

The breeding systems of *Hicksbeachia pinnatifolia* and *Triunia youngiana* (Proteaceae)

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Abstract: The breeding systems of the rainforest shrubs *Hicksbeachia pinnatifolia* and *Triunia youngiana* were studied in north-east New South Wales, where the former is listed as a vulnerable species. *Hicksbeachia pinnatifolia* flowered in winter and spring, and produced an average of 36 inflorescences per plant with 155 flowers per inflorescence (5 580 flowers per plant). Inflorescences initiated and matured only a small number of fruits (c. 1–2 per inflorescence) in self-pollinated and open-pollinated treatments. This compared to about 17 fruits initiated and 4 fruits matured per inflorescence when cross-pollinated. *Triunia youngiana* flowered in spring and produced an average of 3 inflorescences per plant containing 23 flowers per inflorescence (69 flowers per plant). Plants were unable to initiate any fruit in autogamy and self-pollinated treatments. Plants in a cross-pollination treatment produced over three times as many fruit (3.5 matured per plant) compared to a control treatment (0.8 per plant), suggesting that plants were pollen-limited. Both treatments experienced substantial (80%) fruit abortion. These results indicate that *Triunia youngiana* is self-incompatible whereas *Hicksbeachia pinnatifolia* appears to be partially self-compatible.

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Introduction

Although species within the Proteaceae have been common subjects of pollination studies (Whelan & Goldingay 1986, Harriss & Whelan 1993, Vaughton 1993), most studies have focussed on the genus *Banksia* and *Grevillea* (Goldingay & Carthew 1998). This situation currently precludes a better understanding of the evolution of the breeding and mating systems within the Proteaceae because these genera are typically vertebrate pollinated and occur predominantly in heath and woodland habitats.

Study of the pollination ecology of rainforest Proteaceae is of great interest because these species may be quite primitive. However, *Macadamia* is the only genus of rainforest Proteaceae that has been studied (Heard 1993, Wallace et al. 1996), probably due to the commercial value of the fruits of these plants. There are another 23 genera that include rainforest species (CSIRO 1995), so an adequate understanding of the breeding systems of the Proteaceae is a long way off.

This study aims to examine flowering and the breeding systems of two rainforest Proteaceae, *Hicksbeachia pinnatifolia* F. Muell. and *Triunia youngiana* (C. Moore & F. Muell. ex F. Muell.) L.A.S. Johnson & B.G. Briggs. *Hicksbeachia pinnatifolia* is listed as a vulnerable species in NSW. *Triunia youngiana* is not a threatened species but the related *Triunia robusta* is highly endangered (Shapcott 2002). Data gathered on *Triunia youngiana* may be useful to the management of *Triunia robusta* populations.

Methods

Study area and study species

This study was conducted at three sites located approximately 20 km north of Lismore. The three sites include the Big Scrub Flora Reserve (FR) in Whian Whian State Forest, at Rocky Creek Dam, and along Rocky Creek Dam Road about 3 km from Rocky Creek Dam. All three sites were used in the study of *Hicksbeachia pinnatifolia* but only the first site was used in the study of *Triunia youngiana*. Opportunistic observations were made of animals visiting the flowers of these species during visits to each site. Observations were made during the first few hours of darkness on one occasion at each site in an attempt to observe nocturnal visitors.

Hicksbeachia pinnatifolia

Flowering and fruiting phenologies of *Hicksbeachia pinnatifolia* were recorded between August and December 1998. For this study, 23 trees (2–5 m high) were tagged in the Big Scrub Flora Reserve along a 500 m length of walking trail, and 9 were tagged at Rocky Creek Dam, scattered through a picnic area. The reproductive state of all inflorescences on the study trees was recorded at monthly intervals. In this species, fruits undergo a colour change from green to red as they complete maturation. Inflorescences were classified into one of six categories: in flower (at least one floret was open), completed flowering (all florets had withered), initiated fruit (at least one ovary was swollen), unripe fruit (at least one fruit was fully developed, but was still green), and ripe fruit (at least one fruit had changed to red). A subsample of tagged plants was examined to describe the number of flowers on an inflorescence and the number of fruits produced.

A breeding system experiment was conducted in 1998 on 13 plants located in a strip of roadside vegetation along Rocky Creek Dam Road. These plants contained many inflorescences at accessible heights for bagging whereas plants at the other two sites mostly carried inflorescences too high to use. Inflorescences were selected at the bud stage for each of the following four treatments: open-pollinated control (inflorescences were tagged and left untouched for the duration of the experiment), uncovered cross pollination (flowers were hand-pollinated with pollen from trees located >5 m from the experimental tree on 3–5 days), covered cross pollination (inflorescences bagged with nylon shade-cloth and cross-pollinated as above), self-pollination (inflorescences bagged and a separate cloth used to apply self-pollen from other inflorescences on the same plant to the flowers on 3–5 days), and autogamy (inflorescences bagged but not manipulated, in order to test for automatic self-pollination).

When conducting the cross-pollinations, all visible self-pollen on an inflorescence was removed with a cloth prior to hand-pollination. A separate cloth was used to collect pollen from another plant and the pollen was rubbed gently onto the treatment flowers. The timing of stigma receptivity is unknown. Florets on inflorescences opened over a period of approximately seven days and then withered. Thus, multiple hand-pollinations within this period should ensure that some pollen is applied at an appropriate time.

Several treatments were placed on the same plant. There were insufficient plants with accessible inflorescences to use whole-plant treatments. Plants carried large numbers of inflorescences (>20) which enabled more than one treatment to be placed on the same plant with a low chance of interfering with assessment of the breeding system. Of 13 plants used, four contained a single tagged inflorescence, six contained two tagged inflorescences (each a different treatment), one contained four tagged inflorescences (three treatments represented), and two contained five tagged inflorescences (four treatments represented).

The number of fruits developing on each of the experimental inflorescences was counted four and seven weeks after the completion of flowering (since the last flower had withered).

Triunia youngiana

Nineteen plants were assessed so that the average number of inflorescences could be described. A subsample of these was examined to describe the number of flowers on an inflorescence.

A breeding system experiment was conducted in October 1999 on 18 plants. We used whole-plant treatments and reduced the number of inflorescences to three per plant. Plants were selected and tagged when inflorescences were still in bud. Plants were assigned at random to four treatments: open-pollinated control (plants were tagged and left untouched for the duration of the experiment), uncovered cross pollination (flowers were hand-pollinated with pollen from trees located

>5 m from the experimental tree on three occasions), self-pollination (inflorescences were bagged and a separate cloth used to apply self-pollen to the flowers on three occasions) and autogamy (inflorescences bagged but not manipulated, in order to test for automatic self-pollination).

A count of the number of fruit initiated (indicated by swelling of the ovary) was conducted approximately 4 weeks after hand-pollinations were completed. A final count of mature fruit was conducted 3 months after this.

Results

Hicksbeachia pinnatifolia

Hicksbeachia pinnatifolia that flowered in 1998 at the three sites had an average of 36.3 ± 11.6 (s.e.) inflorescences per plant ($n=24$). Inflorescences contained an average of 154.5 ± 5.4 ($n=51$) flowers (range 76–242). Plants produced an average of 22.0 ± 7.9 infructescences ($n=22$). This equates to 61% of inflorescences producing fruit. Infructescences ($n=50$) averaged 3.1 ± 0.4 fruits each with a range of 0–11 (Fig. 1). This equates to a final fruit-set (fruits: flowers \times 100) of 1.2%.



Fig. 1. *Hicksbeachia pinnatifolia* showing low fruit-set per inflorescence.

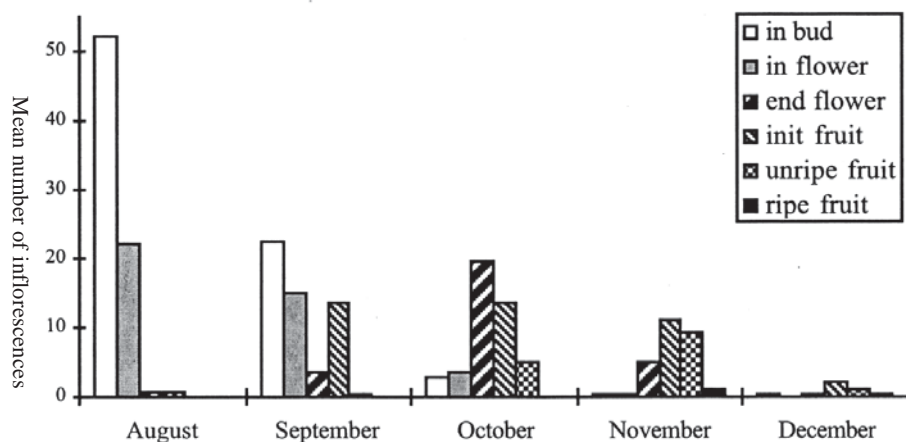


Fig. 2. Flowering phenology of *Hicksbeachia pinnatifolia* (n = 9) located at Rocky Creek Dam, 1998. Inflorescences were scored into one of six categories shown in legend (end flower = flowering completed; init fruit = initiated fruit).

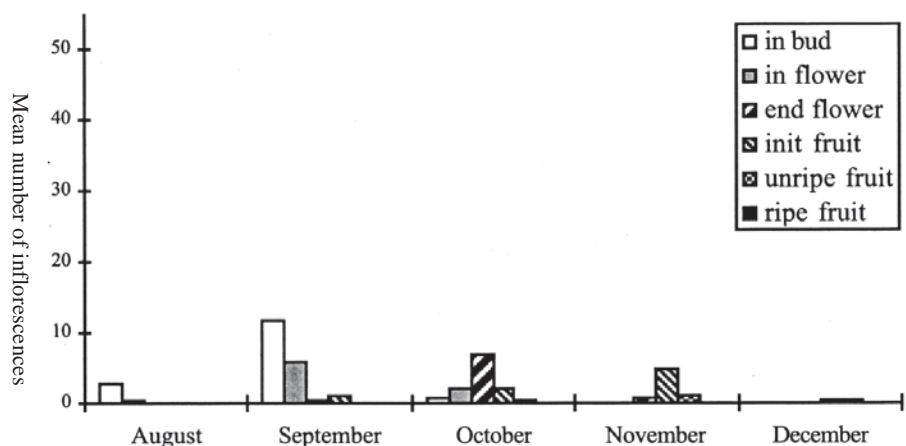


Fig. 3. Flowering phenology *Hicksbeachia pinnatifolia* (n = 23; in August n = 20) located at the Big Scrub Flora Reserve, 1998.

Table 1. Fruit-set per inflorescence in *Hicksbeachia pinnatifolia* in five pollination treatments.

Fruit-set values are means \pm se; n = the number of plants; the number of inflorescences is shown in brackets.

Treatment/ pollination	n	Fruits initiated	Fruits matured	% aborted
Autogamy	4	–	0.4 \pm 0.4 (5)	–
Self	6	2.0 \pm 1.3 (5)	1.0 \pm 0.7 (8)	50
Open/cross	7	14.6 \pm 4.7 (7)	3.3 \pm 1.3 (7)	77
Bagged/cross	3	23.3 \pm 2.6 (3)	6.3 \pm 3.4 (3)	73
Control (open)	6	1.3 \pm 0.9 (6)	1.1 \pm 0.6 (7)	15

The study trees had high numbers of inflorescences at the beginning of the phenology monitoring period in August (Figs 2, 3). The intensity of flowering was greater at Rocky Creek Dam compared to the Big Scrub FR. At Rocky Creek Dam, peak flowering occurred in August and the flowering period extended over at least three months. At the Big Scrub FR, peak flowering occurred in September and the flowering

period extended little over two months. The uneven match between inflorescences in bud and subsequently in flower suggests large numbers of inflorescences were aborted. Few infructescences produced red fruits at either site, and these did not appear until November and December.

Inflorescences in the autogamy, self-pollination and control treatments initiated a small number of fruits (Table 1). This contrasts with the cross-pollination treatments in which the number of fruits initiated was an order of magnitude higher. A greater number of fruits was initiated in the bagged compared to the unbagged cross-pollination treatment but sample sizes were too small to test for any difference. When considered together, these treatments averaged 17.2 ± 3.6 (s.e.) fruits per inflorescence. A large number of fruits (>70%) aborted in the cross-pollination treatments compared to the self-pollination and control treatments (Table 1). Final fruit-set among treatments (excluding autogamy) was compared using the combined cross-pollination treatments. These latter treatments averaged 4.2 ± 1.3 fruits per inflorescence.

All data were $\ln(x + 1)$ transformed. Fruit-set among treatments was significantly different ($F_{2,22} = 4.68$, $P = 0.02$), showing that crossing produced significantly higher numbers of fruit when compared to open pollination and self-pollination. This experiment indicates that plants are capable of producing fruit from self-pollination. Following self-pollination, 4 of 6 plants initiated fruits and 3 of 6 plants matured fruits. Fruit-set in control inflorescences was equivalent to that of selfed inflorescences. Final fruit-set in the control treatment for an average inflorescence of 36 flowers was about 3%.

Native and introduced honey bees were observed visiting *Hicksbeachia pinnatifolia* but mostly did not contact pollen presenters correctly for pollen transfer. Small moths visited for nectar at night but also failed to contact flowers in an effective way.

Triunia youngiana

Plants that flowered in 1999 had an average of 2.7 ± 0.5 (s.e.) inflorescences per plant ($n = 19$). Only 48% of plants flowered at the site ($n = 40$). Inflorescences contained an average of 23.2 ± 0.9 ($n = 44$) flowers (range 12–36). Fruiting data were only collected for experimental plants.

Table 2. Fruit-set in *Triunia youngiana* in four pollination treatments.

Three inflorescences were treated per plant. Fruit-set values are means \pm se; n = the number of plants.

Treatment/ pollination	n	Fruits initiated per plant	Fruits matured per plant	% aborted
Autogamy	4	0	0	–
Self	6	0	0	–
Open/cross	4	17.3 ± 3.3	3.5 ± 1.9	80
Control (open)	4	5.0 ± 2.7	0.8 ± 0.5	84

No plants in the autogamy or self-pollination treatments initiated any fruits (Table 2). This contrasts with the cross-pollination treatment in which all inflorescences initiated some fruit. Only 5 of 12 inflorescences that were crossed matured any fruit (1.2 ± 0.7 per inflorescence) but all plants in this treatment matured some fruit. Control plants initiated significantly ($P < 0.05$) fewer fruit per plant than the cross-pollinated treatment (Table 2). Control plants matured only 0.3 ± 0.2 fruits per inflorescence. The number of fruits matured per plant was substantially lower for the controls compared to the crossed plants but this was not significant. However, 2 of 4 control plants matured no fruits, and only 2 of 12 inflorescences matured fruits. Fruit abortion was high (80–84%) in both treatments. Final fruit-set in the control treatment for an average plant with 70 flowers was 1.2%.

Triunia youngiana was observed for a short period at night and it was visited by two moth species, one < 1 cm long and the other 2 cm long. The larger species made some contact with the pollen presenter. Small flies, ants and beetles were

observed on inflorescences during the day but did not contact the pollen presenter. At other times butterflies (c. 3 cm) visited and made contact with the pollen presenter.

Discussion

The pollination ecology of rainforest Proteaceae is poorly known. *Macadamia integrifolia* has been studied in some detail but essentially from a commercial perspective (Heard 1993; Wallace et al. 1996). This study describes the breeding system of two rainforest species. For *Hicksbeachia pinnatifolia*, the breeding system experiment could only be conducted at the inflorescence level because a small roadside population was the only one of three populations that produced inflorescences at a height where they could be readily manipulated. Sample sizes were also limited because flowering had passed its peak. This experiment demonstrated a small amount of fruit-set from self-pollination (1–2 fruits initiated and matured per inflorescence) which was equivalent to that observed on control inflorescences. In contrast, inflorescences that were cross-pollinated initiated 17 fruits and matured 4 fruits per inflorescence. A self-compatibility index (ratio of selfed to crossed fruit-set) based on initiated fruit (Hermanutz et al. 1998) was 0.16, suggesting partial self-compatibility in *Hicksbeachia pinnatifolia*. Open-pollinated plants monitored in the phenology study averaged more than 60 fruits per plant.

The experiment on *Triunia youngiana* was conducted at the whole-plant level, which is a more appropriate level for conducting this experiment (Goldingay & Whelan 1990, Goldingay et al. 1991). This showed that no fruits were produced by autogamy or self-pollination, indicating self-incompatibility. An assessment of pollen tubes following these pollination treatments is likely to reveal what type of incompatibility mechanism is present in this species (Hermanutz et al. 1998; Matthews & Sedgley 1998).

Due to the uneven coverage of Proteaceae in breeding system studies it is difficult to determine how widespread self-compatibility is in the family, or even to make predictions of its likely frequency among genera or habitats. Self-compatibility seems to be relatively widespread in *Grevillea* but less common in *Banksia* (Goldingay & Carthew 1998). Percentage fruit-set was low ($< 5\%$) in *Hicksbeachia pinnatifolia* and *Triunia youngiana*, though of a similar magnitude to that in many other Proteaceae (Goldingay & Carthew 1998). Denham & Whelan (2000) recently described self-compatibility in *Lomatia silaifolia* that had approximately 6% fruit set. Further studies are required to understand the high fruit abortion levels in cross-pollinated treatments (70–85%) in these rainforest species and to explore factors determining the low fruit-set.

Though not assessed specifically, it appears that populations of both species were pollen-limited (Goldingay & Whelan 1990, Vaughton 1991, Goldingay 2000). Cross-pollinated *Hicksbeachia pinnatifolia* initiated 13 times as many fruits and matured 4 times as many fruits as open-pollinated

inflorescences. This is consistent with the apparent ineffectiveness of visitors observed at inflorescences. This species produces a pungent smell at dusk (CSIRO 1995), suggesting a nocturnal pollinator. The small size of the flowers (c. 1 cm long) and delicate attachment of the inflorescence suggests an insect pollinator. The current study did not aim to describe the pollinators of this species. Furthermore, it is not known how indicative visitors to the road-side plants used in the breeding system study would be. The disturbed location may account for the poor pollination success. Cross-pollinated *Triunia youngiana* initiated 3 times as many fruits and matured 4 times as many fruits as open-pollinated inflorescences. Appropriate contact with the pollen presenter by some moths and butterflies was observed.

A detailed understanding of the pollinators of these two plant species is required. This will be fundamental to the conservation of the threatened *Hicksbeachia pinnatifolia*. Low fruit-set in both species may be a direct consequence of reduced abundance of their pollinators. Moreover, despite the widespread occurrence of insect pollination in the Proteaceae, this type of pollination system remains poorly studied (Bernhardt & Weston 1996). It would also be beneficial to conduct studies to determine the extent to which pollen-limitation occurs in these species. This might provide important insights into aspects of the population ecology of these species (Goldingay 2000). Further research is needed to confirm that *Hicksbeachia pinnatifolia* shows partial self-compatibility. Finally, there is a dearth of ecological studies of rainforest Proteaceae that must be rectified if we are to increase our understanding of the evolution of floral systems in this family. Such studies will also provide important data for conserving these species given that many rainforest Proteaceae are recognised as threatened species.

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