

Pollinator-mediated selfing erodes the flexibility of the best-of-both-worlds mating strategy in *Bulbine vagans*

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Summary

1. The best-of-both-worlds hypothesis proposes that delayed autonomous selfing assures reproduction when pollinators are absent, but allows outcrossing when pollinators are present without incurring seed and pollen discounting. However, because self-compatibility is necessary for delayed selfing, discounting can be incurred if pollinators mediate selfing concurrently with outcrossing. The extent that pollinator-mediated selfing erodes the best-of-both-worlds mating strategy is largely unknown.

2. In this study, we assess how delayed selfing and pollinator-mediated selfing contribute to reproductive output under contrasting pollination environments in *Bulbine vagans* (Asphodelaceae).

3. We manipulated floral phenotype under contrasting weather conditions, plant densities and floral display. We emasculated flowers at early-flowering (allowing only outcrossing) or late-flowering (eliminating delayed selfing) stages and compared reproductive output with intact flowers (outcrossing + pollinator-mediated selfing + delayed selfing).

4. During inclement weather, few or no pollinators visited flowers. Reproductive output was severely limited by cross pollen, and delayed selfing provided reproductive assurance. During fine weather, cross pollen was also limiting, but pollinator-mediated selfing boosted reproductive output before delayed selfing could occur.

5. On isolated plants, flowers received fewer pollinator visits and reproductive output was limited more by cross pollen compared with grouped plants. Despite these differences, reproductive output of both isolated and grouped plants was boosted to similar levels by pollinator-mediated selfing before delayed selfing could occur.

6. One- and three-flowered plants were similarly limited by cross pollen. On three-flowered plants, facilitated and geitonogamous selfing boosted reproductive output before delayed selfing could occur. By contrast, on one-flowered plants geitonogamy was eliminated, and pollinator-mediated selfing was reduced. This provided mating flexibility by retaining opportunities for subsequent outcrossing, although delayed selfing boosted reproductive output.

7. Reproductive output was consistently limited by cross-pollination. Yet, delayed selfing provided reproductive assurance only during inclement weather and on one-flowered plants. Under other conditions, pollinators mediated selfing that could render ovules unavailable for outcrossing as selfing occurred and for outcrossing that might occur subsequently. We conclude that best-of-both-worlds flexibility will often be eroded by pollinator-mediated selfing that inevitability occurs with outcrossing.

Key-words: delayed autonomous selfing, facilitated selfing, floral emasculation, geitonogamy, mixed mating, pollen-limitation, self-pollination, reproductive assurance

Introduction

Selection for self-fertilization in animal-pollinated hermaphroditic plants is a compromise between the costs incurred by

the reduced value of selfed progeny (i.e. inbreeding depression) and the benefits achieved by either the gene transmission bias or reproductive assurance (Darwin 1876; Fisher 1941; Jain 1976). These benefits depend on the pollination environment, but in different ways. The gene transmission bias requires that pollinators transfer pollen, enabling plants

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with selfing alleles to fertilize ovules on other plants as well as their own ovules. By contrast, reproductive assurance requires that pollinators fail to transfer cross pollen in sufficient quantities and that selfing boosts reproductive output. Theoretical and empirical studies have frequently invoked reproductive assurance to explain the evolution of self-fertilization (Baker 1955; Lloyd 1992; Schoen, Morgan & Bataillon 1996; Kalisz, Volger & Hanley 2004; Moeller 2006), but there are few definitive experimental tests of this hypothesis (reviewed by Eckert, Samis & Dart 2006).

To convincingly demonstrate the benefits of reproductive assurance, two requirements should be satisfied. First, selfing must increase reproductive output per flower when cross pollen is limited, and fertility benefits should correlate with the extent of pollinator failure in populations (Kalisz, Volger & Hanley 2004; Moeller & Geber 2005; Kennedy & Elle 2008). Second, when inbreeding depression renders selfed progeny inferior to cross progeny, selfing must not usurp pollen and ovules, preventing them from engaging in outcrossing (i.e. discounting; Herlihy & Eckert 2002; Eckert & Herlihy 2004). Of relevance to both these issues is the mode of self-pollination because how and when selfing occurs determines the extent to which discounting is incurred and whether reproductive assurance is provided (Lloyd 1992; Schoen, Morgan & Bataillon 1996).

Pollinators can mediate self-pollination by foraging within flowers (facilitated selfing) or between flowers on the same plant (geitonogamy). These selfing modes rely on pollinators and do not provide reproductive assurance when it is most needed, when pollinators are absent. Moreover, because facilitated and geitonogamous selfing occur at the same time as outcrossing, discounting costs can be incurred, particularly following geitonogamy. Accordingly, these selfing modes are regarded as non-adaptive consequences of attracting animals that cross-pollinate flowers (Lloyd 1992; de Jong, Waser & Klinkhamer 1993; Harder & Barrett 1995; Eckert 2000). By contrast, autonomous selfing occurs without the aid of pollinators and provides reproductive assurance when pollinators are absent. If pollinators visit flowers, however, autonomous selfing can cause discounting, depending on when self-pollination occurs relative to cross-pollination. Only delayed autonomous selfing (hereafter, delayed selfing), which occurs after opportunities for outcrossing at the end of floral life, incurs no pollen or seed discounting and is almost always beneficial (Lloyd 1992; Schoen & Lloyd 1992; but see Harder & Routley 2006). Indeed, delayed selfing should allow a flexible best-of-both-worlds response to pollinator unpredictability by promoting outcrossing when pollinators are present, but also providing reproductive assurance when outcrossing fails (Becerra & Lloyd 1992; Kalisz & Vogler 2003). Despite the intuitive appeal of this hypothesis, the flexibility of the best-of-both-worlds response relies on pollinators mediating outcrossing only. If they also mediate selfing that discounts opportunities for outcrossing, then the flexibility of the best-of-both-worlds mating strategy will be eroded. Thus, the condition necessary for delayed selfing, self-compatibility, may incur costs when pollinators are available.

The perennial herb *Bulbine vagans* is ideal to examine this issue. Inbreeding depression is sufficiently high to indicate that reproductive assurance rather than gene transmission bias is a factor in the evolution of selfing (Vaughton, Ramsey & Simpson 2008). The pollination environment is variable, which can render pollinator service and cross pollen receipt unreliable (Kalisz & Vogler 2003). Indeed, plants often occur at low densities and flower during summer when weather is frequently inclement. We demonstrated previously that flowers autonomously self-pollinate as they close and that this selfing provided reproductive assurance when pollinators were excluded from flowers. Further, emasculated flowers that were prevented from selfing produced fewer seeds than did intact flowers, but cross-pollinated and intact flowers produced similar numbers of seeds. Collectively, these findings indicate that cross pollen was limited and that selfing boosted reproductive output to maximal levels. However, we did not determine the mode of selfing, but because pollinators visited flowers frequently, we speculated that pollinator-mediated selfing provided reproductive assurance (Vaughton, Ramsey & Simpson 2008).

As our approach above, most studies that assess reproductive assurance emasculate flowers as they open by removing undehisced anthers and compare reproductive output with intact flowers. This technique has been successful, identifying species in which selfing provides reproductive assurance (reviewed by Schoen & Lloyd 1992; Eckert, Samis & Dart 2006). Here, we extend this technique and emasculate flowers either as they open or as they close. Comparison with intact flowers allows contributions to reproductive output by delayed selfing and pollinator-mediated selfing to be estimated. Further, contributions of facilitated and geitonogamous selfing can be differentiated by manipulating floral display. *Bulbine vagans* is suited for emasculation experiments because removal of anthers does not affect pollinator visits, providing that staminal hairs are not damaged (Vaughton, Ramsey & Simpson 2008).

Here we use floral emasculations to assess the extent to which different modes of selfing occur in *B. vagans* under contrasting pollination environments. Specifically, we assess reproductive output under inclement vs. fine weather, low vs. high plant densities, and small vs. large floral displays. We find that cross pollen was consistently limited, but that delayed selfing provided reproductive assurance only when pollinators were scarce. Under other conditions, pollinator-mediated selfing co-occurred with outcrossing, eroding the flexibility of the best-of-both-worlds mating strategy.

Materials and methods

STUDY SPECIES

Bulbine vagans (Asphodelaceae; Fig. 1) is restricted to rocky escarpments within dry sclerophyll forest habitats in northern New South Wales and southern Queensland, Australia (Godden 1993). We studied the LP population from Vaughton, Ramsey & Simpson (2008) located near Armidale, NSW (30°39'3" S, 151°57'1" E, 944 m a.s.l.).



Fig. 1. A recently opened flower of *Bulbine vagans* showing the two levels of stamens with clavate hairs surrounding the anthers and the curved style. Flower diameter ranges between 15–25 mm.

This population contained several hundred plants arranged along cliff tops and in rock crevices. Because plants are often isolated on rocky outcrops and difficult to access, we used potted plants from this population. We excavated individuals growing at least 5 m apart to ensure they were unrelated and placed them in 1.5 L pots containing sand, loam and peat (1 : 1 : 1). After 10 months in a glasshouse, we then used these plants for this study.

The use of potted plants provided two advantages. First, potted plants produced more scapes and thus opened more flowers per day than did naturally occurring plants. This allowed us to manipulate flower number, while ensuring that plants were representative of those growing under natural conditions; most plants open either one (56%), two (30%) or three (13%) flowers per day ($n = 98$ plants). Second, natural populations often contain groups of 2–20 plants and many isolated plants. Within groups, plants can be separated by 1–10 m. Thus, using potted plants allowed us to manipulate plant densities within this range.

Under natural conditions, plants produce one or two scapes, each with about 30 flowers that open acropetally over several weeks. Flowers are open for about 8 h, are 15–25 mm in diameter and have three short and three long stamens (Fig. 1). Stamens initially cluster together on one side of the flower, and the style curves away in the opposite direction. As flowers close, filaments wilt and styles straighten, bringing anthers of the short stamens and stigmas into contact. This causes delayed selfing, but seed set is less than that following manual self- or cross-pollination. Inbreeding depression, estimated in the glasshouse, is moderately high (0.45). Flowers produce no nectar and clavate hairs surrounding the anthers attract insect pollinators. Several species of native bees, bee flies and syrphid flies visit flowers. However, a single syrphid fly species is the most common floral visitor. These flies move between flowers, but spend long periods on individual flowers (Vaughton, Ramsey & Simpson 2008).

INCLEMENT WEATHER

For 2 years, we evaluated the extent that selfing provided reproductive assurance under different weather conditions by examining

reproductive output of emasculated (EE, outcrossing only) and intact (IN) plants. In each year, we assigned different plants to either EE or IN treatments ($n = 10$ plants/treatment). We arranged pots 2 m apart in a grid about 5 m upslope from where *B. vagans* occurred naturally, ensuring that each EE plant had two or three IN neighbours. We assessed each plant under fine and inclement weather conditions (7–8 fine days, maximum temperatures of 21–26 °C; 4–5 inclement days, 9–15 °C). We removed flowers from potted plants to ensure that each plant had one or two open flowers per day, mimicking flower number under natural conditions. On each day, we emasculated EE flowers before anthers dehisced and left IN flowers untouched. On each plant, we tied red or yellow thread around pedicels to mark flowers that opened on fine or inclement days.

After 4 weeks, we scored fruit set as: fruits/flowers (fine, EE and IN: 8–11 flowers/plant; inclement, EE and IN, 4–7 flowers/plant). In the second year, we also scored seed set as: seeds/(seeds + ovules) of 3–5 fruits/plant/treatment; seed set was assessed only for flowers that produced seeds. We integrated fruit and seed set for each plant as an index of reproductive output as: (fruit set/plant) \times (mean seed set/plant). Following Eckert, Samis & Dart (2006), we use mean reproductive output to calculate an index of reproductive assurance (RA) as: $1 - (EE/IN)$. Values vary between 0–1. Lower reproductive output of EE flowers relative to that of IN flowers provides higher RA estimates, indicating that selfing contributes more to reproductive output because cross pollen limitation is greater.

Plants were initially considered as blocks because they experienced both weather conditions. However, herbivory by kangaroos reduced sample sizes of plants and flowers. Consequently, we pooled flowers over plants, ensuring sufficiently high binomial sample sizes for each weather \times emasculating combination (> 30 flowers). We examined fruit set using analysis of deviance with year, emasculating and weather as factors. Year \times emasculating and year \times weather interactions were not significant, and we omitted them from analyses (both $P > 0.52$). EE flowers did not produce fruits on inclement days in the second year, and we compared seed set of the inclement-IN, fine-IN and fine-EE treatments with a one-way ANOVA, using plant means.

In the second year, we monitored pollinator visits to IN flowers on three plants in each of two plots over four inclement days and four fine days, observing different plants each day (total $n = 48$ plants). Each plant had one open flower. We observed each plant separately for four, 15-min periods from 08:00–14:00 h on each day. No flowers were visited on inclement days, and we did not analyse the data statistically. For fine days, we calculated the mean number of visits per flower per h by pooling over all plants and days ($n = 24$ plants).

PLANT ISOLATION

We randomly allocated 54 plants to isolated or grouped treatments and to one of three emasculating treatments: EE, flowers emasculated early in the day before anthers dehisced; EL, emasculating late in the day just before flowers closed; and IN, flowers left intact ($n = 9$ plants for each of the six isolation \times emasculating combinations). We ensured that all plants had two open flowers on each day. We conducted the study on fine days only ($n = 14$) because on inclement days pollinators are inactive and only IN plants can produce seeds. We used nine blocks, each with one plant for each isolation \times emasculating combination ($n = 6$ plants/block). Blocks were ≥ 15 m apart. Within blocks, the isolated and grouped treatments were separated by 11 m. Isolated and grouped plants were placed in separate triangular arrays so that interplant distances were 10 m and 1 m, respectively. We moved plants within isolation treatments each day to

avoid position effects, caged plants at night to prevent herbivory, and within blocks removed flowers on naturally occurring plants.

We scored fruit set of plants, assessed seed set of five fruits/plant and used mean seed set/plant in analyses. We calculated reproductive output and RA for grouped and isolated plants. We used analysis of deviance to examine the effects of block, isolation, emasculation and the isolation \times emasculation interaction on fruit set. For seed set, we used ANOVA with isolation and emasculation as fixed factors, and block as a random factor.

Intact plants produce seeds by outcrossing, pollinator-mediated selfing and delayed selfing; EL plants produce seeds by outcrossing and pollinator-mediated selfing; and EE plants produce outcrossed seeds only. Thus, greater reproductive output of IN compared with EL plants reflects delayed selfing, and greater reproductive output EL compared with EE plants reflects pollinator-mediated selfing.

We counted pollinator visits to isolated and grouped plants on five fine days. Each day, we observed two flowers on one isolated plant and one of three grouped plants in each of two blocks 20 m apart. We counted visits to flowers during four, 15-min periods between 08:00–14:00 h. We used the mean number of visits per flower per h for each plant to calculate a mean for each treatment ($n = 10$ plants). We compared the number of visits per h between treatments with an ANOVA, with block and day as random factors and isolation as a fixed factor.

FLORAL DISPLAY

We randomly allocated 54 plants to two floral display treatments (1 or 3 flowers per plant per day) and three emasculation treatments (EE, EL, IN), resulting in nine plants for each floral display \times emasculation combination. We had too few plants to include density as a factor and used isolated plants only. We set up nine blocks, each with one plant for each combination ($n = 6$ plants/block). Within blocks, we positioned plants 10 m apart in a hexagonal array, so that each plant had two equidistant neighbours. However, because EE plants do not contribute pollen, we ensured that they were not placed adjacently to reduce potential cross-pollen limitation. Blocks were ≥ 15 m apart. For reasons outlined above, we conducted the study on fine days ($n = 14$), moved plants each day and caged plants at night.

We scored fruit set, assessed seed set of five fruits/plant, used mean seed set/plant in analyses, and calculated reproductive output and RA. For fruit set, we used analysis of deviance to examine the effects of block, display, emasculation and the display \times emasculation interaction. For seed set, we used an ANOVA with display and emasculation as fixed factors, and block as a random factor. Interpretation is similar to that provided above, except that on three-flowered EL and IN plants geitonogamous and facilitated pollinator-mediated selfing can occur, but only the latter can occur on one-flowered plants.

To assess the likelihood of geitonogamy, we counted the number of flowers visited by pollinators as they foraged on three-flowered plants. Plants were observed opportunistically over 6 days ($n = 86$ pollinator visits).

STATISTICAL ANALYSES

We computed analyses of deviance using generalized linear models (GLMStat 6.0, Beath 2004). Analysis of deviance uses appropriate error distributions to describe the data and likelihood ratio tests to compare nested models. Reductions in deviance have an approximate chi-square distribution with the degrees of freedom equal to the difference in the number of parameters in each model (Crawley 1993). We

assumed fruit set was binomially distributed and used logit link functions. We used scaled deviances when required to remove overdispersion (Crawley 1993). We computed ANOVAS using JMP 5.0.1 (SAS Institute Inc., Cary, NC, USA). When required, we transformed seed set (arcsin square-root) and pollinator visits (square-root) to ensure ANOVA assumptions were met. Means (\pm SE) are given.

Results

INCLEMENT WEATHER

Fewer EE flowers produced fruits than did IN flowers, and fewer fruits were produced on inclement than on fine days (Fig. 2; emasculation, $\chi^2 = 40.45$, d.f. = 1, $P < 0.001$; weather, $\chi^2 = 31.70$, d.f. = 1, $P < 0.001$). Fruit set did not differ between years ($\chi^2 = 1.93$, d.f. = 1, $P = 0.165$). The emasculation \times weather interaction was not significant ($\chi^2 = 0.25$, d.f. = 1, $P = 0.617$). For seed set, IN flowers produced 21% more seeds than did EE flowers on fine days and IN flowers on inclement days (fine: IN, $78.4\% \pm 2.7$; EE, $57.8\% \pm 3.9$; inclement: IN, $56.9\% \pm 2.3$; $F_{2,38} = 19.37$, $P < 0.001$). Reproductive output for EE and IN plants was 0.31 ± 0.05 and 0.68 ± 0.06 , respectively on fine days, and 0.0 and 0.28 ± 0.04 , respectively on inclement days. RA was 0.54 on fine days and 1.0 on inclement days, indicating that reproductive output was severely limited by cross pollen on inclement days. We observed 7.2 ± 0.7 pollinator visits per flower per h on fine days, but zero visits on inclement days ($n = 24$ plants).

PLANT ISOLATION

Block, isolation, emasculation and the isolation \times emasculation interaction significantly affected fruit set (Table 1). Fruit set of grouped and isolated IN and EL plants was similar ($\approx 80\%$), and all were greater than that of EE plants. Grouped

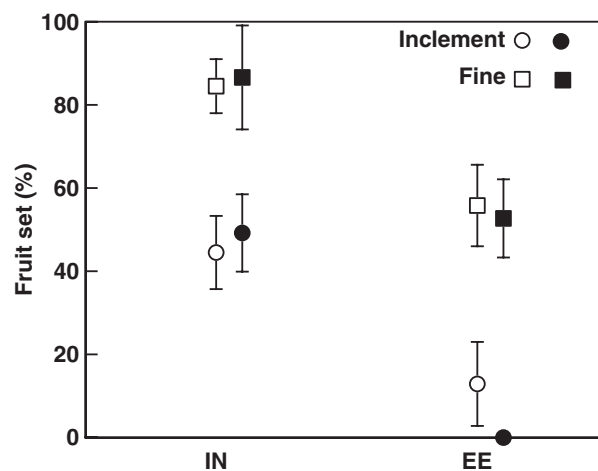


Fig. 2. Mean (\pm SE) percent fruit set of intact (IN, outcrossing, pollinator-mediated and delayed selfing) and emasculated (EE, outcrossing only) flowers under fine and inclement weather conditions in *Bulbine vagans*. Open and filled symbols represent year one and two, respectively.

Table 1. Results of an analysis of deviance (χ^2) and an ANOVA (F) examining the effects of block, isolation, emasculation and the isolation \times emasculation interaction on fruit and seed set in *Bulbine vagans*. For the ANOVA, numerator and denominator degrees of freedom are given. Data are given in Fig 3

Source	Fruits			Seeds		
	d.f.	χ^2	P	d.f.	F	P
Block	8	25.06	0.002	8,40	2.66	0.019
Isolation	1	4.98	0.026	1,40	0.24	0.630
Emasculation	2	67.87	<0.001	2,40	15.90	<0.001
I \times E	2	8.17	0.017	2,40	0.63	0.539

EE plants, however, produced 26% more fruits than did isolated EE plants (Fig. 3a). Block and emasculation significantly affected seed set, but isolation and the isolation \times emasculation interaction did not (Table 1). Seed set of IN and EL plants was similar ($\approx 74\%$) and about 19% greater than that of EE plants (Fig. 3b). Reproductive output of all EL and IN plants was similar (range, 0.58 ± 0.04 – 0.61 ± 0.03), but that of grouped and isolated EE plants was 0.36 ± 0.04 and 0.19 ± 0.03 , respectively. RA of isolated

plants was greater than that of grouped plants, indicating that the former were more cross pollen limited (0.68 vs. 0.39).

Flowers on isolated plants received significantly fewer pollinator visits than did flowers on grouped plants ($F_{1,4} = 29.74$, $P = 0.005$). Averaged over days, flowers on isolated plants received 2.6 ± 0.6 visits per h compared with 9.0 ± 1.1 visits per h on grouped plants ($n = 10$ plants). Block, day and the day \times isolation interaction did not affect visitation (block: $F_{1,9} = 0.90$, $P = 0.368$; day: $F_{4,4} = 1.87$, $P = 0.279$; day \times isolation: $F_{4,9} = 1.16$, $P = 0.391$).

FLORAL DISPLAY

Floral display and emasculation significantly affected fruit set, but their interaction and block did not (Table 2). Fruit set of three-flowered IN and EL plants and one-flowered IN plants was similar ($\approx 77\%$) and about 20% greater than that of one-flowered EL plants. Fruit set of one- and three-flowered EE plants was similar and about 23% less than that of one-flowered EL plants and about 43% less than that of IN plants and three-flowered EL plants (Fig. 4a).

Emasculation significantly affected seed set, but floral display, the floral display \times emasculation interaction and block did not (Table 2). Seed set of IN and EL plants was similar ($\approx 75\%$) and about 23% greater than that of EE plants (Fig. 4b). Reproductive output of IN plants was similar (3-flowered, 0.61 ± 0.03 ; 1-flowered, 0.58 ± 0.03), as was that of EE plants (0.20 ± 0.03 ; 0.16 ± 0.03). Reproductive output of three-flowered EL plants was greater than that of one-flowered EL plants (0.58 ± 0.04 vs. 0.42 ± 0.04). RA was similar for three- and one-flowered plants (0.67 , 0.72). Within plant foraging by pollinators on three-flowered plants was high; 88.4% of pollinators ($n = 86$) visited two or three flowers.

Discussion

We found that reproductive output was consistently limited by cross pollen and that reproductive output was boosted by several modes of selfing. Selfing, therefore, should play a key role in the mating system of *B. vagans*. Consistent with the best-of-both-worlds hypothesis (Becerra & Lloyd 1992; Kalisz & Vogler 2003), delayed autonomous selfing provided

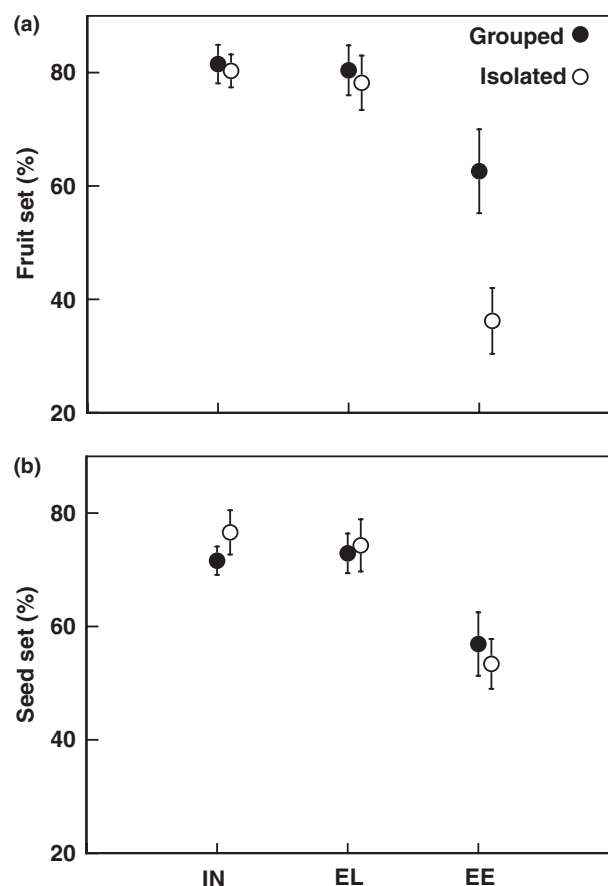


Fig. 3. Mean (\pm SE) percent fruit set (a) and seed set (b) of flowers that were intact (IN, outcrossing, pollinator-mediated and delayed selfing), emasculated late (EL, outcrossing and pollinator-mediated selfing) or early (EE, outcrossing only) in the day on grouped and isolated plants in *Bulbine vagans*.

Table 2. Results of an analysis of deviance (χ^2) and an ANOVA (F) examining the effects of block, floral display, emasculation and the floral display \times emasculation interaction on fruit and seed set in *Bulbine vagans*. For the ANOVA, numerator and denominator degrees of freedom are given. Data are given in Fig 4

Source	Fruits			Seeds		
	d.f.	χ^2	P	d.f.	F	P
Block	8	13.85	0.086	8,40	1.38	0.237
Floral display	1	7.54	0.006	1,40	0.02	0.889
Emasculation	2	105.40	<0.001	2,40	11.84	<0.001
Fd \times E	2	3.24	0.198	2,40	0.091	0.913

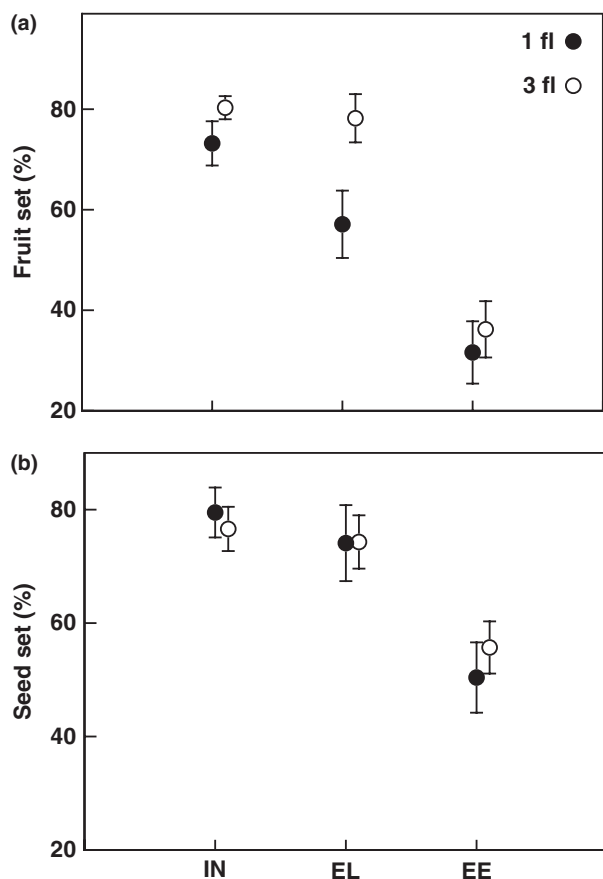


Fig. 4. Mean (\pm SE) percent fruit set (a) and seed set (b) of flowers that were intact (IN, outcrossing, pollinator-mediated and delayed selfing), emasculated late (EL, outcrossing and pollinator-mediated selfing) or early (EE, outcrossing only) in the day on one-flowered plants (geitonogamy eliminated) and three-flowered plants (geitonogamy permitted) in *Bulbine vagans*.

reproductive assurance when flowers received few or no pollinator visits. Under other conditions, flowers were selfed by pollinators before delayed selfing could occur. This is likely to have discounted seeds, a costly by-product of self-compatibility that renders ovules unavailable for cross-fertilization. Below we consider the context-dependent nature of different selfing modes in *B. vagans*, and the extent to which pollinator-mediated selfing erodes the flexibility of the best-of-both-worlds mating strategy.

Throughout this article we assume that reproductive output (fruits \times seeds) of an individual treatment is the additive combination of the reproductive outputs of the different emasculation treatments. Thus, reproductive output of IN plants consists of contributions by outcrossing, estimated from EE plants, pollinator-mediated selfing, estimated as the difference between EL and EE plants, and delayed selfing, estimated as the difference between IN and EL plants. Our approach estimates maximal outcrossing, which may be an overestimate if pollinator-mediated selfing discounts seeds (e.g. Herlihy & Eckert 2002). Biparental inbreeding will not affect our conclusions because we assess reproductive output rather than genetically determined selfing rates. Genetic estimates of selfing

would be required to verify our assumptions. Despite this potential bias, our estimates offer insight into how different modes of selfing can contribute to reproductive output under different pollination conditions. Ultimately, however, the reproductive assurance benefit of selfing will depend upon whether selfed progeny survive to reproduce. Herlihy & Eckert (2002) addressed this question for autonomously selfing *Aquilegia canadensis* and found that selfed progeny did not survive. Such an analysis would be insightful for *B. vagans*, as well as for other autonomously selfing species.

INCLEMENT WEATHER AND POLLINATORS

Inclement weather can render pollinator service unreliable and has been proposed as a selective factor favouring autonomous self-pollination (e.g. Motten 1982; Schoen & Brown 1991; Ruan *et al.* 2009). Our results support this proposal. During inclement weather when pollinators were inactive, selfing provided all of the reproductive output. Averaged over both years, selfing boosted fruit set of intact (IN) flowers 8-fold compared with emasculated (EE) flowers on inclement days, and in the second year, boosted reproductive output to 0.28 from 0.0. We did not determine the mode of selfing on inclement days, but because flowers received few or no visits by pollinators, delayed selfing would have predominated. Interestingly, reproductive output of IN plants on inclement days was 30% less than in our previous study when we excluded pollinators from plants on fine days (Vaughton, Ramsey & Simpson 2008). This difference can probably be attributed to inclement conditions that reduce pollen function or otherwise lessen the effectiveness of delayed selfing (Corbet 1990). Although delayed selfing provided reproductive assurance, its reduced effectiveness during inclement weather may indicate that other factors that reduce pollinator availability have also played a role in the selection of this selfing mode in *B. vagans*.

Contrasting with inclement days, on fine days reproductive output was most likely boosted by pollinator-mediated selfing rather than by delayed selfing. Pollinators readily visited flowers (>7 visits per flower per h), and RA on fine days was reduced to almost half that on inclement days (RA, 0.54 vs. 1.0), indicating that cross pollen was less limiting than on inclement days. But, cross pollen was still limited, as indicated by the lower reproductive output of EE compared with IN plants. The major floral visitor, a syrphid fly species, comprises almost 90% of visits to flowers. These flies move between flowers on the same and different plants, carry pollen on their bodies, but spend long periods at individual flowers. This foraging behaviour probably results in both self and cross pollen being deposited onto stigmas. As we further discuss below, self pollen that is deposited by pollinators probably competes with cross pollen to fertilize ovules. Although this pollinator-mediated selfing boosted reproductive output, the magnitude of reproductive assurance that was provided will depend upon the extent that seed discounting directly reduced outcrossing and assuming that inbreeding depression is less than 1.0 (Vaughton, Ramsey & Simpson 2008).

PLANT ISOLATION AND POLLINATOR-MEDIATED SELFING

Isolation exacerbated cross pollen limitation of outcrossed EE plants, and reproductive output of isolated plants was only 53% that of grouped plants. The difference between isolated and grouped plants can be readily explained by the lower pollinator visitation to flowers on isolated plants (2.6 vs. 9.0 visits per flower per h), which is likely to have resulted in less cross pollen being deposited onto stigmas. In addition, visitors to isolated plants may have been carrying less *B. vagans* pollen. For example, in *Dianella revoluta* pollinator visits to isolated plants was similar to that of grouped plants, but visitors to the former carried less conspecific and more heterospecific pollen, decreasing their capacity to cross-pollinate flowers (Duncan *et al.* 2004). Negative effects of plant isolation on pollination and reproduction have been widely reported (e.g. Kunin 1993; Knight 2003; Scobie & Wilcock 2009), and isolation has been proposed as a factor in the evolution of autonomous selfing (Moeller & Geber 2005).

Our study demonstrates that pollinator-mediated selfing contributed to reproductive output of isolated and grouped EL and IN plants, although the contribution was substantially greater on isolated plants. Relative to EE plants, reproductive output of isolated EL and IN plants increased 3.0-fold, but only 1.6-fold for the grouped plants. Because all plants had two open flowers per day, pollinators could have mediated both facilitated and geitonogamous selfing. Reproductive output of isolated and grouped EL plants was similar to that of isolated and grouped IN plants, indicating that pollinator-mediated selfing, and not delayed selfing, boosted reproductive output of IN plants. Despite equal reproductive output, the fitness of isolated plants could have been less than that of grouped plants. This is because they produce fewer crossed seeds and more selfed seeds and inbreeding depression likely exceeds 0.5, the value at which the gene transmission advantage of selfed seeds relative to crossed seeds is negated (Cheptou & Schoen 2007; Vaughton, Ramsey & Simpson 2008). However, because seed discounting can directly reduce crossed seed set, the greater fitness of grouped plants is contingent upon seed discounting being the same or less than that of isolated plants.

FLORAL DISPLAY: POLLINATOR-MEDIATED AND DELAYED SELFING

Floral display had no effect on EE plants, and reproductive output was only 30–43% that of EL and IN plants, indicating severe cross pollen limitation. By contrast, although reproductive output of one- and three-flowered IN plants was similar, that of EL plants differed, indicating that floral display affected how the different modes of selfing contributed to reproductive output. On three-flowered EL and IN plants, geitonogamy and facilitated selfing increased reproductive output to about 0.60. By contrast, reproductive output of one-flowered EL plants was increased, but only to 0.42, a consequence of geitonogamy being eliminated and low levels of

facilitated self-pollination, which left many ovules unfertilized. As demonstrated for one-flowered IN plants, the availability of unfertilized ovules allowed delayed selfing to further increase reproductive output to that of three-flowered plants. Clearly, this delayed selfing on one-flowered plants provided reproductive assurance. Further, these plants retained the potential to increase outcrossing, if the opportunity had arisen, whereas no such increase was possible in three-flowered plants, owing to geitonogamy. Our findings for one-flowered plants are consistent with the best-of-both-worlds hypothesis, while recognizing that facilitated selfing eroded some of the flexibility.

Our comparison of one-flowered and three-flowered EL plants indicates that geitonogamy accounts for much of the pollinator-mediated selfing on plants with more than one open flower. This geitonogamy is not surprising considering that we observed 88% of pollinators visiting more than one flower on three-flowered plants. The contribution of geitonogamy to overall levels of selfing has been highlighted in other species and is considered disadvantageous, a by-product of self-compatibility and adaptations for outcrossing (Lloyd 1992). Although geitonogamy is often associated with mass flowering displays (de Jong, Waser & Klinkhamer 1993; Eckert 2000), this is not always the case. Schoen & Lloyd (1992) reported that geitonogamy accounted for most of the selfing in the annual *Impatiens pallida* that has small numbers of chasmogamous flowers open concurrently. Similarly, Leclerc-Potvin & Ritland (1994) found that one-third of all selfing was attributed to geitonogamy in *Mimulus guttatus*, even though plants had only two open flowers.

Plants of *B. vagans* have traits that should reduce geitonogamy. Flowers are short-lived (8 hr), which would prevent floral overlap between days. Further, about 50% of plants open only one flower per day, although this is partly determined by environment. Nevertheless, floral display is small, and plants rarely open more than three flowers concurrently (Vaughton, Ramsey & Simpson 2008). Not only would these traits reduce geitonogamy, and ovule discounting, they would also reduce pollen discounting, thus benefiting both female and male fitness (Harder & Barrett 1995). Contrasting with *B. vagans*, some *Campanula*, *Solanum* and *Leptosiphon* species avoid all pollinator-mediated selfing through floral age-dependent self-incompatibility (SI). This mechanism physiologically delays self-compatibility, and hence self-fertilization, until flowers are older (Volger & Stephenson 2001; Stephenson *et al.* 2003; Goodwillie, Partis & West 2004). However, whether floral age-dependent SI conveys reproductive assurance when pollinators are absent or scarce remains to be established (Goodwillie, Partis & West 2004).

IMPLICATIONS FOR A BEST-OF-BOTH-WORLDS MATING STRATEGY

The best-of-both-worlds hypothesis proposes that if pollinators fail to deliver sufficient cross pollen, then delayed autonomous selfing boosts reproductive output, providing reproductive assurance (Becerra & Lloyd 1992; Kalisz &

Vogler 2003). We demonstrate that reproductive output in *B. vagans* was limited consistently by cross pollen and was boosted by selfing under all of the conditions that we examined. However, delayed selfing provided reproductive assurance only under two conditions: inclement weather when pollinators were inactive; and on one flowered plants when isolated. Under other conditions, flowers were selfed by pollinators before delayed selfing could occur. This pollinator-mediated selfing has two related consequences that diminish the flexibility of the best-of-both-world mating strategy.

First, pollinator-mediated selfing is likely to cause direct seed discounting (Lloyd 1992). Pollinator-mediated selfing and crossing probably occur together, and self and cross pollen are likely to compete directly to fertilize ovules, as occurs in self-sterile *B. bulbosa* (Owen, Vaughton & Ramsey 2007). Consequently, self-fertilization and seed discounting would be proportional to the number of self and cross pollen grains deposited onto stigmas. Large floral display is likely to exacerbate direct seed discounting because geitonogamy increases overall selfing. Second, pollinator-mediated selfing can cause potential seed discounting (Lloyd 1992). This also was exacerbated by large floral display because high levels of pollinator-mediated selfing fertilized all ovules that were not outcrossed. Thus, no unfertilized ovules were available to realize additional outcrossing, if such opportunities became available. By contrast, potential discounting was reduced on plants with one flower. On these plants geitonogamy was eliminated, and a substantial proportion of ovules remained unfertilized. Such ovules provide opportunities to increase outcrossing, but if such opportunities do not arise, then delayed selfing can occur. This flexibility is a key element of the best-of-both-worlds mating strategy. Genetic studies are now a priority in *B. vagans* to assess the amount of pollinator-mediated selfing and the extent to which ovules are discounted (e.g. Schoen & Lloyd 1992; Herlihy & Eckert 2002).

We conclude that the mating strategy of *B. vagans* may not be as flexible as might be expected for a species that exhibits delayed selfing. This reduced flexibility is a dilemma confronting many self-compatible plants. Self-compatibility can allow beneficial delayed selfing, but also allows pollinator-mediated selfing, which can be a costly by-product, realized when pollinators are present.

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