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Experimental evidence for pollination of *Banksia* spp. by non-flying mammals

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Summary. The importance of non-flying mammals as pollinators of *Banksia integrifolia* and *B. spinulosa* was analysed by examining the effect of pollinator exclusions on fruit-set. Visitation by potential pollinators was also measured by observation and by indirect methods. Non-flying mammals were frequent visitors to inflorescences of both *Banksia* species. The aluminium sleeves used to exclude non-flying mammals from *B. integrifolia* trees were associated with a reduction in both the number of infructescences produced and the number of fruit per infructescence, indicating that non-flying mammals were important pollinators. Bird-nets over trees also significantly reduced the number of fruit per infructescence, but had no significant effect on the number of infructescences produced. The results of exclusion experiments using single inflorescences were inconclusive due to low fruit-set. No conclusions could be drawn from these experiments with *B. spinulosa*. However, results for *B. integrifolia* support the conclusions of whole-tree experiments. Analysis of the genotype frequencies in seed from *B. integrifolia* provided no support for the hypothesis that the relatively limited mobility of non-flying mammal pollinators would cause inbreeding.

Key words: Pollination – Mammals – Fruit-set – *Banksia* – *Cercartetus nanus*

The role of non-flying mammals as pollinators is controversial. They are frequently assumed to be pollinators (Recher 1981), their own pollination syndrome has been described (Carpenter 1978), and the relative merits of flying and non-flying pollinators have been discussed (Sussman and Raven 1978). However, there has been no empirical evidence that non-flying mammals actually influence levels of fruit-set.

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The ability of non-flying mammals to exploit floral resources is widely documented. There are reports from the tropics and southern temperate regions that non-flying mammals visit a variety of plant species (Lumer 1980; Wiens and Rourke 1978; Goldingay et al. 1987), feed on nectar (Turner 1982; Sussman and Raven 1978) and transport pollen (Carpenter 1978; Hopper 1980). However, some authors suggest that non-flying mammal pollination is unlikely to be adaptive because the limited mobility of these mammals, compared to flying vertebrates, would cause inbreeding in the plants they pollinate (e.g. Faegri and van der Pijl 1979).

Here I assess the role of non-flying mammals in the fruit-set of *Banksia integrifolia* and *B. spinulosa* in south-eastern Australia. I first present the evidence that they visit these species, feed on nectar and transport pollen. Results of exclusion experiments then reveal the impact of their activities on the level of fruit-set. Finally, the hypothesis that non-flying mammal pollination leads to inbreeding is tested by examining genotype frequencies in the seed population.

Materials and methods

Observations and experiments were conducted on *Banksia integrifolia* var. *integrifolia* L. (Proteaceae) from June to November 1988, at Wilson's Promontory National Park (38° 58'S, 146° 17'E, 20 m elevation), and on *B. spinulosa* Smith var. *cunninghamii* Sieber ex Reicht, from August to September, 1989, at Gembrook State Forest (37° 54'S, 145° 36'E, 240 m elevation) in southeastern Australia. Flower visiting mammals were present at both sites. These included the Eastern Pygmy-Possum *(Cercartetus nanus)*, Brown Antechinus *(Antechinus stuartii)* and Dusky Antechinus *(A. swainsonii)*. Additionally, the Feathertail Glider *(Acrobates pygmaeus)* was present at the Gembrook site.

Inflorescence visitors

The frequencies of visits by mammals, birds and insects to inflorescences were assessed by both direct and indirect methods.

To observe nocturnal mammals and insects I watched inflorescences at night with a red light and to observe bird visitors I

watched inflorescences during the day. To monitor diurnal insect activity I watched inflorescences from a distance of 1 m.

Removal of nectar by nocturnal mammals was measured directly by comparing at dawn the nectar loads of inflorescences that had been exposed with nectar loads of inflorescences that had been enclosed since dusk in a wire cage (13 × 13 mm mesh). Nectar was sampled from *B. integrifolia* by probing 50 florets from all regions of the inflorescence with 50-µl microcapillary tubes. I did not sample after overnight rain or dew. The total nectar load of *B. spinulosa* was collected rather than sampled, at dawn, regardless of rain or dew. Sugar concentration was measured using a hand-held refractometer.

I examined directly the extent to which visitors removed pollen from *B. spinulosa* inflorescences, and compared overnight and daytime losses. On sequential days I counted the number of pollen presenters with pollen and those missing pollen both before dawn and after dusk; those missing pollen were then removed. From these data it was possible to deduce both the number of pollen presenters that had emerged, and the number that had lost pollen in the intervening period (see Goldingay et al. 1987).

Two indirect methods helped to monitor mammal activities. Mammal visits to *B. spinulosa* inflorescences were recorded by noting the footprints of animals that had passed through wet paint that had been applied to the stem below the inflorescence just before dusk. The following day identifiable footprints could be found around inflorescences. I also placed nest boxes in trees to aid in the detection of small mammals, 4 at the *B. integrifolia* site, and 26 at the *B. spinulosa* site.

Sticky-traps were used both to sample insect visitors to *B. integrifolia,* and to examine whether they were capable of entering wire exclusion cages. Eight sticky-traps (each measuring 15 × 16 cm) were placed beside exposed inflorescences and 11 beside inflorescences inside exclusion cages.

Pollinator exclusions

I used two different pollinator exclusion techniques to investigate the influence on fruit-set exerted by the different classes of pollinator. One approach excluded visitors from all inflorescences on whole trees (*B. integrifolia* only), the other excluded visitors to individual inflorescences on separate plants (both *Banksia* species).

In whole-tree experiments, climbing mammals were excluded by fixing a sheet of aluminium around the trunk to form a barrier below which I applied a viscous paste, either "Tanglefoot" or "B.P. Hyvis". There were no nectarivorous bats or gliding mammals at the site, and no trees in any treatments had canopy-to-canopy contact that would have permitted mammals to bypass the aluminium barrier. I will refer to this treatment as the "mammal exclusion".

Bird netting (23 × 23 mm mesh) was tied over the canopy of each tree to exclude birds. The bottom of the net was restricted to a small opening to allow access to climbing mammals. Rarely, birds were observed to get under nets, so this treatment was not an absolute exclusion. Nonetheless I will refer to this treatment as the "bird exclusion".

Five treatments were used in this experiment: a bird exclusion, a mammal exclusion, a bird and mammal exclusion (using both the bird nets and aluminium sleeves), a control group with no exclusion, and a fifth treatment which excluded all visitors by enclosing each inflorescence on a whole tree in a plastic box with insect-proof mesh in the panels. In total, 50 trees were chosen and randomly assigned to the treatments.

In the single-inflorescence experiments cylindrical cages of wire mesh (13 × 13 mm) were used to exclude animals. To exclude mammals, inflorescences were caged before dusk and exposed after dawn. To exclude birds, inflorescences were caged within 30 min of dawn and exposed within 30 min of dusk. To exclude both birds and mammals inflorescences were caged day and night. Each treatment was applied throughout the flowering of inflorescences (approx. 10 days), except for 2 days when four *B. integrifolia* inflorescences missed out on their period of exposure. The single-inflorescence exclusions were applied to *B. integrifolia* later in the season than the whole-tree exclusions.

Methods of analysis

Fruit-set after the whole-tree experiment was examined in two ways. First, the dependence between treatment and the number of infructescences produced was examined using log-linear models. Second, the number of fruit per infructescence was examined by a two-way analysis of covariance (ANCOVA, Winer 1971), with mammal exclusion and bird exclusion as the two factors. Rachis length was included as a covariate because an infructescence with a longer rachis could potentially accommodate more fruit. Omega squared (ω^2) was calculated to represent the amount of variance explained by the different treatments (Winer 1971). ANCOVA was considered appropriate as the data were not multi-modal and had no outliers, there was no relationship between means and variances, and the homogeneity of variances satisfied Cochran's test. Because each tree had different numbers of infructescences (from 1 to 16), variation due to trees could not be included in the ANCOVA. The variation that exists within or between trees was included in the error term.

Fruit-set was very low in the single inflorescence experiment. Adjusted means could not be calculated because of the small samples, and so the relationship between number of fruit and rachis length was examined by expressing the data as fruit per cm of rachis.

Genotype frequency

To test the hypothesis that non-flying mammal pollination leads to inbreeding I examined genotype frequencies of two allozymes in the seed population of *B. integrifolia.* Eleven infructescences were collected in the field from separate trees and stored at 4° C. Embryos were ground in one drop of buffer (10% sucrose, 0.1% mercaptoethanol and 0.1% bromophenol blue) and applied to cellulose acetate sheets. Electrophoresis was conducted at room temperature, and staining procedures followed Richardson et al. (1986). I chose the allozymes alcohol dehydrogenase and aspartate aminotransferase because they stained well and could be consistently scored. Approximately 10 seeds were scored from each infructescence.

Inspection of individual genotypes indicated that alleles were independently distributed, so the two loci were assumed to occur on different chromosomes. The level of inbreeding was measured using the *F* index (Falconer 1981) with standard error of *F* calculated according to the method of Brown et al. (1975). The *F* index for alcohol dehydrogenase was calculated from triallelic data.

Results

Inflorescence visitors

Indirect and direct evidence indicate that non-flying mammals including Eastern Pygmy-Possums *(Cercartetus nanus)* were frequent nocturnal visitors. Birds, especially honeyeaters (Meliphagidae), were frequent diurnal visitors.

Eastern Pygmy-Possums were observed visiting a *B. integrifolia* inflorescence five times (I observed *B. integrifolia* for 9.5 h over 13 evenings, and *B. spinulosa* for 7 h over 6 evenings). Three visits lasted for approximately 3 min each, while the Pygmy-Possum crawled all over the inflorescence with its belly fur contacting the pollen

Table 1. Emergence of *Banksia spinulosa* flowers, and flowers that have lost pollen. *n* is number of inflorescences in sample

	Flowers presenting pollen		Flowers with pollen removed	
	$\bar{x} \pm SE$	*n*	$\bar{x} \pm SE$	*n*
Day	12.3±2.7	(12)	13 ±2.8	(12)
Night	14.6±4.1	(16)	16.7±4.1	(16)

presenters. Pygmy-Possums occupied all nest boxes at the *B. integrifolia* site but none at the *B. spinulosa* site. However, footprints of the Eastern Pygmy-Possum were found on and around most (19 of 21) inflorescences of *B. spinulosa* marked with paint. Footprints could be traced to other inflorescences up to 2 m from the paint source.

The nocturnal removal of pollen from *B. spinulosa* inflorescences was consistent with evidence of mammal visitation. Pollen was removed at similar rates by day or night (Table 1). Observations indicate that pollen was not removed by wind, rain or ants.

The nocturnal removal of nectar from *B. integrifolia* inflorescences indicates that a high proportion of inflorescences were visited by mammals, but the results from *B. spinulosa* were less clear. Of caged *B. integrifolia* inflorescences, 96% had nectar at dawn ($n=23$), while only 15% of uncaged inflorescences had nectar at dawn ($n=40$). In contrast only 36% of caged *B. spinulosa* inflorescences had nectar at dawn ($n=17$), while 65% of uncaged inflorescences had nectar at dawn ($n=23$). However, the mean mass of sugar collected from *B. spinulosa* inflorescences is consistent with nocturnal nectar loss, with a mean sugar mass of 21.3±19.0 mg from caged inflorescences, and only 1.4±0.46 mg from uncaged inflorescences.

Birds were frequent visitors to inflorescences during the day (I observed *B. integrifolia* inflorescences for 15.5 h over 10 days, and *B. spinulosa* for 74 h over 12 days). Four species, all in family Melaphagidae, took nectar from *B. spinulosa:* the New Holland Honeyeater *(Phylidonyris novaehollandiae),* Crescent Honeyeater *(P. pyrrhoptera),* Eastern Spinebill *(Acanthorhynchus tenuirostris)* and Little Wattlebird *(Anthochaera chrysoptera).* Birds visited inflorescences at a rate of approximately 1 visit per inflorescence every 48 min, and removed pollen (Table 1). The same four species were observed feeding on inflorescences of *B. integrifolia,* along with a fifth, the Rainbow Lorikeet *(Trichoglossus haematodus). Banksia integrifolia* inflorescences were visited at an approximate rate of 1 visit per inflorescence every 40 min. Comparison between caged and uncaged inflorescences at dusk revealed pollen was removed from inflorescences during the day.

Insect activity was low at both sites. During daytime I only once observed an insect *(Apis mellifera)* contacting the stigma of any *B. integrifolia* flower. One *Apis mellifera* was also observed on *B. spinulosa.* At night, an Emperor Gum Moth (Saturniidae) was seen feeding on a *B. integrifolia* inflorescence. Sticky traps showed that insects could enter exclusion cages.

Fruit-set

The exclusion of non-flying mammals from *B. integrifolia* trees significantly reduced fruit-set. This reduction affected both the number of infructescences produced, and the number of fruit per infructescence.

Numbers of infructescences were reduced significantly by mammal exclusions ($G=13.92$, df$=5$, $P=0.016$). Bird exclusion had no significant effect ($G=10.78$, df$=5$) and there was no significant interaction between the effects of birds and mammals ($G=6.42$, df$=3$, Table 2).

The number of fruit per infructescence was significantly less when mammals were excluded, and the exclusion of birds also caused a significant reduction. The interaction between these factors was not significant. Mammal exclusion had less effect on the number of fruit per infructescence than bird exclusion; bird exclusion explained approximately 5 times as much of the total variation than was explained by mammal exclusion (Table 3).

No fruit was produced in the treatment excluding all visitors to *B. integrifolia,* which included 34 inflorescences distributed over ten trees.

Few infructescences were produced after exclusion experiments on single inflorescences of *B. integrifolia,* but the pattern of fruit-set was consistent with the results from whole-tree exclusions despite the different techniques used (Fig. 1). The most fruitful treatment was the control, with 9 of 30 inflorescences producing fruit. After mammal exclusion 5 of 28 fruited, after bird exclusion 5 of 29, and after bird and mammal exclusion 1 of 32 fruited. Fruit-set in inflorescences of *B. spinulosa* was extremely low, and shed little light on the role of pollinators.

Table 2. *Banksia integrifolia* fruit-set after whole-tree exclusion experiment; adjusted mean fruit per infructescence ± SE, proportion of inflorescences that produced fruit and total number of inflorescences (*n*)

	Mammals present			Mammals excluded		
	$\bar{x}$(SE)	Proportion	*n*	$\bar{x}$(SE)	Proportion	*n*
Birds present	55.5(0.54)	0.71	(45)	44.9(0.67)	0.51	(43)
Birds excluded	34.8(0.65)	0.74	(38)	23.5(1.30)	0.54	(22)

Table 3. Two-way ANCOVA on fruit per infructescence of *Banksia integrifolia* subjected to whole-tree exclusions, with rachis length as a covariate

Source	DF	Mean square	*F* ratio	*P*	ω^2
Mammal exclusion	1	2 393.718	5.670	0.019	0.027
Bird exclusion	1	8 764.821	20.760	0.000	0.114
Interaction	1	2.126	0.005	0.944	0.000
Rachis length	1	24 183.773	57.281	0.000	0.324
Error	89	422.192	–	–	0.535

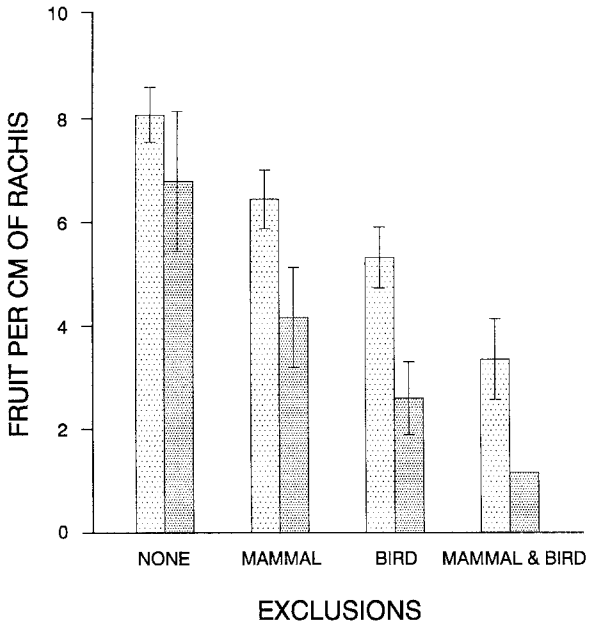

Fig. 1. Number of fruit per cm of rachis (± SE) for *Banksia integrifolia* infructescences from whole-tree exclusions *(lighter)* and single-inflorescence exclusions *(darker)*

Genotypic analysis of wild population *B. integrifolia* seed provided no evidence to support the inbreeding hypothesis. Both allozymes had F indices that did not depart significantly from zero (aspartate aminotransferase, $F = -0.0922 \pm 0.1031$ SE; alcohol dehydrogenase, $F = 0.2208 \pm 0.2339$ SE). The sample size in this survey was small, contributing to the large standard error. Therefore only a major deviation from panmixis would have been detected.

Discussion

Non-flying mammals were frequent visitors to inflorescences of both *Banksia* species, and pollinator exclusion demonstrated that they significantly influenced fruit-set of *B. integrifolia* on Wilson's Promontory.

Mammals observed directly and indirectly on inflorescences at night were clearly responsible for nocturnal nectar losses from *B. integrifolia* and probably *B. spinulosa*. Absence of nectar in many *B. spinulosa* inflorescences was probably due to the frequent rain which washed nectar away. This was not a problem with *B. integrifolia* because nectar was not sampled after rain. I attribute the nocturnal loss of pollen from *B. spinulosa* to non-flying mammals rather than insects. Mammals made frequent visits to inflorescences as revealed by their footprints. It is unlikely that insects were responsible because first, nocturnal insects were uncommon, and second, the recurved style of the *B. spinulosa* flower and the open nectar channels of the inflorescence make it possible for insects to collect nectar without dislodging pollen. Goldingay et al. (1987) also found non-flying mammals visiting and removing pollen from *B. spinulosa* inflorescences.

The low fruit-set in the single-inflorescence exclusion experiments was probably due to seasonal effects. The single-inflorescence experiments on *B. integrifolia* were conducted later in the season than the whole-tree experiments, and the single-inflorescence control group produced a substantially lower proportion of infructescences than the whole-tree control. Therefore the overall low fruit-set in the single-inflorescence experiment may simply reflect the decline in fruit-set as the season progressed beyond the peak of flowering. This pattern of decline has been noted in other *Banksia* species (Copland and Whelan 1989) and may help to explain the extremely low fruit-set in *B. spinulosa,* which was also experimentally examined after the peak of flowering had passed.

Pollinator exclusions indicate that non-flying mammals and birds were both major contributors to *B. integrifolia* fruit-set, and that insects were of minor importance. The fruit-set in the whole-tree mammal and bird exclusion cannot be wholly attributed to insects because of the failure of nets to completely exclude birds. The permanently caged treatment of the single-inflorescence experiment did allow access to insects alone and this treatment produced very low fruit-set.

Mammal visitation to *B. integrifolia* increased both the numbers of infructescences initiated and the fruits per infructescence. Bird visitation only increased the number of fruits per infructescence, but to a greater extent than mammals. The occasional visit by birds to inflorescences under the bird-nets may have lead to an underestimate of the importance of birds, but this is unlikely to affect the overall pattern. The pattern suggests that birds were important pollinators, but only pollinated a small portion of inflorescences. This pattern may result from the different foraging modes of non-flying mammals and birds.

Because they can fly, birds are likely to be efficient pollinators, visiting inflorescences frequently and carrying pollen from a wide pool of inflorescences. Nectarivorous birds forage primarily by sight and have a poor sense of smell (Stiles 1978), therefore one might expect them to favour visually well-advertised inflorescences such as those on the periphery of the canopy. *Banksia integrifolia* at this site had many inflorescences hidden deep within the foliage that may have been less attractive to the birds. This pattern of visitation has been described for Hummingbird visitors to *Centropogon valerii* (Colwell et al. 1974).

Non-flying mammals are probably less efficient at causing outcrossed pollinations because they are not as wide ranging (Sussman and Raven 1978). However, they have a highly developed sense of smell (Gunderson 1976) and can climb throughout a tree to locate inflorescences. Indeed odorous inflorescences positioned close to branches, as in these *Banksia* species, could be a feature of plants pollinated by non-flying mammals (Holm 1978). Therefore mammals may have pollinated a greater proportion of inflorescences, including those hidden within foliage, but achieved fewer pollinations per inflorescence than birds. This hypothesis on the effect of inflorescence position on pollination could be tested in the field.

Genotypic analysis failed to provide evidence of high

levels of inbreeding due to non-flying mammal pollination. Two biological explanations are possible; either non-flying mammals are carrying pollen from a larger than expected pool, or they carry pollen from a local pool but inbreeding is not being expressed in the seed. Ayre and Whelan (1989) have suggested that *Banksia* may be able to exercise "mate choice" by selectively developing only the "good" pollinations. It may be that the capacity to exercise mate choice neutralises what is otherwise a limitation in pollination by non-flying mammals.

Why is non-flying mammal pollination not more common? The similar floral morphology found in many Proteaceae presents the possibility that the phenomenon is widespread in Australia, especially in southwest Australia where the Honey-Possum *(Tarsipes rostratus)* feeds on nectar from many *Banksia* species (Hopper 1980). To assess the true incidence of non-flying mammal pollination will require further examination of the assumption that they achieve a poor rate of outcrossing, and experimental assessment of the pollination systems of other plant species that are visited by non-flying mammals. It may be that we are not familiar with the phenomenon because so few such experiments have been done.

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