

## Mating cost of large floral displays in hermaphrodite plants

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HERMAPHRODITISM compromises outcrossing whenever the proximity of male and female organs allows self-fertilization<sup>1</sup> and interference between sexual functions<sup>2</sup>. Many floral traits of animal-pollinated angiosperms encourage cross-fertilization<sup>3</sup>, as recognized by Darwin<sup>4-6</sup>; however, these characteristics may also allow pollination between flowers on the same individual (geitonogamous self-pollination)<sup>7,8</sup>. Simultaneous display of many flowers exemplifies this conflict. Although large floral displays promote outcrossing through enhanced pollinator attraction<sup>9</sup>, they could be costly in terms of lost mating opportunities<sup>10,11</sup> if geitonogamy decreased outcrossed siring success by reducing pollen transfer between plants (pollen discounting<sup>12</sup>). We report here that, after manipulating the flower number of bee-pollinated *Eichhornia paniculata*

plants, we observed the predicted higher selfing and lower outcrossed siring success for larger inflorescences. Given the reduced fitness resulting when pollen receipt by one flower interferes with pollen export by another, we propose broadening traditional interpretations of floral design and display to recognize their roles in reducing geitonogamous pollen discounting.

To assess the effect of daily inflorescence size (3, 6, 9 or 12 flowers) on male- and female-fertility variation, we estimated the selfing frequency and outcrossed siring success in *Eichhornia paniculata* (Pontederiaceae), using allozyme markers. This species is annual, self-compatible, heterostylous and native to aquatic habitats in the Neotropics<sup>13</sup>. Each day, plants produce up to 20 flowers per inflorescence which open synchronously and last for about 6 h. Self and intramorph pollen have equivalent pollen-tube growth and siring ability and flowers seldom abort fertilized seeds<sup>7</sup>. As a result, fertility estimates are not biased by post-pollination discrimination against selfed progeny.

The experiment was conducted in Etobicoke, Ontario, and involved arrays of 35 or 36 long-styled plants (depending on treatments) with one inflorescence each. During a trial, an array included either plants of only one inflorescence size (pure arrays), or plants of two sizes (pairwise arrays). The experiment included all four pure arrays and all six possible pairwise arrays, with four replicates per combination. For pairwise arrays, we adjusted the number of plants for the two inflorescence-size treatments so that both were represented by the same total num-

ber of flowers (that is, equivalent pollen and ovule production per treatment). During the weeks before the experiment, we placed *E. paniculata* plants at the array locations so that resident bees (primarily *Bombus fervidus* and *B. vagans*) were familiar with this species.

For daily inflorescence size to affect reproductive success, the number of flowers visited per inflorescence by individual pollinators must increase with display size. Observations of bumblebee foraging during the experiment indicated that individual bees visited more flowers on larger inflorescences, although this response was context-dependent as the number of flowers visited on large inflorescences depended on the sizes of competing inflorescences in an array (Fig. 1a). The increase in number of flowers visited did not keep pace with increases in inflorescence size, so that the proportion of flowers visited per pollinator declined with inflorescence size. This decline was counter-balanced by the preference by pollinators for large inflorescences (Fig. 1b), so that the cumulative number of visits received per flower did not differ significantly among inflorescence size treatments ( $F_{5,21} = 2.40$ ,  $P > 0.05$ ).

Female fertility did not vary significantly with daily inflorescence size, as all inflorescence-size treatments produced equivalent numbers of seeds ( $F_{3,17} = 0.11$ ,  $P > 0.9$ ), regardless of the size of competing inflorescences (flower number  $\times$  competitor size interaction,  $F_{9,17} = 0.66$ ,  $P > 0.7$ ). This uniform female success probably reflects the equal cumulative visitation of individual flowers within inflorescences of all sizes.

In contrast to female fertility, the incidence of self-fertilization varied positively with inflorescence size, so that selfing occurred twice as frequently in 12-flowered inflorescences as in 3-flowered inflorescences (Fig. 2a). The mechanism responsible for this increase can be inferred from the increase in selfing from bottom to top flowers within inflorescences (Fig. 2b). Such a pattern is consistent with geitonogamous movement of pollen by pollinators that habitually begin visiting inflorescences on bottom flowers and then move upwards<sup>7,8,14</sup>, as did the bumblebees visiting *E. paniculata*<sup>7</sup>. In contrast, the alternative possibility that pollinators alter intrafloral self-pollination as they move up

inflorescences seems unlikely, because the duration of bee visits to flowers did not differ between the top and bottom halves of inflorescences (repeated-measures ANOVA,  $F_{1,5} = 0.08$ ,  $P > 0.75$ : based on video-taped records of a related experiment). Hence, geitonogamous pollination is strongly implicated as the mechanism responsible for the pattern of selfing within inflorescences. This geitonogamy, coupled with the tendency for bees to visit more flowers on large inflorescences (Fig. 1a), also provides the most likely explanation for the observed positive relation of selfing to inflorescence size.

The increased selfing by large inflorescences was accompanied by reduced outcrossed siring success (Fig. 2c), indicating that pollen discounting varied directly with inflorescence size. Indeed, the smaller inflorescences in pairwise arrays realized a significant outcrossing advantage over larger inflorescences (paired *t*-test,  $t_{23} = 2.74$ ,  $P < 0.02$ : mean  $\pm$  s.e. difference in outcrossed siring success,  $0.077 \pm 0.028$ ). We interpret this negative relation between selfing and outcrossed siring success as a direct outcome of geitonogamous pollination: such a mating cost associated with large floral displays has not previously been demonstrated empirically.

The occurrence of pollen discounting and its significance for the ecology and evolution of plants have been questioned<sup>11,15</sup> because "[p]ollen grains are much more numerous than ovules, and . . . [c]onsequently, an increase in self-fertilization can often be achieved with minimal effect on the outcrossing pollen pool" (ref. 11). Although pollen grains are plentiful and individually inexpensive, the important consideration is the number of mating opportunities, rather than pollen production. Typically <1% of a plant's pollen reaches stigmas<sup>16</sup>, so we should ask how much of the pollen that might have reached other plants is used up in selfing, rather than merely how much pollen is involved in selfing. Unlike intrafloral selfing, geitonogamous self-pollination may often limit opportunities for outcrossing because it probably removes pollen directly from the restricted pollen pool that pollinators transport between flowers<sup>11</sup>. As a result, geitonogamous pollen discounting should be a common consequence of displaying flowers simultaneously.

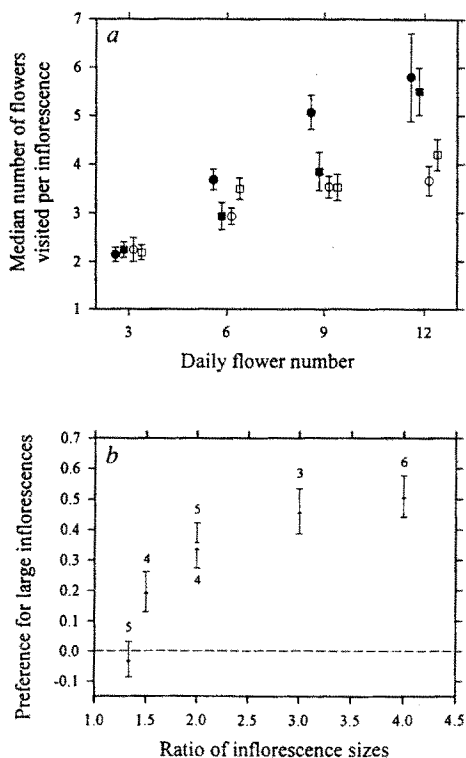


FIG. 1 The number of flowers per inflorescence affects the behaviour of pollinating bumblebees. In all trials we observed pollinator behaviour for 15 min during each of the first 3 h after flowering began. For each inflorescence visited by focal bees we recorded the inflorescence position in the array and number of flowers visited. a, Relation of the number of flowers visited per inflorescence to the size of visited and competing inflorescences. The median number of flowers visited per bee served as individual observations in a two-factor design with sizes of visited and competing inflorescences as classification variables. ANOVA of these data revealed a significant interaction in the effects of the sizes of the two inflorescence classes in an array ( $F_{8,137} = 2.73$ ,  $P < 0.01$ ): the general positive relation between the number of flowers visited and inflorescence size was most pronounced when competing inflorescences were small. Means and standard errors for 3 to 12 bees per treatment combination are shown. Symbols indicate size of competing inflorescences: solid circles, 3 flowers; solid squares, 6 flowers; open circles, 9 flowers; open squares, 12 flowers. b, Relative preference by bumblebees for large inflorescences in pairwise arrays. For focal bees that visited >10 inflorescences we estimated preference as  $\ln\left(\frac{\text{odds that the bee visited a large inflorescence}}{\text{odds that any inflorescence was large}}\right)$ , where the odds of an event is the ratio of the probability of the event occurring to the probability of the event not happening. The horizontal line indicates indifference, whereas positive preference values indicate preference for large inflorescences. Trend analysis<sup>26</sup> indicates that increases in the ratio of inflorescence sizes increased the preference by bees for large inflorescences (linear contrast,  $F_{1,21} = 31.00$ ,  $P < 0.001$ ), but reduced the incremental advantage of larger inflorescences (quadratic component of linear + quadratic contrast,  $F_{1,21} = 9.04$ ,  $P < 0.01$ ). In this analysis each observation was weighted by the inverse of the expected variance of the  $\log_e$  of the numerator of the preference index<sup>27</sup> (the denominator is constant for trials involving the same combination of inflorescence sizes).

Our results provide insight into strategies for allocating flowering effort during the blooming period. Both inflorescence treatments in pairwise arrays had the same total number of flowers, which were deployed differently on individual plants. Such a situation occurs if plants produce the same numbers of flowers during their entire flowering period, but differ in their daily inflorescence sizes and thus flower for different periods. In our experiment, 'few-flowered' plants sired more outcrossed seeds than 'many-flowered' plants. Thus the strategy of presenting few flowers each day and flowering for a protracted period can maximize the outcrossing component of male fertility.

The mating implications of pollen discounting depend on the relative susceptibility of self pollen to post-pollination losses<sup>8,11</sup>. If the plant is self-compatible and not subject to inbreeding depression, then discounting lowers the average heterozygosity of offspring by reducing the siring of outcrossed seeds. In contrast, if selfed seeds suffer inbreeding depression, geitonogamy reduces both the parent plant's female and male fertility. Self-incompatibility protects plants from losses of female fertility through selfing; however, it exacerbates male losses associated with pollen discounting because self pollen sires no seeds.

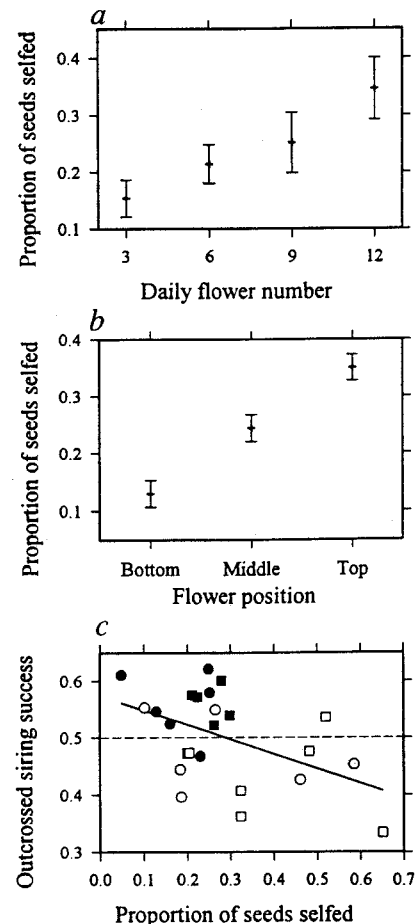
Pollen discounting bears two main consequences for the evolution of plant reproduction. First, discounting may influence the evolution of self-fertilization. With no discounting, selfing indi-

viduals realize a fitness advantage, as they contribute an average of three gene copies to the next generation (two through each selfed seed and one through paternal contributions to outcrossed seed) for every two copies contributed by outcrossing individuals<sup>17</sup>. Discounting diminishes this advantage because gametes involved in selfing reduce the number of gametes available for outcrossing<sup>12,18</sup>. Recognition of pollen discounting as a potential constraint on the evolution of selfing has prompted recent attempts to measure discounting, with differing results<sup>15,19-22</sup>. In contrast to earlier work, our study provides the first demonstration of a potentially widespread mechanism for discounting, namely geitonogamy. This mode of discounting may explain the disparity between previous studies, as studies of multiflowered plants demonstrated discounting<sup>19,20,22</sup>, whereas those of single-flowered plants did not<sup>15,21</sup>.

Lost outcrossing opportunities associated with large floral displays may also promote the evolution of floral mechanisms that decouple the benefits of large displays for pollinator attraction from the costs of geitonogamous pollen discounting. Consider two examples. First, when male and female functions are temporally separated in individual flowers (dichogamy) and inflorescence structure causes pollinators to visit female- before male-phase flowers, inflorescences can display many flowers without causing much geitonogamy<sup>23</sup>. This interpretation may explain

FIG. 2 Daily inflorescence size influences the trade-off between selfing and outcrossed siring success. Plants in all arrays contained known electrophoretic markers, which allowed us to identify the inflorescence size of the father of each seed and to quantify the fraction of seeds that was self-fertilized for each inflorescence-size treatment ( $s_i$  and  $s_j$ , respectively for the two treatments in an array). a, Relation of the proportion of selfed seeds (mean  $\pm$  s.e.) to inflorescence size. The frequency of selfing ( $s_i$ ) increased linearly with increasing inflorescence size (test for linear trend,  $F_{1,16} = 9.47$ ,  $P < 0.01$ ): this result did not vary with the size of competing inflorescences in the array (flower number  $\times$  competitor size interaction,  $F_{9,16} = 0.51$ ,  $P > 0.8$ ). b, Relation of the proportion of selfed seeds (mean  $\pm$  s.e.) to the position of a flower within an inflorescence. Self-fertilization ( $s_i$ ) increases significantly from bottom to top flowers ( $F_{2,32} = 23.75$ ,  $P < 0.001$ ), regardless of inflorescence size (flower position  $\times$  inflorescence size interaction,  $F_{6,32} = 0.99$ ,  $P > 0.4$ ), presumably because of geitonogamous pollination resulting from the vertical structure of *E. paniculata* inflorescences and the tendency of bees to move upwards on inflorescences<sup>7</sup>. c, Dependence of relative outcrossed siring success on the frequency of selfing ( $s_i$ ) in pairwise arrays. Outcrossed siring success ( $O_i$ ) is the fraction of all outcrossed seeds per array that were sired by inflorescence treatment  $i$ ,  $O_i = (1 - s_i)T_i - B_i + B_j / (1 - s_j)T_j + (1 - s_j)T_j$ , where  $T_i$  and  $T_j$  are the total numbers of seeds produced by the two inflorescence-size treatments in an array,  $B_i$  is the number of seeds produced by treatment  $i$  that were sired by treatment  $j$ , and  $B_j$  is the number of seeds sired by treatment  $i$  on treatment  $j$  plants. In the absence of pollen discounting, both treatments in an array should realize equivalent outcrossed siring success (that is,  $O_i = 0.5$ ; dashed line). Instead, the negative relation between outcrossed siring success and selfing ( $O_i = 0.573 - 0.254s_i$ ; solid line) indicates significant pollen discounting ( $F_{1,22} = 18.43$ ,  $P < 0.001$ ,  $R^2 = 0.239$ ). Pure arrays were excluded from this analysis because outcrossing is constrained to be the exact complement of selfing when only one treatment is involved. Furthermore, we included data for only one inflorescence treatment per array (randomly selected) to avoid the lack of independence caused by the siring success of one treatment being the complement of that for the competing treatment in an array. Symbols indicate inflorescence sizes: solid circles, 3 flowers; solid squares, 6 flowers; open circle, 9 flowers; open squares, 12 flowers.

**METHODS.** In pairwise arrays, plants with one inflorescence size were homozygous for the fast AAT-3 allele whereas plants in the competing treatment were homozygous for the slow AAT-3 allele. Pure arrays contained equal numbers of plants with one of these homozygous AAT-3 genotypes. Plants in all arrays were polymorphic for *PGI-2*. Once flowers wilted after an experimental trial, one flower from each of the top, middle and bottom thirds of the inflorescence was marked with paint. Capsules produced by marked flowers were collected after the seeds had ripened (11–12 days) and stored in separate envelopes until the seeds could be counted and assayed electrophoretically. On the basis of the genotypes of three randomly selected seeds per capsule (that is,



nine seeds per plant) we estimated the fraction of selfed seed ( $s_i$ ) and the associated standard error (based on 100 bootstrap samples) according to flower position and inflorescence size with Ritland's<sup>26</sup> multilocus selfing rate program. For a and b, the influences on  $s_i$  were assessed with a repeated-measures ANOVA in which individual estimates of  $s_i$  were weighted by the inverse of their squared standard error<sup>27</sup>. Supplemental hand pollination indicated that seed production was not resource limited<sup>7</sup>.

the paradox that although dichogamy is traditionally interpreted as an anti-selfing mechanism<sup>2</sup>, many dichogamous species also possess physiological self-incompatibility<sup>24</sup>. The apparent redundancy of two mechanisms that prevent selfing<sup>2</sup> is resolved by recognizing inter-floral dichogamy as a mechanism that promotes outcrossed siring success by limiting pollen discounting, a role that self-incompatibility cannot serve. The second example involves heterostyly, a genetic polymorphism in which plants of each morph have anthers and stigmas in dissimilar positions, with the other morph(s) producing the reciprocal arrangement(s). The pronounced separation of sex organs in heterostylous plants reduces pollen transfer within and between plants of the same morph<sup>25</sup> and, consequently, more pollen remains on the pollinator until it visits the alternative morph(s). Like dichogamy, heterostyly may function, in part, to reduce the mating cost of large floral displays. However, whereas dichogamy typically functions within inflorescences, heterostyly limits pollen dispersal between inflorescences of the same morph. These examples imply that the negative relation between selfing and outcrossed siring success demonstrated by our experiment may be an important, though largely unexplored, influence on the evolution of floral design and display. □

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1. Jarne, P. & Charlesworth, D. A. *Rev. Ecol. Syst.* **24**, 441–446 (1993).
2. Lloyd, D. G. & Webb, C. J. *New Zeal. J. Bot.* **24**, 135–162 (1986).
3. Richards, A. J. *Plant Breeding Systems* (Allen and Unwin, London, 1986).

4. Darwin, C. R. *The Effects of Cross and Self Fertilisation in the Vegetable Kingdom* (Murray, London, 1876).
5. Darwin, C. R. *The Different Forms of Flowers on Plants of the Same Species* (Murray, London, 1877).
6. Darwin, C. R. *The Various Contrivances by Which Orchids are Fertilised by Insects* 2nd edn (Murray, London, 1877).
7. Barrett, S. C. H., Harder, L. D. & Cole, W. W. *Funct. Ecol.* **8**, 526–535 (1994).
8. de Jong, T. J., Waser, N. M. & Klinkhamer, P. G. L. *Trends Ecol. Evol.* **8**, 321–325 (1993).
9. Klinkhamer, P. G. L., de Jong, T. J. & de Bruyn, G.-J. *Oikos* **54**, 201–204 (1989).
10. Charlesworth, D. & Charlesworth, B. *Evolution* **41**, 948–968 (1987).
11. Lloyd, D. G. *Int. J. Pl. Sci.* **153**, 370–380 (1992).
12. Holsinger, K. E., Feldman, M. W. & Christiansen, F. B. *Am. Nat.* **124**, 446–453 (1984).
13. Barrett, S. C. H. *Biol. J. Linn. Soc.* **25**, 41–60 (1985).
14. Crawford, T. J. in *Evolutionary Ecology* (ed Shorrocks, B.) 135–173 (Blackwell Scientific, London, 1984).
15. Holsinger, K. E. & Thomson, J. D. *Am. Nat.* **144**, 799–812 (1994).
16. Harder, L. D. & Barrett, S. C. H. in *Floral Biology: Studies on Floral Evolution in Animal-Pollinated Plants* (eds Lloyd, D. G. & Barrett, S. C. H.) (Chapman and Hall, New York, in the press).
17. Fisher, R. A. *Ann. Eugenics* **11**, 53–63 (1941).
18. Nagylaki, T. *J. theor. Biol.* **58**, 55–58 (1976).
19. Holsinger, K. E. in *Ecology and Evolution of Plant Reproduction* (ed. Wyatt, R.) 169–191 (Chapman and Hall, New York, 1992).
20. Kohn, J. R. & Barrett, S. C. H. *Evolution* (in the press).
21. Rausher, M. D., Augustine, D. & VanderKooi, A. *Evolution* **47**, 1688–1695 (1993).
22. Ritland, K. *Am. Nat.* **138**, 1049–1057 (1991).
23. Klinkhamer, P. G. L. & de Jong, T. J. *Oikos* **66**, 180–184 (1993).
24. Bertin, R. I. *Am. J. Bot.* **80**, 557–560 (1993).
25. Lloyd, D. G. & Webb, C. J. in *Evolution and Function of Heterostyly* (ed. Barrett, S. C. H.) 179–207 (Springer, Berlin, 1992).
26. Kirk, R. E. *Experimental Design* 2nd edn (Brooks/Cole, Belmont, 1982).
27. Neter, J., Wasserman, W. & Kutner, M. H. *Applied Linear Statistical Models* 3rd edn (Irwin, Homewood, 1990).
28. Ritland, K. *J. Hered.* **81**, 235–237 (1990).

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