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The influence of floral display size on selfing rates in *Mimulus ringens*

JD Karron[1], RJ Mitchell[2], KG Holmquist[1], JM Bell[1] and B Funk[1]
[1]*Department of Biological Sciences, PO Box 413, University of Wisconsin-Milwaukee, Milwaukee, WI 53201, USA;* [2]*Department of Biology, University of Akron, Akron, OH 44325, USA*

Pollinators often visit several flowers in sequence on plants with large floral displays. This foraging pattern is expected to influence the rate of self-fertilization in self-compatible taxa. To quantify the effects of daily floral display on pollinator movements and selfing, we experimentally manipulated flower number in four replicate (cloned) arrays of *Mimulus ringens* (Scrophulariaceae), each consisting of genets with unique combinations of homozygous marker genotypes. Four display classes (two, four, eight and 16 flowers) were present in each array. Pollinator visitation rate per flower and seed set per fruit were unaffected by display. However, flower number strongly influenced the frequency of within-plant pollinator movements, which increased from 13.8% of probes on two-flower displays to 77.6% of probes on 16-flower displays. The proportion of within-plant movements was significantly correlated with selfing ($r = 0.993$). The increase from 22.9% selfing on two-flower displays to 37.3% selfing on 16-flower displays reflects changes in the extent of geitonogamous self-pollination. We estimate that approximately half of all selfing on 16-flower displays resulted from geitonogamy. Selfing also varied dramatically among fruits within display classes. Nested ANOVA indicates that differences among flowers on two-flower ramets accounted for 45.4% of the variation in selfing, differences among genets accounted for 16.1% of the variation, and statistical and sampling error accounted for 38.5% of the variation. Differences among flowers within ramets may reflect the order of sequential floral probes on a display.
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Introduction

When pollinators visit plants with large floral displays, they frequently probe several flowers in sequence (Darwin, 1876; Dudash, 1991; Robertson, 1992; de Jong *et al*, 1993; Robertson and Macnair, 1995; Snow *et al*, 1996). These within-plant foraging movements are likely to influence the composition of transported pollen, and the fraction of self pollen deposited on stigmas (Lloyd, 1992; Lloyd and Schoen, 1992; Cruzan *et al*, 1994; Harder and Barrett, 1996; Eckert, 2000). In self-compatible species, pollen load composition may affect plant-mating system phenotypes and the evolutionary stability of mixed mating systems (Holsinger, 1991; Lloyd, 1992). However, surprisingly little is known about the relationship between patterns of pollinator movement and the realized mating system of flowering plants (Barrett *et al*, 1994; Harder and Barrett, 1996; Holsinger, 1996; Snow *et al*, 1996, Eckert, 2000).

The strong tendency for pollinators to visit more flowers on large displays should increase the rate of geitonogamous (among-flower) selfing, but have little impact on autogamous (within-flower) selfing. Few studies have quantified the relative contributions of geitonogamy and autogamy to overall selfing rates (Schoen and Lloyd, 1992; LeClerc-Potvin and Ritland, 1994; Eckert, 2000), or how the rate of geitonogamy changes with floral display size.

Although selfing rates have predominantly been characterized at the population level, there can be substantial variation among individuals, and even among fruits within individuals (reviewed in Karron *et al*, 1997; Cruzan, 1998). Pollinator behaviour and pollen carryover may contribute to this variation; when lengthy foraging bouts occur on individual plants, the composition of pollen loads deposited on adjacent flowers may differ considerably. For example, the first flower probed by a pollinator may receive both outcross pollen and autogamous self pollen, but cannot receive geitonogamous self pollen. Therefore, this flower may have a low rate of self-fertilization. By contrast, the fourth flower probed sequentially on this display may primarily receive geitonogamous and autogamous self pollen, and therefore may have a high rate of self-fertilization.

These among-flower differences in visitation history will tend to increase the genetic similarity of progeny within fruits, and decrease the genetic similarity of progeny from adjacent fruits (Ritland, 1989). Under these conditions, self progeny from individual fruits are especially likely to compete with inbred siblings. Such differences in the genetic structure of sibships may influence the expression of inbreeding depression (Schmitt and Ehrhardt, 1990). In addition, these patterns of genetic relatedness may influence the response to selection within sibships (Ritland, 1989). Due to technical limitations associated with procedures for estimating

Correspondence: JD Karron, Department of Biological Sciences, PO Box 413, University of Wisconsin-Milwaukee, Milwaukee, WI 53201, USA. E-mail: karron@uwm.edu
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family selfing rates (Ivey and Wyatt, 1999), few studies have explored how variation in selfing rates of individual fruits is partitioned within and among plants.

To quantify the effects of floral display on selfing rates, we experimentally manipulated flower number in four replicate (cloned) arrays of *Mimulus ringens* (square-stemmed monkeyflower), each consisting of genets with unique combinations of homozygous marker genotypes. This design facilitated the measurement of selfing rates of individual fruits and daily floral displays, since we could unambiguously classify each sampled seed as self or outcross (Karron *et al*, 1995a, 1997). Therefore, we could readily partition variation in selfing rates within and among plants. Ramets of each genet received a different floral display treatment in each array. This enabled us to examine how floral display influences selfing rates on a common genetic background.

We address the following questions: (1) Does floral display influence the proportion of intra-plant pollinator movements? (2) Does floral display influence selfing rates? (3) Are the effects of floral display on selfing rates consistent across genets? (4) Is the extent of self-fertilization correlated with patterns of pollinator movement? (5) Do the proportions of geitonogamous and autogamous selfing vary among display classes? (6) How is the variation in selfing rates of individual fruits partitioned within and among plants?

Materials and methods

Mimulus ringens L. (Scrophulariaceae) is a diploid perennial herb native to the wetlands of central and eastern North America. Populations are usually small and frequently have fewer than 50 individuals (J. Karron, pers. obs.). Fecundity is often high. Nearly every flower yields a fruit containing 700–6000 seeds (unpublished data).

Daily floral displays vary widely among plants, ranging from one to more than 15 flowers. Although flower buds are produced in pairs at leaf nodes, paired flowers are often open on different days. Flowers in a single display are usually scattered across several adjacent stems.

The showy purple hermaphroditic flowers open and dehisce their anthers before dawn. Stigmas usually close within 7–10 h following anthesis (Mitchell *et al*, in press) and corollas fall off by late afternoon. In contrast to some *Mimulus* species, which close stigmas in 3–12 s following pollination (Ritland and Ritland, 1989), stigma closure in *Mimulus ringens* occurs gradually over a period of 15–90 min (unpublished data).

Mimulus ringens flowers are primarily visited by three species of worker bumblebees (*Bombus fervidus*, *B. griseocollis* and *B. impatiens*; Karron *et al*, 1995a, b; Mitchell *et al*, in press), which gather pollen and/or nectar. Pollinators readily fly between flowers, both on a single ramet and between neighbouring genets.

Mimulus ringens is self-compatible and has a mixed-mating system (Karron *et al*, 1995a), like many of its congeners (Ritland and Ritland, 1989; Dole, 1990; Dudash and Ritland, 1991). Both selfing rates and neighbourhood size vary with population density due to changes in patterns of pollinator movement (Karron *et al*, 1995a, b). Controlled self and outcross pollinations do not differ in seed set, seed mass, germination rates or seedling survival (Karron *et al*, unpublished MS). However, self progeny have lower fitness at later stages of the life cycle, especially flower and fruit production. The overall fitness of self progeny is 20–30% lower than the overall fitness of outcross progeny (Karron, unpublished data).

Breeding of genets with unique marker genotypes

To facilitate complete paternity exclusion, we bred a set of 16 *M. ringens* genets with unique multilocus combinations of homozygous genotypes at four unlinked allozyme loci (Karron *et al*, 1995a; Karron, 1997). We produced these homozygous lines by outcrossing 22 multilocus heterozygotes, all derived from a single natural population. This breeding program ensured that the homozygous genotypes did not result from a history of close inbreeding. Details of the breeding procedure may be found in Karron *et al* (1995a). Three of the 16 genets in our earlier studies had very low pollen production and seed set (Karron *et al*, 1995a). Therefore, in 1999, we bred fertile replacement genets with each of these three marker genotypes. We also bred replacements for two clonal lines that had become infected with a virus. Pollen production for all 16 genets used in the present experiment exceeded 65 000 grains per flower, and preliminary analyses indicate no significant differences among genets (Mitchell, unpublished data).

Mimulus ringens occasionally produces vegetative offshoots in natural populations, and is readily cloned in the greenhouse. Clonal propagation enables us to replicate specific genotypes for our experiments (Karron *et al*, 1995a). We stored vegetative propagules from each of the 16 genets over the winter at 4°C. In early May 2000, we moved the propagules to a cool greenhouse, where they were hardened off for planting in the field.

Planting of experimental arrays

On June 7, 2000 we planted replicate arrays of genets in four gardens at the UW-Milwaukee Field Station (Saukville, WI, USA). Each array was separated from all others by at least 75 m of vegetation that included several unrelated bumblebee-pollinated taxa. (Our previous work has shown that these conditions minimize the movement of bumblebees between arrays; Karron *et al*, 1995a, b). Also, gene flow from natural populations is unlikely, since the nearest known natural population of *M. ringens* is more than 15 km away.

For each array, we planted 36 ramets in a square grid with 0.8 m spacing (Figure 1a). In the centre were single ramets of 15 different genets ('central genets'), which we arranged in a different random order in each array. To minimize the edge or boundary effects on patterns of pollinator visitation to these 15 genets, we surrounded them with a buffer row of 21 ramets of genet 'D' (Figure 1a).

Floral display treatments

Traplining bees may exhibit behaviours related to a previous day's floral display (Thomson, 1999). To ensure that pollinators acclimated to displays, we assigned the same flower number treatments to individual ramets on two consecutive sunny days (August 10–11, 2000). We permitted pollinators to acclimate to the displays on August 10, but did not quantify the patterns of visitation

a

D	D	D	D	D	D
D	C	H	A	D	D
D	B	M	O	K	D
D	P	G	F	N	D
D	Q	J	E	L	D
D	D	D	D	D	D

b

4	2	8	16	4	2
16	4	2	8	16	4
8	16	4	2	8	16
2	8	16	4	2	8
4	2	8	16	4	2
8	4	2	8	16	4

Figure 1 (**a**) Arrangement of genets in one of the experimental arrays. Single ramets of 15 genets are shown on a white background. Multiple ramets of border genet 'D' are shown on a grey background. (**b**) Arrangement of floral display treatments in one of the experimental arrays.

or tag developing fruits. On August 11, we observed patterns of pollinator visitation and then tagged fruits for mating system analyses.

To determine the effects of floral display on selfing rates, we manipulated displays in all four arrays. Plants in our experimental arrays grew larger displays than in natural populations. During peak bloom in mid-August, 2000, most plants produced 25 or more flowers every day. We used scissors to cut off excess flowers on each plant in the early morning, before pollinators became active. We removed flowers evenly across the plant to generate displays that matched the general appearance of natural displays.

In each array, we trimmed the floral display of all 36 plants to one of four sizes (two, four, eight or 16 flowers). We selected these displays because they spanned much of the range observed in natural populations of *M. ringens*. A minimum display of two flowers ensured that if stem breakage occurred prior to fruit harvest, at least one fruit could be sampled on every ramet. We used a regular spatial arrangement of floral displays so that every central genet was surrounded by two plants with each display size (Figure 1b). We rotated flower number treatments among ramets so that each genet experienced all four floral displays (Steel and Torrie, 1980).

Pollinator observation

We initiated pollinator observations at 0620 h, immediately following completion of floral display manipulations. We used two teams of three observers each to record the patterns of pollinator visitation. During a series of 20-min observation periods, we noted the location and identity of each visited plant, and the number of flowers visited sequentially on that plant (Karron *et al*, 1995a, b; Mitchell *et al*, in press). The two teams observed all four arrays in rotation until 1100 h, when nearly all of the stigmas were closed and pollinator activity had markedly declined. We estimate that we observed and recorded approximately 40% of all effective floral visits in the arrays. From these data, we determined the frequency of within-plant pollinator movements, and the visitation rate experienced by flowers on each ramet (probes/flower/h).

At 3 h after pollinator observations ended, we tied labelled plastic tags to pedicels of open flowers. On September 14, 2000, we harvested fruits and stored them individually in centrifuge tubes at 4°C. We used a dissecting microscope to count the number of seeds in each of two fruits on all 60 central genets.

Determination of selfing rates

To genotype progeny at the four allozyme loci, we used the tissue extraction and electrophoretic methods of Karron *et al* (1995a) with one modification: we resolved shikimate dehydrogenase and aconitase on horizontal starch gels with a morpholine citrate pH 6.1 buffer (Ritland and Ganders, 1987). We ran the morpholine citrate gels for 7.5 h at 35 mA.

We established progeny arrays for individual fruits from all 60 central genets (15 plants per array × four arrays). We germinated seeds from all harvested fruits on two- and four-flower displays, and from four randomly chosen fruits from eight- and 16-flower displays. Germination rates were uniformly high in all four display classes (exceeding 85%). We transplanted 2-week-old seedlings into 5 cm square cells in plastic flats, and grew them for three additional weeks, until they were large enough for genotyping. The survival rates of transplanted seedlings were high in all four display classes. From two-flower displays, we sampled up to 20 seedlings from each of two fruits. We successfully genotyped a mean of 19.4 seedlings from each of these fruits. From larger displays, we sampled up to 10 seedlings from each of the four fruits. We successfully genotyped a mean of 9.9 seedlings from each of these fruits. The mean number of progeny genotyped per ramet was 38.1.

Since each central genet had a unique multilocus combination of homozygous allozyme genotypes, self progeny were homozygous at all four loci. By contrast, outcrossed progeny were heterozygous at one or more loci, and we assigned paternity with a simple exclusion procedure (Karron *et al*, 1995a; Karron, 1997). Self *vs* outcross paternity was unambiguously determined for a total of 2285 progeny from 204 fruits. The number of progeny arrays for each display class were: two-flower displays $N = 28$; four-flower displays $N = 57$; eight-flower displays $N = 59$; 16-flower displays $N = 60$.

Statistical analysis

To analyse the effect of floral display on the proportion of within-plant pollinator movements, we used generalized linear models under SAS PROC GENMOD (Agresti,

1996; SAS Institute, 2000), using binomial errors and a logit link function.

To examine the effect of floral display on both visitation rate (probes/flower/h; $N = 60$ plants) and seed set ($N = 120$ fruits), we used one-way ANOVA with type III (simultaneous) sums of squares. We used fixed effects ANOVA to examine the effects of both floral display treatment and genet (PROC GLM; SAS Institute, 2000) on selfing rate, using individual fruits, nested within ramets, as the unit of observation. Genets were not replicated within arrays, so we were unable to test for effects of both genet and array in one analysis. Since we used array as a blocking variable, we present the analysis of the effects of display treatment and genet. Our conclusions remain unchanged if we test for array instead of genet.

We used Pearson correlation to examine the relationship between the proportion of within-plant pollinator moves and the extent of self-fertilization across display classes.

To examine how variation in selfing rates of individual fruits is partitioned within and among plants, we quantified the variance components using SAS PROC VARCOMP (SAS Institute, 2000). This analysis could only be performed on two-flower displays, since the larger 20 seed samples from each fruit on these displays enabled us to estimate the sampling error associated with samples of 10 seeds. Seeds from each fruit were randomly assigned to two groups (seeds 1–10 and seeds 11–20), and selfing rates were calculated for each group. Differences among fruits could then be partitioned into the following categories: (a) sampling error; (b) differences among flowers within ramets; and (c) differences among genets.

Estimation of selfing components

To estimate the contributions of autogamy and geitonogamy to the overall selfing rates, we used a slight modification of one of the procedures developed by Schoen and Lloyd (1992). This methodology, labelled 'procedure 2' by Schoen and Lloyd (p. 383), compares the selfing rate of a group of plants with numerous flowers (s_n) and the selfing rate of a group of plants with all but one flower removed or emasculated (s_r). Flowers on single-flower displays are subject to both autogamy and outcrossing, but do not experience geitonogamy. By contrast, flowers on large floral displays are subject to geitonogamy as well as autogamy and outcrossing. The rate of autogamous selfing (a_n) may be estimated as:

$$a_n = s_r(1 - s_n)/(1 - s_r)$$

The estimated rate of geitonogamous selfing (g_n) is then:

$$g_n = s_n - a_n$$

As noted earlier, we utilized a minimum floral display of two to ensure at least one fruit from every ramet. Therefore, our experimental design estimates how much additional geitonogamous self-fertilization occurs in displays with more than two flowers. In the equations above, we used the selfing rate of two-flower displays as s_r and the selfing rate of larger display classes as s_n. We then estimated the selfing components separately for the four-, eight- and 16-flower display classes. Note that our approach slightly underestimates the extent of geitonogamous self-fertilization, since all selfing in two-flower displays was attributed to autogamy. However, since pollinators rarely visited both flowers in sequence on two-flower displays (see Results), little geitonogamous pollination should occur on two-flower displays.

As the values of a and g are point estimates, we derived their standard deviations and tested their significance using bootstrap resampling (see Eckert, 2000). For each of 1000 resampled data sets, we calculated a and g for the four-, eight- and 16-flower display classes, and then used the resulting distributions to estimate the standard deviations. If $>95\%$ of the resampled estimates were >0, we considered the component to differ significantly from zero.

Results

Floral display size strongly influenced the relative frequency of within-plant pollinator movements (generalized linear model; χ^2 3 df $= 171$, $P < 0.0001$; $N = 1240$ interflower moves). The proportion of within-plant moves increased from 13.8% of probes on two-flower displays to 77.6% of probes on 16-flower displays (Figure 2a).

Pollinator visitation rate per flower did not vary significantly among floral display treatments ($F_{3,56} = 0.5$, $P > 0.6$), and means showed no clear relationship to display. Every flower produced a fruit, and the seed set per flower was unaffected by floral display

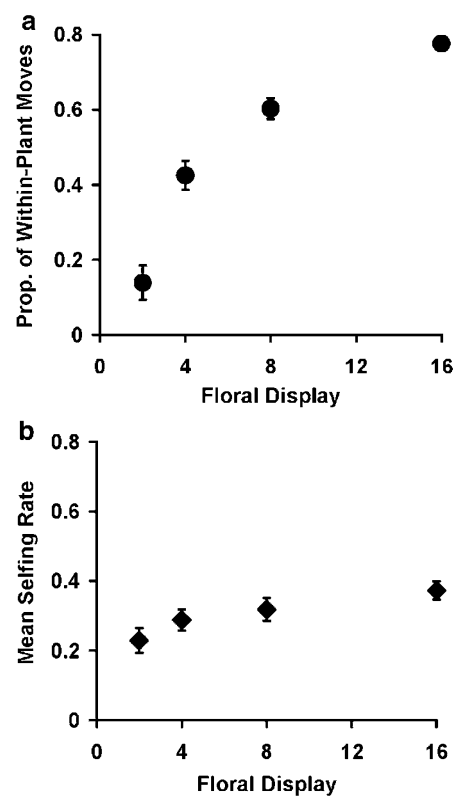

Figure 2 (**a**) Effect of floral display on the proportion of within-plant pollinator movements. Values were backtransformed from LS mean logit values estimated by a generalized linear model (GENMOD) ± 1 SE. N interflower movements for two-, four-, eight- and 16-flower displays are respectively, 72, 167, 300 and 701. SE for 16-flower display is smaller than the symbol. (**b**) Effect of floral display on the mean selfing rates (± 1 SE). $N = 204$ fruits, 2285 progeny.

Table 1 ANOVA of the effects of floral display treatment and genet on selfing rate

Source	*df*	*MS*	*F*	P
Floral display	3	0.153	3.25	0.024
Genet	14	0.073	1.55	0.099
Genet*Floral display	42	0.053	1.12	0.310
Error	144	0.047		

We used individual fruits, nested within ramets, as the unit of observation. The model explains 35.7% of the variation in selfing rate.

Table 2 Estimated levels of autogamy and geitonogamy on floral displays with four, eight and 16 flowers

Selfing component	*Display size*	*Mean*	*SD*	P
Autogamy	4	0.216	0.044	<0.001
	8	0.207	0.043	<0.001
	16	0.190	0.039	<0.001
Geitonogamy	4	0.071	0.058	0.12 (NS)
	8	0.111	0.060	<0.03
	16	0.182	0.052	<0.001

Standard deviation (SD) and probability that estimates differ significantly from zero (*P*) were calculated by bootstrapping. Due to the experimental design, selfing components could not be calculated for displays with two flowers. However, most of the selfing in two-flower displays is likely to result from autogamy (see text for details).

($F_{3,116}=0.2$, $P>0.8$; means again did not systematically vary with floral display, and averaged 4108 ± 106 seeds per flower).

The mean rate of self-fertilization varied significantly with floral display, increasing from 22.9% on two-flower displays to 37.3% on 16-flower displays (Figure 2b; Table 1). The nonsignificant genet by floral display interaction in Table 1 suggests that response to display treatment was consistent across genets. Selfing rate tended to increase with floral display in most genets; in particular, the correlation between floral display and selfing rate was positive in 13/15 genets (mean correlation coefficient = 0.47).

Differences in selfing rates among display classes closely corresponded to patterns of pollinator behaviour. There was a strong positive correlation between the proportion of within-plant pollinator moves and total selfing rate ($r=0.993$, $N=4$ display classes, $P<0.01$).

Estimated rates of autogamy were substantial, and significantly different from zero in the four-, eight- and 16-flower displays (Table 2). The estimated rates of geitonogamy also differed significantly from zero in the eight- and 16-flower displays.

The estimated fraction of total selfing attributable to geitonogamy increased with floral display (Table 2). In 16-flower displays, nearly half of all self-fertilization could be attributed to geitonogamy.

Within floral display classes, selfing rates of individual fruits varied widely, ranging from 0% to more than 70% (Figure 3). Selfing rates also varied among fruits on a single plant. For example, the four fruits sampled from the four-flower display of genet E had selfing rates of 0, 44, 60 and 80%. Similar variation in the selfing rates of fruits from a single plant was also evident in the eight-flower display of genet *N* (selfing rates of 0, 10, 70 and 70%).

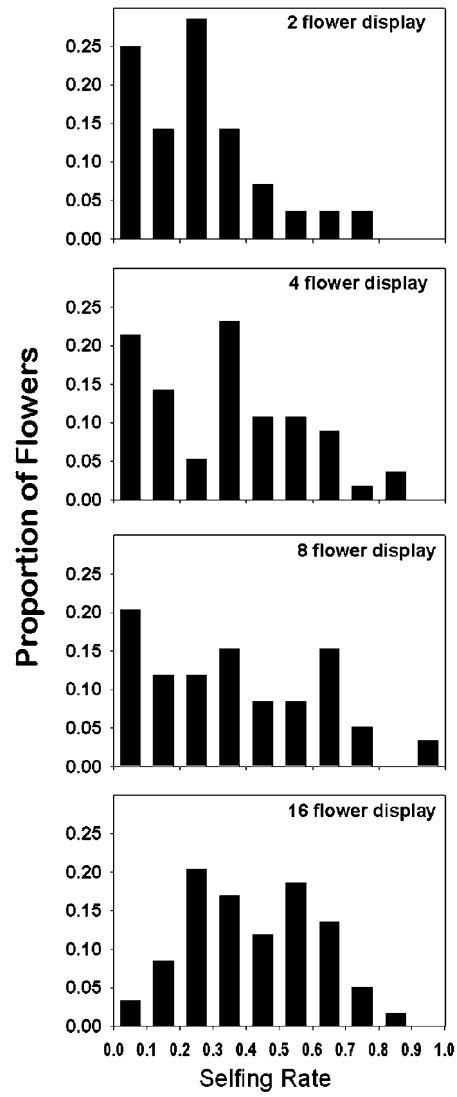

Figure 3 Frequency distributions of selfing rates for individual fruits on plants with displays of two, four, eight or 16 flowers ($N=15$ ramets for each display class).

Since we sampled 20 seeds/fruit from the two-flower displays, we were able to assess the components of variation in selfing rates in the two-flower display class. Nearly half (45.4%) of the variation in selfing rates of individual fruits was due to differences among flowers within ramets. An additional 16.1% of the variation was due to differences among genets. The remaining 38.5% of the variation was due to sampling error.

Discussion

Experimental manipulation of *Mimulus ringens* floral display had strong and significant effects on pollinator behaviour and the frequency of self-fertilization. These results have important implications for understanding how pollinators affect plant-mating systems, and we explore these implications below.

Pollinator behaviour and selfing rates

Although several authors have suggested that increased selfing in large floral displays results from within-plant pollinator movements (e.g. Crawford, 1984; Schoen and Lloyd, 1992; LeClerc-Potvin and Ritland, 1994), few studies have tested this assertion (Barrett *et al*, 1994; Harder and Barrett, 1995; Snow *et al*, 1996). Harder and Barrett (1995) established experimental arrays of *Eichhornia paniculata* with all pairwise combinations of four floral display sizes (three, six, nine, or 12 flowers). Selfing rates were quantified for the two display classes in each array. Manipulation of floral display in *E. paniculata* did not influence the rate of pollinator visitation or seed set, but did affect both the proportion of within-plant pollinator movements and selfing rates. Snow *et al* (1996) also examined the effects of floral display on pollinator movements and selfing rates in a study of *Hibiscus moscheutos.* A central target plant homozygous for a unique allele was surrounded by 12 neighbours lacking the allele. Flower number (three, six, or 12 flowers) was then manipulated on the central plant. Both the frequency of within-plant pollinator movements and the selfing rate increased with the size of floral display. Our research adds to this growing consensus that floral display primarily influences the patterns of pollinator movement, rather than the rate of visitation, and that pollinators probe more flowers sequentially on large displays, leading to an increase in the extent of geitonogamous self-fertilization.

Contributions of geitonogamy and autogamy to the overall selfing rates

Our work with *M. ringens* is among the few studies to quantify the contribution of autogamy and geitonogamy to the overall rates of self-fertilization. We estimate that geitonogamy accounted for approximately 1/4 of the self-fertilization in four-flower displays, and approximately 1/2 of the self-fertilization in 16-flower displays. This increase in geitonogamy is in accord with expectations based on changes in pollinator behaviour. To the extent that there is inbreeding depression, this increase in geitonogamy represents a potential cost to plants of producing larger displays – although more flowers means more seeds, the quality of those seeds may decline.

Our experimental design slightly underestimates geitonogamous self-fertilization in *M. ringens*, since all selfing in two-flower displays was attributed to autogamy. Through extrapolation from a linear regression of selfing rate as a function of the proportion of within-plant pollinator moves (not shown), we estimate that the frequency of selfing in the absence of intra-plant moves would be approximately 20%. Since the observed selfing rate in two-flower displays was 22.9%, we estimate that the geitonogamous selfing rate for two-flowered displays was approximately 3%. LeClerc-Potvin and Ritland (1994) estimated a slightly higher level of geitonogamous selfing (7.9%) in two-flower displays of *Mimulus guttatus*. However, open *M. guttatus* flowers usually occur in pairs at a single node, which may promote within-plant pollinator movements. By contrast, open *M. ringens* flowers are often widely separated, and most pollinators visiting two-flower displays probed a single flower (Mitchell *et al*, in press).

In our system, autogamy accounts for a large part of the overall selfing rate, but further work is required to determine whether this occurs through prior, facilitated, or delayed selfing, each of which may have different consequences for the evolution of plant-mating systems (Lloyd and Schoen, 1992).

Variation in selfing rates among fruits

Nearly all flowers in our experimental arrays must have received at least one pollinator visit, since 99% of the genotyped fruits had at least two outcross seeds. In the terminology of Schoen and Brown (1991), nearly all fruits in our arrays resulted from 'part-flower selfing' (ovules in a flower fertilized by a mixture of self and outcross pollen) rather than 'whole-flower selfing' (all ovules in a flower fertilized by self-pollen). Schoen and Brown suggested that whole-flower selfing is more likely when pollinator activity is limited. Our study of *M. ringens* was undertaken during fair weather, when pollinators were abundant. It is quite possible that a higher frequency of whole-flower selfing would occur if pollinator service were more limited (Dole, 1990).

Selfing rates in our study varied dramatically among fruits within individual ramets, almost solely as a result of part-flower selfing. This variation may potentially be caused by four factors: (1) Technical limitations of mating system estimation procedures at fine spatial scales (Morgan and Barrett, 1990; Cruzan *et al*, 1994; Ivey and Wyatt, 1999; Ritland, 2002). This includes violation of the assumptions of the mixed-mating model. In our study, error due to the mating system estimation procedure is likely to be minimal, since we were able to determine whether each genotyped seed was the product of self or cross-fertilization. (2) Statistical error inherent in sampling a small fraction of the seeds in a fruit (Morgan and Barrett, 1990). (3) Differences among flowers within ramets in patterns of self-pollen deposition (Lloyd and Schoen, 1992; Harder and Barrett, 1996). (4) Differences among genets in self-pollination (Karron *et al*, 1997).

This study provides one of the first demonstrations that selfing rate can vary dramatically among fruits on individual genets. Our variance decomposition for two-flowered displays demonstrates that the majority of variation within a display class represents real differences among fruits, rather than sampling error. These among-fruit differences may largely result from variation in the pollination history of individual flowers. In fact, differences within floral displays were nearly three times as great as differences among floral displays. Such large differences among individual fruits in selfing rate can bias the estimates of selfing rate that are based on bulk seed collections (Schoen and Brown, 1991), and may also affect the expression of inbreeding depression and seedling competition (Schmitt and Ehrhardt, 1990).

Geitonogamous self-fertilization should generally increase with position in the visitation sequence (Barrett *et al*, 1994; Rademaker *et al*, 1999). This relationship will, however, be complicated by subsequent pollinator visits (Dudash and Ritland, 1991). Many flowers in our study received two or more visits prior to stigma closure (stigmas received approximately 0.7 probes/h over a roughly 4-h period of receptivity). Since *M. ringens* flowers are scattered fairly evenly across the plant, the sequence of visitation on a large display is likely to differ

among pollinators. Therefore, individual flowers may receive successive visits with very different proportions of self and outcross pollen. Selfing rate will then depend upon the composition and time of arrival of pollen deposited on the stigma, as well as any postpollination processes (Marshall and Folsom, 1991). Harder and Barrett (1996) emphasize the need for experimental studies that combine observations of pollinator behaviour with measurement of the mating system. Our results indicate that studies of the reproductive events influencing individual flowers will be especially informative.

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