

Causes and consequences of seasonal variation in pollen limitation of seed production in *Blandfordia grandiflora* (Liliaceae)

Mike Ramsey

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Natural seed set was consistently pollen-limited for 5 yr in the perennial herb *Blandfordia grandiflora*. The extent of pollen limitation varied within seasons, depending on when individual plants flowered. In all years, percent seed set was greatest in January (36–41%) and least in March (12–14%). Seed set of hand-pollinated fruits did not differ within flowering seasons (46–52%), indicating that pollination factors caused natural seed set to vary. Variation in natural seed set was positively correlated with frequency of visits to flowers by nectarivorous birds, but not their abundance in the area. Bird abundance increased during the flowering season, but floral visits decreased. *B. grandiflora* flowers received about 2 visits in December, 3 in January, fewer than 1 in February and none in March. In February and March, *B. grandiflora* plants co-flowered and competed for bird pollinators with *Banksia serrata*. In these months, birds preferentially foraged at *Banksia serrata* inflorescences which produced about 10-fold more nectar per 24 h. The consistently greater natural seed set in January suggests that optimizing selection would favour plants that flower in this month.

M. Ramsey, Depts of Botany and Zoology, Univ. of New England, Armidale, N.S.W. 2351, Australia.

Many flowering plants depend upon pollinators for successful reproduction, and consequently the abundance and behaviour of pollen vectors can become an important ecological factor influencing seed production. Pollen limitation occurs when seed production is less than would be achieved if visits by pollinators were more frequent or pollinators deposited more compatible pollen on stigmas (e.g. Waser 1978, Bierzychudek 1981, 1983, Rathcke 1983, Campbell and Motten 1985, Zimmerman and Pyke 1988, Ramsey 1989, Vaughton 1991). Although pollen limitation occurs in a diverse range of species with different life-histories, breeding systems, and pollinators (reviewed by Burd 1994, Vaughton and Ramsey 1994), the frequency, extent and selective importance of pollen limitation require further empirical study. Variation in pollination will affect the strength and direction of selection on reproductive traits that reduce pollen limitation. Only when female reproductive success is consistently pollen-

limited, will selection favour traits that either increase pollen receipt, resulting in plants attracting more or better pollinators, or decrease the dependence on pollinators for pollen receipt (Haig and Westoby 1988, Rathcke and Real 1993, Vaughton and Ramsey 1994).

Plants flowering at different times within a reproductive season may differ in seed production and the extent to which they are pollen-limited (Primack 1980, Augspurger 1981, Gross and Werner 1983, Widén 1991). Temporal variation in pollen limitation may be caused by changes in pollinator abundance or foraging behaviour in response to changes in intra- or interspecific floral density during a flowering season (Gross and Werner 1983, Rathcke 1983, Waser 1983). Sympatric co-flowering plants may compete for pollinators, causing pollen limitation. This occurs when the flowering of one species causes reduced seed production in other co-flowering species sharing its pollinators, by either pollinator prefer-

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ence or the transfer of interspecific pollen (Waser 1983). Temporal changes in floral density or nectar production between co-flowering species may cause pollinators to change foraging preferences to the more rewarding plants or to forage indiscriminantly between species (Waser 1978, Campbell and Motten 1985, Kwak and Jennersten 1991). Pollen limitation caused by competition should selectively favour phenotypes that either flower when other species do not, or are unaffected by the flowering of other species (Mosquin 1971, Waser 1983, Feinsinger et al. 1991).

In the present study, I examine seasonal variation in pollen limitation of plants flowering at different times in the perennial herb *Blandfordia grandiflora* R. Br. (Liliaceae). I use both whole- and partial-plant treatments to assess pollen limitation, and first establish whether there are differences between these methods. Second, I test whether seed set is resource-limited within a reproductive event by examining seed abortion and seed set of flowers that have opened at different times on the same plant. Third, I assess variation in the extent of pollen limitation by comparing seed sets of open- and hand-pollinated plants that flowered at different times during flowering seasons. Lastly, to gain insight into the causes of pollen limitation, I examine the relationships between pollinator abundance, flower visitation and seed set, and examine the flowering phenology and nectar production of *B. grandiflora* and *Banksia serrata* R. Br. (Proteaceae), a possible competitor for pollinators.

Methods

Study site and species

Populations of *B. grandiflora* occur in wet heathland communities on damp sand and/or peaty soils of low pH and nutrient status in coastal areas of north-eastern New South Wales and south-eastern Queensland, Australia (Ramsey et al. 1994). I studied flowering populations of *B. grandiflora* from 1988 to 1992 within Yuraygir National Park, NSW (29° 51'S, 153° 16'E). Study sites were located within a wet heathland area of about 3 km², surrounded by heathland dominated by *Ba. serrata* shrubs. When flowering, *B. grandiflora* is a conspicuous component of the vegetation. About 85% of plants flower in the first year after fires (495 ± 73 flowering plants/50 m², N = 8), but declines to negligible levels after 4–5 yr (M. Ramsey unpubl.). Several fires occurred during the study period, thus permitting different flowering populations to be examined each year. Populations were located 3 km or less from each other.

Most flowering occurs between December and March and individual plants flower for about 3–4 wk. Sexually reproductive plants consist of a corm, basal leaves and a single terminal, racemose inflorescence with 3–12 pendulous, yellow and red hermaphroditic flowers, each 55–60 mm long. Plants are self-infertile, and floral morphology

and nectar production indicate that nectarivorous birds pollinate flowers (Ramsey 1993, Ramsey et al. 1993, 1994). Any competitive interactions for pollinators between *B. grandiflora* and *Ba. serrata* is probably not a recent phenomenon since both species are endemic to eastern Australia and their distributional ranges overlap to a large extent.

Pollen limitation of whole plants

In 1988, I conducted whole-plant treatments on naturally pollinated (hereafter, open-pollinated) plants that flowered during either January or March, and hand-pollinated plants that flowered during March. In January, 50 plants were selected at 5–10 m intervals on arbitrarily chosen transect lines and marked. Fruits from each plant were harvested about 6 wk later. The numbers of aborted seeds, sound seeds and ovules were counted, and percent seed set determined as the ratio of the number of sound seeds to total number of seeds plus ovules per plant. In March, plants were assigned to either hand- (N = 30) or open-pollination (N = 35) treatments. Hand-pollinated inflorescences were covered with net bags (1 mm² mesh). As flowers opened, they were cross-pollinated with pollen from 3–5 plants located at least 10 m away. Open-pollinated plants were marked and left untouched. The numbers of seeds and ovules and percent seed set of plants were assessed. I compared flower, ovule and seed production among the treatments using one-way analyses of variance (ANOVA).

Whole plant and single fruit assessment of seed set

It was not possible to use whole-plant treatments in all years, and I examined whether the percent seed set of single fruits could be used instead. First, I compared percent seed set of single fruits from plants on which one flower was used with seed set of plants on which all flowers were used (whole plant treatments described above). In January 1988, 50 plants were assigned to a single fruit open-pollination treatment. In March, plants were assigned to either hand- (N = 50) or open-pollination (N = 60) treatments. On each plant one floral bud was selected. Buds on hand-pollinated plants were cross-pollinated and bagged as they opened. Open-pollinated flowers were left untouched. I tested for differences in percent seed set between pollination methods (whole plants or single fruits) and among pollination treatments (January and March open- or hand-pollinated) with a two-way model, one ANOVA (unweighted means analysis for unbalanced design; Winer et al. 1991). Non-significant F-ratios for methods and methods × treatments interaction indicate that estimates of seed set using single-fruit and whole-plant pollination methods are comparable.

Second, I examined whether seed set of fruits within

plants was limited by resources. If resources are limiting, seed set should decrease over a plant's flowering period (e.g. Ågren and Willson 1992), and flowers that open last on inflorescences should contain more aborted seeds and fewer sound seeds than those that open earlier. Using hand-pollinated plants, I compared percent seed abortion and seed set of the first and last fruits and another randomly chosen fruit on each plant. Percent abortion was determined as the ratio of the number of aborted seeds to the number of all seeds plus ovules per fruit. I determined seed set as described previously. Single-factor repeated measures ANOVAs were used to test for differences among fruits within plants, using correction factors (Greenhouse-Geisser epsilon) to determine degrees of freedom for F-ratios (LaTour and Miniard 1983).

Finally, I tested whether resources were disproportionately allocated to hand-pollinated fruits on plants with both hand- and open-pollinated flowers by comparing seed sets of open-pollinated fruits on plants with and without hand-pollinated fruits (e.g. Zimmerman and Pyke 1988). In the hand-pollination treatment, I used the open-pollinated fruit on inflorescences that followed the hand-pollinated fruit. From open-pollinated plants, one fruit was selected randomly. I compared percent seed sets of the open-pollinated fruits with one-way ANOVA.

Within and among season pollen-limitation

Natural levels of seed set

Having determined that percent seed set of single fruits and whole plants do not differ significantly (see results), I assessed seasonal variation in natural seed set using single fruits. I determined seed set of open-pollinated fruits from plants that flowered (1) in January or March for 5 yr (1988 to 1992), and (2) in each month from December to March for 3 yr (1988–89, 1989–90, 1991–92). Each month, 50 plants were marked, except in 1991, when 20 plants were marked. One fruit per plant was randomly selected. Two-way mixed model ANOVAs (unweighted means analysis) were used to test for differences in seed set per fruit between January and March for the 5 yr, and among the 4 mo for the 3 yr. Months and years were considered fixed and random effects, respectively, enabling general conclusions about all years to be drawn (Bennington and Thayne 1994). The number of seeds produced by plants was estimated by multiplying the mean number of seeds per fruit by the number of fruits per plant.

Natural vs hand-pollinated seed set

I determined the extent of pollen limitation within seasons by examining seed sets of hand- and open-pollinated fruits. In January and March of 1989 and 1990, one floral bud was marked on each of 50 plants. When flowers opened, they were cross-pollinated and inflorescences bagged. Open-pollinated flowers for each month were used as controls. A three-way mixed model ANOVA (unweighted means analysis) was used to test for differ-

ences in percent seed set of hand- and open-pollinated fruits for January and March of the 2 yr. Pollination and month were fixed effects and year was a random effect.

Pollinator exclusion, abundance, floral visitation and seed set

Pollinator exclusion

I examined the importance of nectarivorous birds as pollinators in 1990 by excluding them from plants in January (N = 10) and March (N = 16). Plants were assigned to either caged or uncaged treatments. Cages of plastic bird netting (1 cm² mesh) were placed over a wire frame, excluding nectarivorous birds, but allowing insects access to flowers. Uncaged plants were left untouched. A two-way model one ANOVA (unequal but proportional subclasses; Winer et al. 1991) was used to test for differences in percent seed set between treatments and months.

Pollinator abundance, floral visitation and seed set

I examined the effect of nectarivorous bird abundance and floral visitation on seed set in 1989 and 1990. Three sites were monitored in January and March in either 1989 (DC) or 1990 (Bea, Qua). Another site (Sub) was monitored each month from December to March in 1989–90. Sites were 1–2 km apart.

I assessed relative bird abundance while walking along 40 × 200 m fixed-strip transects for 15 min shortly after sunrise, at midday and shortly before dusk, for three d each month. The numbers of birds counted each day were summed and treated as replicates. I assessed floral visitation by observing patches of flowering plants and recording visits to flowers by birds. Numbers of flowering plants varied among sites and months, and I varied patch sizes to observe the largest number of flowers possible. Between 800–2000 flowers were observed for three 1-h periods each day, for 3 d each month. I estimated the number of visits to flowers over their lifespan, assuming a 6-d floral lifespan (Ramsey 1993) and 12-h foraging days. At each site, seed set was assessed for each month from 50 tagged plants (1 fruit/plant).

Sites were analysed separately. Numbers of birds on transects and numbers of visits to flowers were compared between months with either non-parametric two-sample H-tests (DC, Bea, Qua sites) or multi-sample H-tests (Sub site). Non-parametric a posteriori tests were used to examine differences among months at the Sub site (Meddis 1984). Percent seed set of months was compared with one-way ANOVAs. Product-moment correlations were calculated on pooled data from all sites to examine the relationships between bird abundance, floral visitation and seed set.

Seasonal variation in floral nectar production

Because seasonal variation in floral rewards might affect pollinator visitation, I examined 24-h nectar production

Table 1 Number of flowers, ovules and seeds, and percent seed set for open-pollinated plants flowering during either January (N = 50) or March (N = 35) and hand-pollinated plants flowering during March (N = 30) in 1988. Data are means (\pm se) and were analysed with one-way ANOVAs. Means within rows having different superscripts differed significantly ($P < 0.05$, SNK tests).

	Hand-pollinated	Open-pollinated		F	P
		January	March		
Number of flowers	5.4 ± 0.3	5.6 ± 0.2	5.3 ± 0.2	0.79	NS
Number of ovules	678.0 ± 47.1	695.5 ± 26.8	637.3 ± 31.8	1.55	NS
Number of seeds	351.2 ^a ± 30.6	281.7 ^b ± 13.8	84.6 ^c ± 6.0	117.71	<0.001
Percent seed set	52.2 ^a ± 2.0	41.0 ^b ± 1.4	13.5 ^c ± 0.9	148.85	<0.001

using 1 flower per plant in January (N = 50) and March (N = 40) in 1992. Flowers had been open for 24–72 h. I measured nectar volumes of bagged flowers using 50 μ l capillary tubes. Concentration was measured in sucrose equivalents with a pocket refractometer and converted to mg sucrose per volume. Nectar parameters were compared using one-way ANOVAs.

Flowering phenology of *Blandfordia grandiflora* and *Banksia serrata*

Banksia serrata is a shrub found in the heathland surrounding *B. grandiflora* habitats. Flowering commences in late January and overlaps with that of *B. grandiflora*. Plants produce several inflorescences, each producing copious amounts of nectar. As with other banksia species, inflorescences are visited by nectarivorous birds that probably act as pollinators (e.g. Ramsey 1988, 1989). The mean (\pm se) number of shrubs is $7.7 \pm 0.6/100 \text{ m}^2$ (N = 20).

Because *Ba. serrata* and *B. grandiflora* are the major nectar-producing plants from November to April at the study site, I examined flowering overlap between them to gain insight into factors affecting natural seed set of *B. grandiflora*. For *Ba. serrata*, I counted flowering inflorescences on 15 shrubs each month from January to April in 1990 and 1992. The number of inflorescences per 50 m^2 was estimated each month by multiplying plant density by the mean number of inflorescences per plant. Nectar production was estimated by multiplying this value by the 24-h nectar production per inflorescence (367 mg sucrose, Armstrong 1991).

For *B. grandiflora*, I counted flowering plants in two, 2 \times 25 m plots each month from November to April for 4 yr from 1988 to 1992. Flowering for the first 3 yr was

similar (results not presented) and 1989–90 data were used for analyses. In 1991–92, flowering was delayed by 1 mo, probably because the winter was drier than other years. To assess whether flowering differed between years, I compared flowering intensity among months and years using a two-way, model one ANOVA. Nectar production per 24 h per 50 m^2 was estimated by multiplying the mean nectar production per flower (24 mg sucrose, see results) by two, the mean number of flowers producing nectar per plant, and by the number of plants flowering each month.

Flowering overlap of the species for the 2 yr were examined using Renkonen's index (Krebs 1989). Indices range from 0 to 100 (no to complete overlap), and were calculated from monthly flowering converted as a percentage of total flowering for each species.

Data transformation and statistical analyses

Data were transformed to improve normality and homoscedasticity when necessary. Counts and percentages were transformed using natural logarithm and arcsine transformations, respectively. Nectar data were transformed using natural logarithms. Student-Newman-Keuls a posteriori multiple range tests were used to compare individual treatment means following significant ANOVAs. In mixed model ANOVAs, residual error terms to construct F-ratios were calculated following preliminary tests and using the pooling criteria of Winer et al. (1991). With these analyses, nonsignificant interactions are not given. Means (\pm se) are presented.

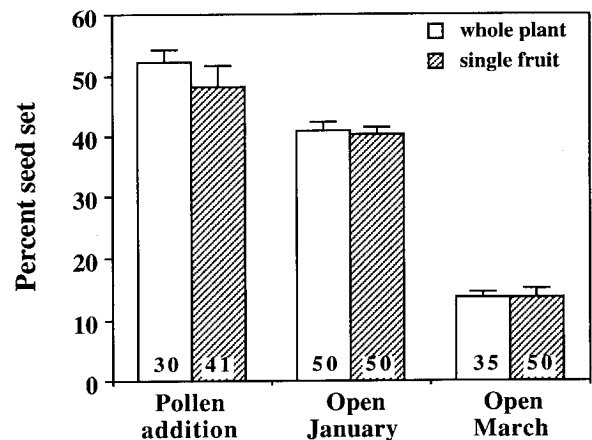


Fig. 1. Percent seed set of whole-plant and single-fruit pollination methods for open- and hand-pollination treatments (mean \pm se). There were no differences between whole-plant and single-fruit methods, although treatments differed from each other (SNK tests, all $P < 0.05$). Sample sizes are given at the bottom of bars.

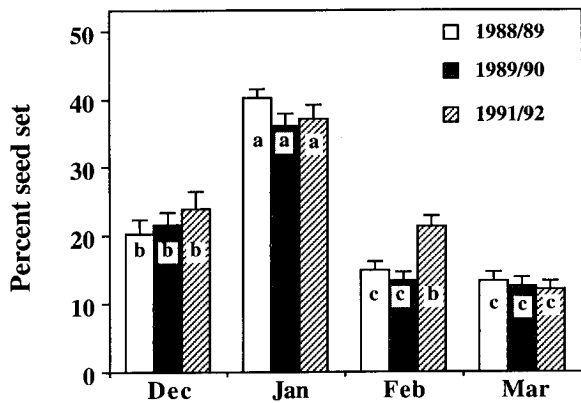


Fig. 2. Percent seed set of open-pollinated fruits (mean + se) for December to March in 1988-89, 1989-90 and 1991-92. Each month 50 fruits were examined. There were no differences between years, although months within years differed. Bars with different letters differed significantly (SNK tests, $P < 0.05$).

Results

Pollen and resource limitation of whole plants

Seed production per plant was pollen-limited and varied during the flowering season. Plants in all treatments produced similar numbers of flowers and ovules, but seed set differed significantly, depending on when they flowered and the pollination treatment they received (Table 1). Open-pollinated seed set for January and March plants was about 80% and 24% that of hand-pollinated plants, respectively. Open-pollinated January plants produced about 196 more seeds than open-pollinated March plants (Table 1).

Whole plant and single fruit assessment of seed set

Using single-fruit instead of whole-plant pollination treatments seed set was justified. Percent seed set determined from single-fruit and whole-plant pollination treatments did not differ significantly ($F_{1,250} = 0.19$, $P > 0.50$, Fig. 1). The significant pollination effect ($F_{2,250} = 226.24$, $P < 0.001$) indicates that percent seed set depended on whether plants flowered in January or March and whether they were open- or hand-pollinated. The interaction was not significant ($F_{2,250} = 0.04$, $P > 0.50$).

Resources did not limit seed set of fruits within plants. Neither percent seed abortion nor percent seed set differed significantly among successively developing fruits on hand-pollinated plants (abortion: first fruit, 10.0 ± 1.6 ; middle, 8.9 ± 1.3 ; last, 9.4 ± 1.4 ; $F_{2,58} = 0.90$, $P > 0.50$; seed set: first, 50.7 ± 3.5 ; middle, 48.8 ± 3.7 ; last, 44.9 ± 2.6 ; $F_{2,58} = 1.50$, $P > 0.20$). Similarly, resources were not disproportionately allocated to hand-pollinated fruits at the expense of open-pollinated fruits. Percent

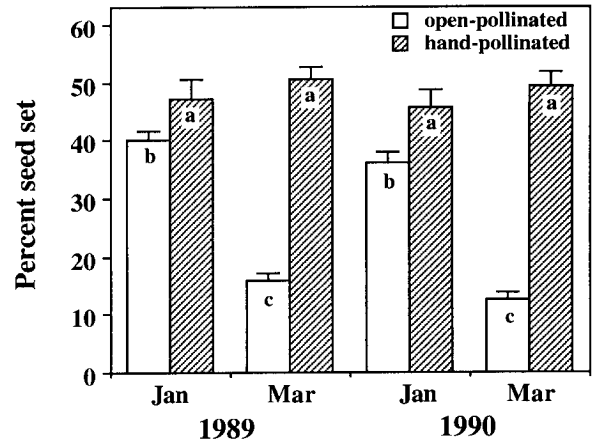


Fig. 3. Percent seed set of fruits that were open- or hand-pollinated during January or March in 1989 or 1990 (mean + se). Bars with different letters differed significantly (SNK tests, $P < 0.05$). Sample sizes varied between 35 and 50.

seed set of open-pollinated fruits on plants with and without hand-pollinated fruits did not differ significantly, and was $15.9 \pm 1.2\%$ and $13.5 \pm 1.2\%$, respectively ($F_{1,95} = 2.75$, $P > 0.20$).

Within and among season pollen-limitation

Natural levels of seed set

Percent seed set of plants flowering in January was greater than in March (range of values; January, $38.7 \pm 5.3 - 43.2 \pm 4.5$; March $12.3 \pm 3.7 - 15.6 \pm 4.2$; $F_{1,434} = 75.26$, $P < 0.001$) for the 5 yr from 1988 to 1992 ($F_{4,434} = 1.96$, $P > 0.20$). Similarly, seed set differed each month from December to March ($F_{3,588} = 128.75$, $P < 0.001$), but did not vary among the 3 yr ($F_{2,588} = 2.86$, $P > 0.10$; Fig. 2). The significant month \times year interaction was due to higher seed set during February 1992 compared to the other years ($F_{6,588} = 3.09$, $P < 0.02$).

The number of seeds per fruit was significantly greater in January than in March ($F_{1,434} = 570.54$; $P < 0.001$) for each of the 5 yr ($F_{4,434} = 0.98$; $P > 0.50$). The numbers of seeds per fruit pooled over the 5 yr for January and March

Table 2. Three-way ANOVA (mixed model) for percent seed set of hand- vs open-pollinated fruits on plants that flowered during January or March of 1989 or 1990. The residual error MS was calculated by pooling the nonsignificant interactions with the error term using the criteria given by Winer et al. (1991). Corresponds to data in Fig 3.

Source	df	MS	F	P
Pollination	1	19269.7	272.20	<0.001
Month	1	5307.4	74.67	<0.001
Year	1	147.3	2.08	NS
Pollination \times Month	1	8768.8	123.87	<0.001
Residual error	356	70.8		

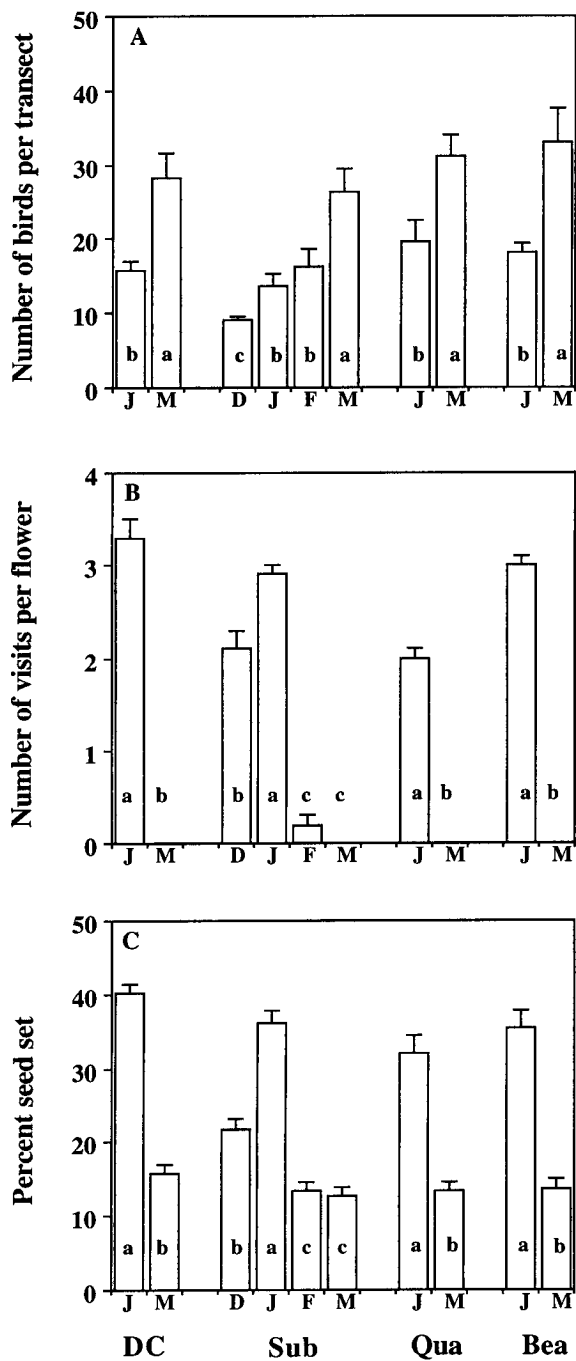


Fig. 4. Seasonal changes in (A) number of nectarivorous birds counted on transects, (B) number of visits by birds per flower, and (C) percent seed set for January and March at the DC, Qua and Bea study sites, and for December to March at the Sub study site. Data are means (+ se). Within sites, bars with different letters differed significantly ($P < 0.05$). Transects and patches of flowers were monitored for 3 d and seed set was assessed from 50 fruits each month.

were 47.0 ± 1.2 and 14.3 ± 0.6 , respectively ($N = 220$). The estimated numbers of seeds produced per plant in January and March were 282.0 ± 7.2 and 85.8 ± 3.6 seeds, respectively, which is similar to the actual seed set of whole plants in the open-pollination treatments in 1988 (Table 1). Thus, in all years January flowering plants had a 3-fold female fecundity advantage compared to March plants.

Natural vs hand-pollinated seed set

Similar to the whole-plant treatments, percent seed set of hand-pollinated fruits was significantly greater than open-pollinated fruits in January and March 1989 and 1990 (Fig. 3, Table 2). Seed set of hand-pollinated fruits did not differ between months, whereas natural seed set was significantly greater in January than March. Seed set between years did not differ (Fig. 3, Table 2). The ratio of open- to hand-pollinated seed set was 82% and 29% in January and March, respectively, causing a significant pollination \times month interaction (Table 2).

I calculated an index of pollen limitation by pooling monthly seed set data in all years (excluding February 1992), and then calculating the ratio of open- to hand-pollinated seed set, and subtracting this value from one. Values were 0.54, 0.20, 0.71 and 0.73 for December, January, February and March, respectively, indicating that pollen limitation was greatest in February and March. For February 1992, it was 0.56.

Pollinator exclusion, abundance, flower visitation and seed set

Pollinator exclusion

The effect of excluding pollinators from plants depended on the month that the treatment was conducted (caging, $F_{1,48} = 71.37$, $P < 0.001$; month, $F_{1,48} = 34.49$, $P < 0.001$; caging \times month, $F_{1,48} = 40.98$, $P < 0.001$). Caging significantly reduced percent seed set in January, but had no effect in March. Seed set of caged plants was similar in the 2 mo (January, 13.3 ± 0.7 ; March 11.1 ± 1.0 ; $P > 0.05$, SNK test), whereas seed set of uncaged plants was significantly greater in January (35.1 ± 2.6) than in March (12.0 ± 1.1 ; $P < 0.05$, SNK test).

Pollinator abundance, flower visitation and seed set

The numbers of nectarivorous birds observed on transects, their visits to flowers, and the percent seed set of fruits for the 4 study sites are presented in Fig. 4. Almost 98% of visits to flowers were by white-cheeked honeyeaters (*Phylidonyris nigra*, Meliphagidae). At all sites, the number of birds observed on transects increased during the flowering season (all $P < 0.05$; Fig. 4A). In contrast, the number of visits to flowers was greatest in January, intermediate in December and least in February and March (all $P < 0.05$). In January, flowers were visited about 3 times over their lifespan, whereas March flowers were not visited (Fig. 4B). Percent seed set for all sites was significantly greater in January than in March (all

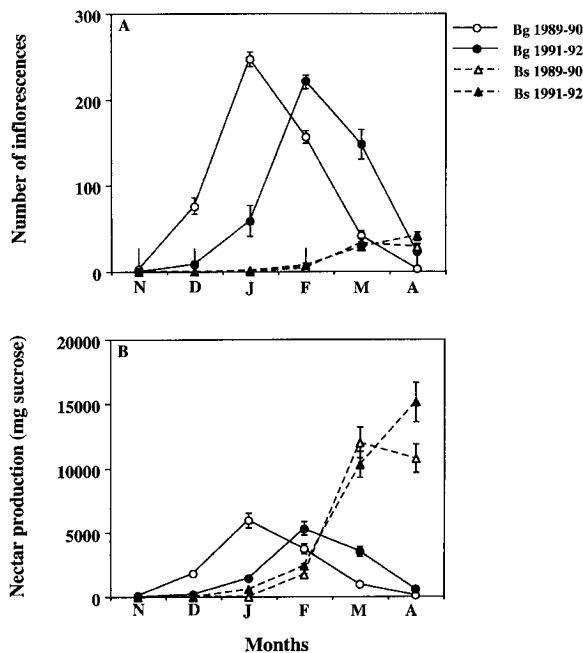


Fig. 5. Flowering phenology and nectar production of *Blandfordia grandiflora* (Bg) and *Banksia serrata* (Bs) from November to April during 1989–90 and 1991–92. Data (mean \pm se) are (A) numbers of flowering inflorescences per 50 m², and (B) mg sucrose equivalents per 24 h per 50 m².

$P < 0.001$; Fig. 4C). Visits to flowers and seed set were positively correlated ($r = +0.96$, $df = 8$, $P < 0.001$). Correlations between the number of honeyeaters on transects and the number of visits per flower ($r = -0.62$) and seed set ($r = -0.49$) were nonsignificant (both $df = 8$, $P > 0.05$).

Seasonal variation in floral nectar production

Floral nectar production over a 24-h period did not differ between January and March (volume in μ l, January 49.0 ± 3.2 , March 52.9 ± 3.6 , $F_{1,88} = 0.95$, $P > 0.50$; concentration in % sucrose equivalents w:v, January 24.6 ± 1.1 , March 24.0 ± 1.1 , $F_{1,88} = 2.45$, $P > 0.20$; mg of sucrose, January 11.6 ± 0.7 , March 12.2 ± 0.8 , $F_{1,88} = 1.28$, $P > 0.50$).

Flowering phenology of *Blandfordia grandiflora* and *Banksia serrata*

The flowering phenologies of *B. grandiflora* and *Ba. serrata* for 1989–90 and 1991–92 are given in Fig 5A. In both years, *B. grandiflora* flowering preceded that of *Ba. serrata*. The total number of flowering *B. grandiflora* plants did not differ between years (1989–90, 526.0 ± 19.5 ; 1991–92, 459.5 ± 11.5 ; $F_{1,6} = 4.56$, $P > 0.20$), but varied among months within years

($F_{3,6} = 24.13$, $P < 0.002$). The pattern of flowering also differed between years (year \times month interaction, $F_{3,6} = 22.59$, $P < 0.005$); peak flowering in 1989–90 occurred in January, but in 1991–92 occurred in February. The number of flowering plants at the peak periods did not differ significantly (SNK test, $P > 0.05$). Renkonen's indices were 20.8% and 47.5% for 1989–90 and 1991–92, respectively, indicating that the later flowering in 1991–92 increased flowering overlap with *Ba. serrata*. The flowering phenology of *Ba. serrata* was similar in both years. Shrubs produced 303 and 320 inflorescences in 1990 and 1992, respectively; 85% had flowered by the end of April.

Estimated nectar production is given in Fig. 5B. In February, *B. grandiflora* produced more nectar per 50 m² than *Ba. serrata*, whereas in March *Ba. serrata* produced more. In 1992, because flowering was delayed, *B. grandiflora* produced more nectar in March than in 1990. Nectar production of *Ba. serrata* was similar during February and March in both years.

Discussion

Natural seed set in different *B. grandiflora* populations was consistently pollen-limited during the December to March flowering season for each of the 5 yr of the study. The extent of pollen limitation, however, depended on when plants flowered. In January, seed set was about 80% that of hand pollination, whereas in March it was 28%. Limitation of seed set by factors unrelated to pollination was unlikely, since seed set of hand-pollinated flowers was similar within seasons. The significance of these results is 2-fold. First, except for some orchid species, consistent pollen limitation of seed production has rarely been documented (e.g. Gill 1989, Primack and Hall 1990). Second, this is one of only a few studies that has documented variation in pollen limitation among plants within a reproductive season (Primack 1980, Augspurger 1981, Gross and Werner 1983, Paige and Whitham 1987, Widén 1991).

In the present study, pollen limitation was examined by comparing seed set in natural and hand-pollinated flowers. If increased seed production in response to hand pollination decreases future fitness more than current fitness gains (i.e. costs of reproduction), then lifetime reproductive success may be limited by resources rather than pollen (e.g. Ackerman and Montalvo 1990, Primack and Hall 1990, Calvo 1993). However, this is unlikely in *B. grandiflora*. As for several other species (e.g. Reekie and Bazzaz 1987, Horvitz and Schemske 1988, Jennersten 1991), no detectable costs of reproduction were found when survival, growth and reproduction of plants in high and low reproductive treatments (seed set, 47.8% and 0.8%, respectively) were compared over a 4-yr period (M. Ramsey unpubl.).

Causes of pollen limitation

In *B. grandiflora*, seasonal variation in pollen limitation was related to floral visitation. Seed set was correlated with the number of visits to flowers by nectarivorous birds; both were greatest in January and least in March. Changes in visitation were not due to seasonal differences in floral nectar rewards at the plant level, since nectar production per flower was similar within a season. Instead, visitation depended on bird abundance in the area and competition for pollinators with *Ba. serrata*, a co-occurring shrub. As with other pollinators (Rathcke 1983), Australian nectarivorous birds are often attracted to areas of high floral density (e.g. Ramsey 1989). In December, both bird abundance and visitation were low probably because flowering density was low. Too few birds were in the area to adequately visit and pollinate flowers. In January, bird abundance and floral visitation increased in response to the greater *B. grandiflora* flowering. In February and March, flowering of *B. grandiflora* and *Ba. serrata* overlapped and bird abundance was greatest. However, in these months, *B. grandiflora* competed for pollinators with *Ba. serrata* and birds visited flowers less frequently.

Competition for pollinators is demonstrated by showing that (1) pollinators are required to achieve maximal seed set, (2) sharing pollinators with another species causes reduced seed set, and (3) reduced seed set is caused by either fewer visits by pollinators or reduced effectiveness of pollinator visits (Waser 1978, 1983). In the present study, excluding birds from plants reduced seed set in January, but not March. This indicates that in January, floral visits by birds were important for high seed production, but that in March seed set was low because birds did not visit flowers. In February and March, as *Ba. serrata* flowering increased, birds switched their foraging from *B. grandiflora* to *Ba. serrata*, indicating that plants competed via pollinator preference (Waser 1983). Foraging preference was probably influenced by differences in nectar production. *Ba. serrata* produces about 10-fold more nectar per inflorescence per day than *B. grandiflora*. In February, despite similar nectar production per unit area by both species, birds probably preferentially forage at *Ba. serrata* because inflorescences produce more nectar and foraging is energetically less costly, or the shrub habitat affords greater protection during foraging than the open heathland. In March, preferential foraging on *Ba. serrata* is due to its greater nectar production per unit area.

In 1991–92, *B. grandiflora* flowering was delayed and peak flowering was in February, and not January as in other years. This resulted in greater flowering overlap with *Ba. serrata*, and provided an opportunity to assess the effect of greater *B. grandiflora* flowering on its ability to compete for pollinators. In February 1992, seed set was greater than for the same month in other years, suggesting that plants competed for pollinators more successfully because flowering was greater. Nevertheless, February

seed set was less than peak flowering of other years, suggesting that competition for pollinators negatively affected pollination during this period. In March, *B. grandiflora* flowering was almost 4-fold greater than in other years, but seed set was similar, indicating birds preferentially foraged on *Ba. serrata* plants. Overall, these findings suggest that pollination and seed set in *B. grandiflora* are affected by density-dependent interactions with *Ba. serrata*.

The present study examined the effect of variation in *B. grandiflora* flowering, but variation in *Ba. serrata* flowering may also affect reproduction. For example, years of low *Ba. serrata* flowering should result in increased pollination and seed set in *B. grandiflora* in February and March, providing birds do not leave for alternative areas of greater flowering. Experiments that manipulate flower density and hand-pollinate flowers with interspecific pollen (Waser 1983, Feinsinger and Tiebout 1991, Feinsinger et al. 1991) are now needed to more closely examine the competitive interactions between these plant species.

Evolutionary consequences of pollen limitation

When female reproductive success is consistently pollen-limited, such as found for *B. grandiflora*, selection should favour floral traits that increase the probability and amount of pollination. (Haig and Westoby 1988, Rathcke and Real 1993, Vaughton and Ramsey 1994). The findings of the present study suggest that increased pollination in *B. grandiflora* has been achieved by maintaining divergent flowering times with *Ba. serrata*. Competition for pollination has been suggested as a potential selective force promoting and maintaining divergence in flowering times of co-occurring plant species that share pollinators. Providing pollinators are present throughout the flowering seasons, divergent flowering times would reduce competition for pollinators, and increase pollen receipt and seed set (Mosquin 1971, Waser 1978, 1983, Lack 1982, Rathcke 1983). Because both pollinator visits and seed set in *B. grandiflora* were reduced when *Ba. serrata* shrubs flowered, selection should act to maintain flowering time divergence by favouring *B. grandiflora* plants that flower before *Ba. serrata*. Such selection, however, will depend upon whether there are fitness differences between seeds produced at different times within the flowering season and whether flowering time is heritable. Seed fitnesses are similar; no differences in seed germination, seedling growth and seedling survival were found between January and March seeds (M. Ramsey unpubl.). Flowering time in many species is genetically determined and can respond rapidly to directional selection (e.g. McNeilly and Antonovics 1968, Lack 1982, Pors and Werner 1989, Widén 1991). The relative importance of genetic and environmental factors in *B. grandiflora* flowering remain to be examined. Some degree of genetic control of flowering is likely, although environmental

factors also play a role as indicated by the late 1991–92 flowering.

Overall, *B. grandiflora* seed set was consistently greatest in January, and optimizing selection (sensu Travis 1989) should favour plants flowering in this month. In long-lived organisms, however, one season is not equal to one generation, and each reproductive season constitutes an episode of selection. Temporal variation within or among years in *Ba. serrata* flowering or in factors such as predation of reproductive structures may weaken selection for January flowering and partially explain the presence of plants that flower in other months.

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