required to determine natural levels of outcrossing, and more detailed observations on pollinators and their effectiveness are also needed.

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