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Ovule pre-emption and pollen limitation in a self-fertile perennial herb (*Blandfordia grandiflora*, Liliaceae)

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Abstract The extent, frequency and causes of pollen-limited seed production were examined in partially self-fertile populations of *Blandfordia grandiflora* for 2 years. Percentage seed set of open-pollinated plants (50–57%) did not differ within or between years, and was about 19% less than experimentally cross-pollinated plants (70–75%). Floral visits by honeybees did not differ through the flowering season and the number of pollen grains deposited on stigmas within 12 h of flowers opening exceeded the number of ovules per flower, indicating that the quality rather than the quantity of pollination limited seed set. Pollen limitation was caused by concurrent self- and cross-pollination and the subsequent abortion of some selfed ovules due to inbreeding depression. Natural seed set (55%) was intermediate between selfed (43%) and crossed (75%) flowers and was not increased when flowers that had been available to pollinators for 24 h were hand cross-pollinated, suggesting that ovules were already fertilized. Similarly, experimental pollination with both cross and self pollen within 24 h of flowers opening did not increase seed set relative to natural seed set, indicating that both cross- and self-fertilizations had occurred. In contrast, when selfing followed crossing by 48 h, or vice versa, seed set did not differ from crossed-only or selfed-only flowers, respectively, indicating that ovules were pre-empted by the first pollination. Collectively, these results indicate that under natural conditions self pollen pre-empted ovules, rendering them unavailable for cross-fertilization. This selfing reduces fecundity by 50%, as estimated from the natural production of cross seeds when selfing was prevented. Consequently, selection should favour floral traits, such as increased stigma-anther separation or

protandry, that reduce interference between male and female functions that leads to selfing.

Key words Self-pollination · Facilitated selfing · Ovule pre-emption · Pollen limitation · *Blandfordia grandiflora*

Introduction

Pollen limitation occurs when seed production is less than would be achieved if the overall quantity or quality of pollen deposited onto stigmas was increased. Although pollen-limited seed set has been demonstrated in a diverse range of plant species, questions remain concerning the extent, frequency, causes and overall selective importance of pollen limitation (Johnston 1991; Burd 1994; Vaughton and Ramsey 1994; Wilson et al. 1994). Most pollen limitation studies have examined hermaphroditic, obligately outcrossing plants that produce few or no seeds following selfing (e.g. Sutherland 1987; Zimmerman and Pyke 1988; Lawrence 1993; Ramsey 1995). In obligately outcrossing plants, the quantity of pollen deposited onto stigmas often depends on the number of visits to flowers by pollinators and their foraging behaviour, both of which can vary within and between seasons (e.g. Ramsey 1995). Because pollinators often deposit both compatible cross pollen and incompatible self pollen onto stigmas, seed set may be limited by pollen quality even though overall deposition is high. In some plants self-pollination may exacerbate pollen limitation if it pre-empted cross pollen in reaching stigmatic surfaces, stilar transmitting tissues or ovules (Ockendon and Currah 1977; Seavey and Bawa 1986; Galen et al. 1989; Waser and Price 1991; Broyles and Wyatt 1993).

Few studies have examined pollen limitation in self-fertile plants (Sih and Baltus 1987; Jennersten 1988; Johnston 1991; Vaughton 1991; Karoly 1992). Many of these plants require pollinators and, as for

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obligately outcrossing plants, pollen quantity can limit seed set although pollen quality is generally considered to be less important. In partially self-fertile plants, however, pollen quality may limit seed set because a proportion of self-fertilized ovules are aborted, thus rendering them unavailable for cross-fertilization. Following selfing, abortion occurs because unfavourable recessive or partially recessive genes become homozygous (i.e. inbreeding depression; Charlesworth and Charlesworth 1987). Because many important developmental functions occur during seed formation, inbreeding depression may be particularly high at this life stage (Charlesworth and Charlesworth 1987; Levin 1989). The extent of pollen limitation caused by selfing will depend on the magnitude of inbreeding depression, the amount of self-pollination, and the relative timing of self- to cross-pollination.

In the present study, I assess the extent, frequency and causes of pollen-limited seed production in populations of the partially self-fertile, perennial herb *Blandfordia grandiflora* R. Br. (Liliaceae). Specifically, I examine whether: (1) natural seed set, pollen limitation and pollinator visitation vary within and between flowering seasons, (2) the timing of self-pollination relative to cross-pollination affects seed set, and (3) self-pollination reduces female fecundity.

Materials and methods

Study site and species

I examined tableland populations of *B. grandiflora*, within Gibraltar Range National Park, in north-eastern New South Wales, Australia (29° 36' S, 152° 16' E; 1000 m a.s.l.). Tableland populations are restricted to a small area in the vicinity of the Gibraltar Range, and are peripheral and disjunct to the range of the more widespread coastal populations. Populations occur in wet heathland communities on peaty soils of low pH and nutrient status overlying granite (Ramsey et al. 1994). *B. grandiflora* plants flower predominantly after fires. In the first year after a fire about 86% of plants flower (506 ± 23 flowering plants/50 m²). Flowering declines to negligible levels after 4–5 years. I studied two populations 5 km apart for 1 year each in the first year after a fire.

In tableland populations, most flowering occurs from December to March, with peak flowering in January. Flowering plants consist of a corm, with basal leaves and a single terminal, racemose inflorescence with 1–4 pendulous, red hermaphroditic flowers (mean \pm SE = 1.8 ± 0.1 , $n = 133$), although about 95% have 1–2 flowers. Plants bloom for 1–3 weeks, depending on how many flowers they have. Flowers remain attractive for about 8 days, after which they begin to wither. Stigmas become receptive about 24 h after flowers open and remain receptive for a further 7 days. After anther dehiscence, pollen remains viable for at least 8 days (Ramsey 1993). Plants are partially self-fertile, but pollinators are required for pollination (Ramsey et al. 1993). Floral morphology and nectar production suggest bird pollination, but bird visitation is infrequent (Ramsey 1993; Ramsey et al. 1994; M. Ramsey, unpublished data). Introduced honeybees (*Apis mellifera*) are presently the most important pollinators. Pollen-collecting honeybees visit newly opened flowers, removing pollen within 12 h. Nectar-collecting honeybees occasionally visit newly opened flowers, but mostly visit flowers that have been open for 24 h or longer. Except for an undescribed ant species

(*Iridomyrmex* sp.), other insects have rarely been seen visiting flowers (M. Ramsey, unpublished data).

Seasonal variation of seed set

Natural seed set

I assessed variation in natural seed set within and between flowering seasons (1988–1989 and 1989–1990) using open-pollinated plants that flowered during either December, January, February or March. Each month randomly selected plants with one flower were marked and their fruits ($n = 25$) harvested about 6 weeks later. The numbers of seeds and ovules were counted, and percentage seed set determined. Percentage seed set was used because this measure avoided potential bias due to the variable number of ovules per flower (range 109–241). I used a two-way mixed model ANOVA to compare seed set among months (fixed factor to reflect seasonal changes) for the 2 years (random factor; Bennington and Thayne 1994).

Frequency and extent of pollen limitation

I examined pollen limitation within seasons by comparing seed set of cross-pollinated and open-pollinated plants. In January and March of 1989 and 1990, single-flowered plants were randomly assigned to either cross- ($n = 20$) or open- ($n = 25$) pollination treatments. The use of plants with one flower ensured all experiments had whole plant treatments (Zimmerman and Pyke 1988). The peak and the end of the flowering season occurs during January and March, respectively, and are periods when pollen limitation may occur. As flowers opened on cross-pollinated plants, anthers were removed, stigmas were cross-pollinated with a mixture of pollen from 3–5 plants located at least 10 m away and inflorescences were covered with net bags (1 mm² mesh). Anther removal and bagging do not affect seed set (Ramsey et al. 1993). Open-pollinated plants were left untouched. I compared percentage seed set of cross- and open-pollinated plants for January and March of the 2 years using a three-way mixed model ANOVA (unweighted means analysis; Winer et al. 1991); pollination and month were fixed factors and year was a random factor (Bennington and Thayne 1994).

Causes of pollen limitation

Pollinator visitation and pollen deposition

To examine whether the number of pollinator visits to flowers limited seed set, I assessed floral visitation by honeybees during the flowering season of 1989–1990. I observed patches of flowers and recorded the number of visits to flowers over three 1-h periods at 0900 hours, 1200 hours and 1700 hours on 3 consecutive days each month from December to March. The density of flowering plants varied among months, and different numbers of flowers were observed each month (range 38–106). One flower per plant was observed. A visit occurred when a honeybee alighted on a flower. I used two analyses to examine whether floral visitation varied during the flowering season. First, I tested whether the frequency of flowers that were visited was dependent on month using a 4×2 G-test of independence. Second, I tested whether the number of visits per flower varied among months. I considered flowers as replicates and pooled observations for the 3 days, and compared months with a one-way ANOVA. I estimated the number of visits received by a flower over its lifetime by multiplying the mean number of visits per 9 h (the average number of hours available for honeybee foraging) by 8 days, the length of time stigmas are receptive (Ramsey 1993).

I examined whether pollen quantity limited seed set by comparing the number of pollen grains deposited onto stigmas with the number of ovules per flower. I marked 20 flowers on different plants as they opened at about 0700 hours. At 1800 hours, anthers were removed and flowers were bagged. Bagging excluded pollinators but allowed pollen to germinate and adhere to stigmas (Ramsey 1993). The next morning (0700 hours), pistils were stored individually in 70% ethanol. Later, stigmas were excised, placed on a microscope slide in a drop of acetocarmine and glycerol solution, squashed under a coverslip, and the number of pollen grains counted at 40 × magnification. I compared the number of pollen grains per stigma with the number of ovules per flower using an approximate *t*-test for unequal variances (Sokal and Rohlf 1981). Ovule number was determined from fruits of 25 open-pollinated plants.

Effects of self-pollination on seed set

Negative effects of selfing on seed set are termed costs of self-pollination, and occur when opportunities for cross-pollination and seed set are reduced because self pollen pre-empted stigmas, styles or ovules (Galen et al. 1989). This can occur when self and cross pollen are deposited concurrently or when self precedes cross-pollination. Self-pollination in *B. grandiflora* occurs within 12 h of flowers opening, and to determine if seed set can be increased after opportunities for selfing, I supplemented natural pollination with cross pollen in 1990. Flowers on single-flowered plants that had been open to pollinators for 24 h were cross-pollinated and bagged in January ($n = 30$) and March ($n = 20$). I compared seed set with that of flowers that were either open-pollinated or cross-pollinated and bagged upon opening ($n = 25$ and $n = 20$, respectively, in both months) using a two-way model I ANOVA (unweighted means analysis; Winer et al. 1991); pollinations and months were considered fixed factors (Bennington and Thayne 1994).

To examine further whether selfing caused pollen limitation, in March 1990 I compared seed set of flowers that were open-pollinated ($n = 25$), cross-pollinated plus bagged upon opening ($n = 20$) and cross-pollinated plus bagged 24 h after opening ($n = 20$) with that of flowers pollinated with either self pollen only or self and cross pollen. In the selfing-only treatment 20 plants were marked, and when flowers opened they were self-pollinated and bagged. In other treatments, flowers upon opening were either selfed and then crossed or vice versa. The first pollination preceded the second by either 24 h ($n = 30$) or 48 h ($n = 15$). The 24 h treatments are analogous to simultaneous pollinations because stigmas become receptive about 24 h after flowers open and remain receptive for about 7 days, and pollen is viable for at least 8 days (Ramsey 1993). Similar amounts of self and cross pollen were applied to stigmas by transferring only the pollen on the marked tip of a dissecting needle to stigmas. Percentage seed set of all treatments was compared using a one-way ANOVA.

I examined whether natural cross-pollination and seed set can occur following opportunities for selfing during January and February 1990. I randomly assigned 100 plants with one flower to one of three treatments:

- 1) Open: 30 newly opened flowers were marked and then left untouched to assess natural seed set.
- 2) Open plus emasculated plus bagged: 30 newly opened flowers were marked and left untouched until about 12 h later when anthers were removed. Inflorescences were then bagged until fruits were harvested. This seed set assesses the pollination that occurs during the removal of self pollen by pollen-collecting honeybees.
- 3) Emasculated plus bagged plus open: undehiscent anthers were removed from 40 newly opened flowers and inflorescences were bagged. Bags were removed 24 h later and left off, allowing pollinators access to flowers. This seed set estimates the cross seed set that could be produced if selfing was prevented. Emasculated flowers are not visited by pollen-collecting honeybees, but visits by nectar-collecting honeybees are unaffected (M. Ramsey, unpublished data).

Percentage seed set of treatments was compared with a one-way ANOVA.

Data transformation and statistical analyses

Percentages and counts were transformed using arcsine and square-root transformations, respectively. Student-Newman-Keuls a posteriori tests (SNK) were used to compare means following significant ANOVAs. Means (\pm SE) are presented. Sample sizes in treatments and analyses differed because insects ate some flowers.

Results

Seasonal variation in seed set

Natural seed set

Percentage seed set per plant ranged from 50.1 to 57.2% and did not differ significantly between months or years (Fig. 1; month: $F_{3,195} = 1.19$; year: $F_{1,195} = 0.03$, both $P > 0.50$). In preliminary analysis, the month × year interaction was not significant ($P > 0.50$), and it was pooled with the error term to test the significance of the main effects (Winer et al. 1991).

Frequency and extent of pollen limitation

Natural seed set was consistently pollen-limited. Preliminary analyses indicated that all interactions (pollination × month × year, pollination × month, pollination × year, month × year) were not significant (all $P > 0.20$), and they were pooled with the error term to test the significance of the main effects (Winer et al. 1991). The percentage seed set of flowers that were hand cross-pollinated upon opening was significantly greater than open-pollinated flowers in January and March in 1989 and 1990 (Fig. 2; $F_{1,173} = 28.94$, $P < 0.001$). The extent of pollen limitation did not vary for the 2 years.

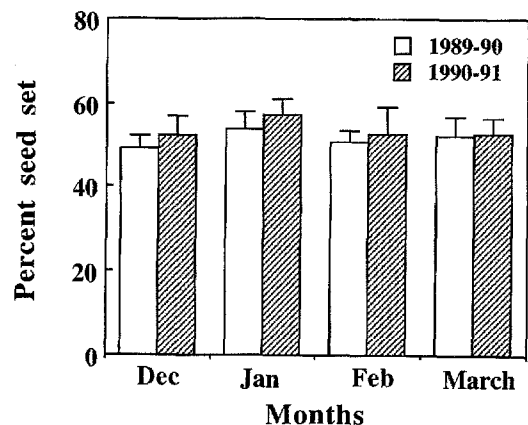


Fig. 1 Percentage seed set of open-pollinated plants (mean \pm SE) for December–March in 1988–1989 and 1989–1990. Seed set did not differ either among months or between years. Each month 25 fruits were examined

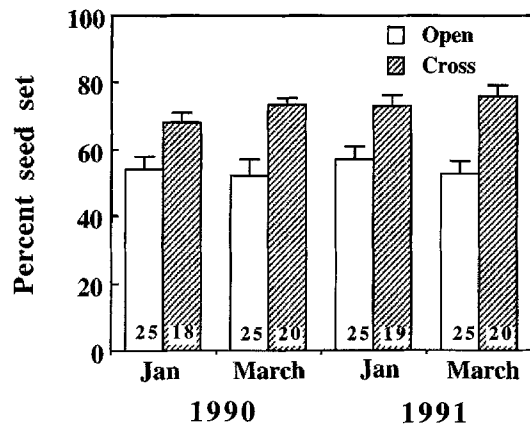


Fig. 2 Percentage seed set of plants that were open- or cross-pollinated as flowers opened in January or March in 1989 or 1990 (mean \pm SE). Seed set did not differ within treatments but was greater for cross-pollinated plants. Sample sizes are given at the bottom of the bars

Neither cross nor open-pollinated seed set differed significantly between months or years (Fig. 2; months: $F_{1,173} = 0.08$, $P > 0.50$; years: $F_{1,173} = 1.67$, $P > 0.20$). Open-pollinated seed set was about 74% that of cross-pollinated flowers.

Causes of pollen limitation

Pollinator visitation and pollen deposition

Floral visitation by honeybees did not differ during the 4 months of the flowering season and between 82–90% of flowers were visited during observations each month ($G_3 = 3.29$, $P > 0.20$). The mean number of visits to flowers ranged from 1.9 ± 0.2 to 2.4 ± 0.3 per 9 h of observation ($F_{3,292} = 1.83$, $P > 0.20$). Estimated lifetime visitation ranged from 15.2 ± 1.6 to 19.2 ± 2.4 visits per flower. The number of pollen grains deposited onto stigmas of flowers that had been open for 12 h significantly exceeded the number of ovules per flower (pollen grains, 198.8 ± 17.4 ; ovules, 152.6 ± 2.8 ; $t' = 2.54$, $P < 0.05$). These findings, together with the lack of increase in seed set in the pollen supplementation experiment (see below), indicates that the quantity of pollen deposited onto stigmas did not limit seed production.

Effects of self-pollination on seed set

Percentage seed set of flowers that were either left open and then cross-pollinated 24 h later (pollen supplementation), cross-pollinated only upon opening, or open-pollinated only, differed significantly (Fig. 3; $F_{2,133} = 17.74$, $P < 0.001$). Seed set of the crossed-only flowers was greater than open plus crossed and open-pollinated flowers, which did not differ, indicating that

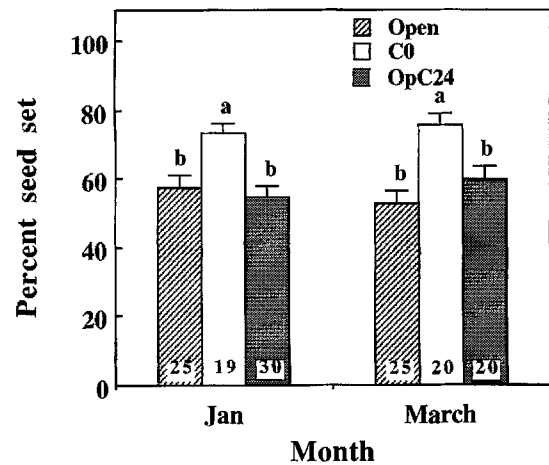


Fig. 3 Percentage seed set of open- and cross-pollinated plants. Plants were cross-pollinated either upon flower opening (C0) or 24 h after opening (OpC24) during January or March. Data are means (\pm SE). Sample sizes are given at the bottom of bars. Bars with different letters differed significantly ($P < 0.05$; SNK tests)

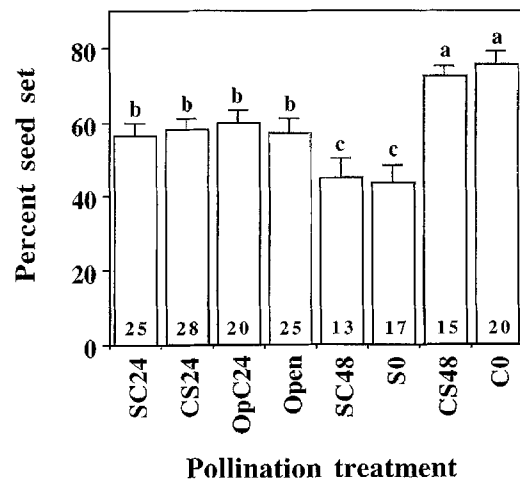


Fig. 4 Percentage seed set of plants in treatments demonstrating ovule pre-emption. Plants that were both self- and cross-pollinated were either selfed and then crossed (SC) or vice versa (CS), with the first pollination preceding the second by either 24 h (SC24, CS24) or 48 h (SC48, CS48). Other treatments were open pollination, selfing or crossing only upon flower opening (S0, C0), and crossing after flowers had been open for 24 h (OpC24). Data are means (\pm SE). Sample sizes are given at the bottom of bars. Bars with different letters differed significantly ($P < 0.05$; SNK tests)

pollen supplementation of flowers previously visited by pollinators did not increase seed set. Seed set within treatments was similar in January and March ($F_{1,133} = 0.04$, $P > 0.50$). The pollination \times month interaction was not significant ($F_{2,133} = 0.10$, $P > 0.50$).

Seed set of flowers in the eight different selfing, crossing and open-pollination treatments varied significantly (Fig. 4; $F_{7,155} = 7.82$, $P < 0.001$). Seed set was greatest in flowers that were either crossed upon opening or crossed and then selfed 48 h later (both about 74%), indicating that all ovules in the latter flowers had been cross-fertilized. Seed set was least in flowers that were

either selfed upon opening or selfed and then crossed 48 h later (both about 44%), indicating that all ovules in the latter flowers had been selfed. Seed set of flowers that were either selfed and then crossed 24 h later, or vice versa, or open plus crossed, or open-pollinated was intermediate (all about 57%). In contrast to flowers that were self- and cross-pollinated within 48 h, seed set of flowers self- and cross-pollinated within 24 h did not differ. This indicates that: (1) the order of pollinations within 24 h did not affect seed set, (2) both self- and cross-fertilizations occurred, and (3) selfing reduced seed set. These findings also suggest that both self- and cross-fertilizations occurred in the open-pollinated flowers and in the open plus crossed flowers before they were cross-pollinated.

Seed set of open-pollinated flowers did not differ from flowers that were either left open for 12 h and then emasculated and bagged or emasculated and bagged for 24 h and then left open (open, 52.4 ± 3.2 ; open plus emasculated plus bagged, 48.3 ± 2.9 ; emasculated plus bagged plus open, 46.0 ± 3.6 ; $F_{2,81} = 0.64$, $P > 0.50$). Similarly to the pollen supplementation results, the lack of differences in seed set between the open and open plus emasculated plus bagged flowers indicates that natural seed set results from pollination that occurs during the period shortly after flowers open when self pollen is removed. Seed set in the emasculated plus bagged plus open flowers indicates that cross-pollination and seed production can occur if selfing is prevented and that selfing causes a substantial loss in fecundity.

Discussion

Seed set in self-fertile populations of *Blandfordia grandiflora* was consistently pollen-limited and was only about 74% that of flowers cross-pollinated upon opening, irrespective of the month or the year of flowering. Limitation of seed set by factors unrelated to pollination was unlikely, since seed set of cross-pollinated flowers did not differ either within or between years. The significance of the present study is two fold. First, this study is only one of a few that has demonstrated that seed production in self-fertile plants is pollen-limited (Sih and Baltus 1987; Jennersten 1988; Johnston 1991; Vaughton 1991; Karoly 1992). Second, the findings clearly indicate that pollen limitation was caused by the quality of pollen deposited onto stigmas rather than the quantity. Pollen quality is not usually tested directly as a factor limiting seed set, although its potential has been suggested (Galen et al. 1989; Vaughton 1991; Waser and Price 1991; Broyles and Wyatt 1993; but see De Jong et al. 1992).

In the present study, pollen limitation was examined by comparing seed set in naturally and hand-pollinated flowers. Increased seed production following hand pollinations may decrease future fitness more than the gain

in current fitness (i.e. costs of reproduction), suggesting that lifetime reproductive success is limited by resources rather than pollen (e.g. Snow and Whigham 1989; Ackerman and Montalvo 1990; Primack and Hall 1990; Calvo 1993). This is unlikely, however, in the present study. Similarly to self-infertile *B. grandiflora* (Ramsey 1995) and several other plant species (e.g. Reekie and Bazzaz 1987; Horvitz and Schemske 1988; Jennersten 1991), costs of reproduction were not detected when plants in high and low reproductive treatments (seed set, 71.9% and 0.6%, respectively) were compared over a 2-year period (M. Ramsey, unpublished data).

Causes of pollen limitation

In *B. grandiflora*, seed production from self-pollination is less than that from cross-pollination. The increase in crossed seed set, however, depends on whether cross-pollination occurs with self-pollination and when crossing occurs relative to selfing. Seed set of flowers that were cross-pollinated 24 h after they opened did not differ from open-pollinated flowers, and both of these treatments did not differ from flowers that were experimentally self- and cross-pollinated within 24 h of flowers opening. The lack of increase in seed set following cross-pollination in these treatments suggests that a proportion of ovules were self-fertilized, and some of these were aborted. This is consistent with previous findings for *B. grandiflora* showing that similar numbers of ovules are fertilized following either self- or cross-pollination and differences in seed set are due to greater seed abortion caused by inbreeding depression following selfing (Ramsey et al. 1993). Overall, these findings indicate that the quality of pollen deposited onto stigmas limited seed set in these populations of *B. grandiflora*. Specifically, pollen limitation was caused by self-fertilization which pre-empted ovules, rendering them unavailable for cross-fertilization. This contrasts with predominantly outcrossing coastal populations of *B. grandiflora* where competition for pollinators limits the quantity of pollen deposited onto stigmas (Ramsey 1995).

In *B. grandiflora*, self-pollination requires pollinators and is mediated by pollen-collecting honeybees (Ramsey et al. 1993). Selfing occurs by autogamy rather than by geitonogamy since honeybees visit only one flower per plant and visit many flowers on different plants during a foraging bout. Honeybees remove pollen from anthers within 12 h of flowers opening, contacting the stigma frequently, and thus depositing self and cross pollen concurrently onto it (M. Ramsey, unpublished data). Because stigmas become receptive about 24 h after flowers open and pollen is viable for several days (Ramsey 1993), self and cross pollen grains would germinate at the same time, resulting in ovules being either self- or cross-fertilized. This selfing causes

pollen limitation, as evidenced by the lack of increase in seed set when natural pollination was supplemented with cross pollen, and when self and cross pollen were applied within 24 h of each other. This mode of self-pollination where self and cross pollen are deposited concurrently onto stigmas by pollinators has been termed facilitated selfing by Lloyd and Schoen (1992).

In self-fertile plants, the potential negative effects of facilitated selfing may be reduced or negated if cross pollen tubes grow faster than and reach ovules before self tubes (e.g. Cruzan and Barrett 1993; Johnston 1993). In *B. grandiflora*, however, the present findings suggest that self and cross pollen tubes grow at similar rates. If cross tubes grew faster, seed set of flowers that were crossed and then selfed 24 h later, or vice versa, would have been greater than observed and similar to flowers that were cross-pollinated only. Equal pollen tube growth rates imply that the number of ovules fertilized by either self or cross pollen is proportional to the number of grains of each type deposited onto stigmas. When the proportions of pollen types are known, this can be tested by comparing observed seed set with expected seed set which is estimated as the product of the number of fertilized ovules and their survivorship. In this study, similar amounts of self and cross pollen was deposited on stigmas of flowers that were selfed and crossed 24 h later, and vice versa. In this study, the mean number of ovules per flower was 150, and 75 ovules were available for selfing and for crossing each. Survivorship of selfed and crossed ovules is the proportion of seeds produced following either selfing only (0.43) or crossing only (0.73). The expected number of selfed and crossed seeds are 32 and 54, respectively, and expected total seed set per fruit is 86 seeds or 57%. This expected seed set is similar to the observed seed set of flowers that were selfed and crossed within 24 h (56%), or vice versa (58%), and open-pollinated (50–57%). This congruency suggests that self and cross pollen tubes grow at equal rates and that under natural conditions both pollen types are deposited onto stigmas in similar proportions. Further work including genetic estimates of selfing rates following mixed pollinations is now needed to corroborate these results (e.g. Cruzan and Barrett 1993; Johnston 1993).

In plants such as *B. grandiflora*, pre-emption of ovules by self-fertilization will be detrimental if selfed ovules could have been cross-fertilized (Lloyd 1992). In the present study, seed set of flowers that were either selfed and crossed within 24 h, or vice versa, or open and then crossed indicate that self-pollination reduces seed set by pre-empting ovules that could have been fertilized by cross pollen deposited during and after self pollen is removed. Also, seed set of flowers that were open plus emasculated plus bagged demonstrates that pollen can adhere to non-receptive stigmas, since these flowers were left open for only 12 h and stigmas do not become receptive until 24 h after flowers open

(Ramsey 1993). Further, the seed set of flowers in the emasculated plus bagged plus open treatment shows that under natural conditions opportunities for cross-pollination occur after self pollen has been removed. The magnitude of the fecundity loss can be determined by estimating the number of seeds produced by natural cross-pollination if selfing was prevented. As estimated above, flowers pollinated concurrently with self and cross pollen produce 86 seeds of which 54 are crossed. The number of seeds that could be potentially produced after opportunities for selfing can be estimated by multiplying percentage seed set of the emasculated plus bagged plus open flowers by the number of ovules per flower (i.e. 0.46×150 ovules = 69 seeds). Consequently, potential cross seed set is 123 seeds (54 + 69) or 82%. This value is 9% greater than the maximum seed set obtained following experimental cross-pollination (109 seeds, 73%), indicating that natural cross-pollination is in excess of that needed for maximum seed set. The cost of selfing, expressed as the reduction in fecundity due to selfing in open-pollinated plants, is about 50% [i.e. (109 seeds of experimentally cross-pollinated plants – 54 crossed seeds of open-pollinated plants)/(109 seeds)]. This value exceeds that reported for *Ipomopsis aggregata* (25.2%) which has a late-acting self-incompatibility system (De Jong et al. 1992). Although several studies have suggested that selfing reduces fecundity (e.g. Galen et al. 1989; Vaughton 1991; Waser and Price 1991; Broyles and Wyatt 1993), few have examined the magnitude of the effect under natural conditions.

Evolutionary consequences

If plants are consistently pollen-limited, selection should favour traits that increase either the quantity or quality or both of the pollen deposited onto stigmas (Haig and Westoby 1988; Johnston 1991; Burd 1994; Vaughton and Ramsey 1994). In self-fertile plants, where facilitated self-pollination occurs and selfing pre-empted ovules, selection should favour traits that reduce selfing. The strength of such selection, however, will depend on the relative amounts of self and cross pollen deposited onto stigmas, and the relative fitness of selfed and crossed seeds. High selfing and low crossing would promote purging of unfavourable recessive or partially recessive genes, providing that the rate of purging exceeds the rate of mutation to detrimental genes (Charlesworth and Charlesworth 1987; Barrett and Charlesworth 1991). Under these conditions, the proportion of selfed ovules that are aborted and the extent that plants are pollen-limited would be reduced, and selection to reduce selfing should be weak. However, if mutation rates are similar to or higher than purging rates, selfed seeds would be less fit than crossed seeds (Barrett and Charlesworth 1991), and selection to reduce selfing may be stronger. If self- and

cross-pollination contribute equally to fertilizations as in *B. grandiflora*, the rate of purging will be reduced and high genetic loads will be maintained over longer periods. Under these conditions, pollen limitation caused by self-fertilization pre-empting ovules will persist and selection should strongly favour traits that reduce either self-pollination or self-fertilization or both.

Selfing can be reduced by preventing interference of male and female functions either spatially or temporally (Lloyd and Webb 1986; Webb and Lloyd 1986). In *B. grandiflora*, flowers are bell-shaped and stigmas are separated from anthers on average by 6.8 ± 0.4 mm with the six anthers located around the style (Ramsey 1993). The simplest morphological change that would reduce self-pollination without affecting cross-pollination would be a reduction in filament length. This would increase stigma-anther separation such that pollen-collecting honeybees would first contact the stigma and then contact the anthers, thereby decreasing self-pollination. Although greater stigma-anther separation could be achieved by increasing carpel length, such a change may not be beneficial since it would increase stigma exertion which may decrease contact with pollinators (e.g. Campbell et al. 1994).

Temporal separation in *B. grandiflora* is achieved by protandry; anthers dehisce shortly after flowers open and stigmas become receptive about 24 h later (Ramsey 1993). Because stigmas are self-pollinated within 12 h of flowers opening but cross-pollinations occur over the lifetime of the flower, increased protandry would allow more cross pollen to be deposited onto stigmas before they become receptive, thereby reducing the proportional contribution of self pollen to the stigmatic pollen load and self-fertilization. Increased temporal separation may be particularly advantageous because self-fertilization is delayed but not prevented and seed set is ensured in the absence of cross-pollination. Similar spatial and temporal traits reduce selfing in other plants and respond to directional selection (e.g. Schoen 1982; Epperson and Clegg 1987; Holtsford and Ellstrand 1992).

Differences between self and cross pollen in the ability to fertilize ovules can also reduce self-fertilization, and can result from differences between self and cross pollen in the timing of germination, in pollen tube growth rate or in pollen tube attrition (Johnston 1993). With concurrent self- and cross-pollinations, self pollen fertilizes fewer ovules leaving more to be cross-fertilized. Similarly to protandry, these mechanisms provide reproductive assurance in the absence of cross-pollination (e.g. Cruzan and Barrett 1993). Differences in fertilization ability require some mechanism of self-recognition, and are likely to be due to weak or cryptic self-incompatibility (Walsh and Charlesworth 1992). As such, they may be difficult to evolve de novo. In *B. grandiflora*, since fertilization ability of self and cross pollen appears to be similar, selection for other

pre-existing floral traits that reduce selfing may be more likely than the evolution of a self-recognition system.

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