Selection through Male Function Favors Smaller Floral Display Size in the Common Morning Glory *Ipomoea purpurea* (Convolvulaceae)

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ABSTRACT: In self-compatible, hermaphroditic plants, display size—the number of flowers open on a plant at one time—is believed to be influenced by trade-offs between increasing geitonogamous selfing and decreasing per-flower pollen export as display size increases. Experimental results presented here indicate that selection through male function favors smaller display sizes in *Ipomoea purpurea*. In small arrays, plant display size was manipulated experimentally, and female selfing rate, male outcross success, and total male fitness were estimated using genetic markers and likelihood and regression analyses. As would be expected if larger displays experience greater geitonogamy, selfing rate increased with display size. However, the per-flower amount of pollen exported to other plants decreased with display size. The magnitude of this effect is more than sufficient to offset the increase in selfing rate, resulting in reduced per-flower total male fitness with increasing display size. The low values of inbreeding depression previously reported for this species would enhance this effect.

Keywords: floral display size, geitonogamy, male fitness, morning glory, plant mating systems, selfing.

It is commonly believed that the dynamics of both pollen export and geitonogamy, as well as the magnitude of in-

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breeding depression, play a central role in shaping the evolution of plant mating systems in general and of floral display size in particular (de Jong et al. 1992, 1993; Harder and Barrett 1995; Barrett 2003; Karron et al. 2004). Empirical evidence indicates that as floral display size (number of flowers open on a given day) increases in a self-compatible species, the average female selfing rate of ovules on a plant typically tends to increase because of increased pollinator movement, and thus increased pollen transfer, between flowers on the same plant (geitonogamy; Crawford 1984; Hessing 1988; de Jong et al. 1992; Snow et al. 1996; Rademaker and de Jong 1998; Eckert 2000; Harder et al. 2004). Increased geitonogamous selfing represents a gain in male fitness because it increases the number of gene copies transmitted to the next generation through the plant's own seeds. Potentially counteracting this benefit are inbreeding depression and the effects of display size on male outcross success.

The effect of display size on male outcross success is expected to be mediated by its effects on pollen removal and on the partitioning of removed pollen to the outcross and geitonogamous pollen pools (fig. 1). Because, in general, larger display sizes tend to be more attractive to pollinators (Ohashi and Yahara 2001), it is expected that larger displays will increase the number of pollinator visits per flower and therefore will increase the proportion, P, of the total pollen produced by a flower that is removed by pollinators. Increased pollinator visitation is expected to increase pollen contribution to both the geitonogamous and the outcross pollen pools and thus increase both male selfing success and male outcross success. On the other hand, because pollinators are expected to visit more flowers on a plant with larger display sizes, a greater fraction $(1 - E)$ of that pollen is expected to be deposited on other flowers on the same plant and therefore be unavailable for outcrossing. Consequently, it is expected that a smaller fraction, E, of the removed pollen is contributed to the outcross pollen pool in plants with larger displays (Lloyd 1992; de Jong et al. 1993; Morris et al. 1994). This effect
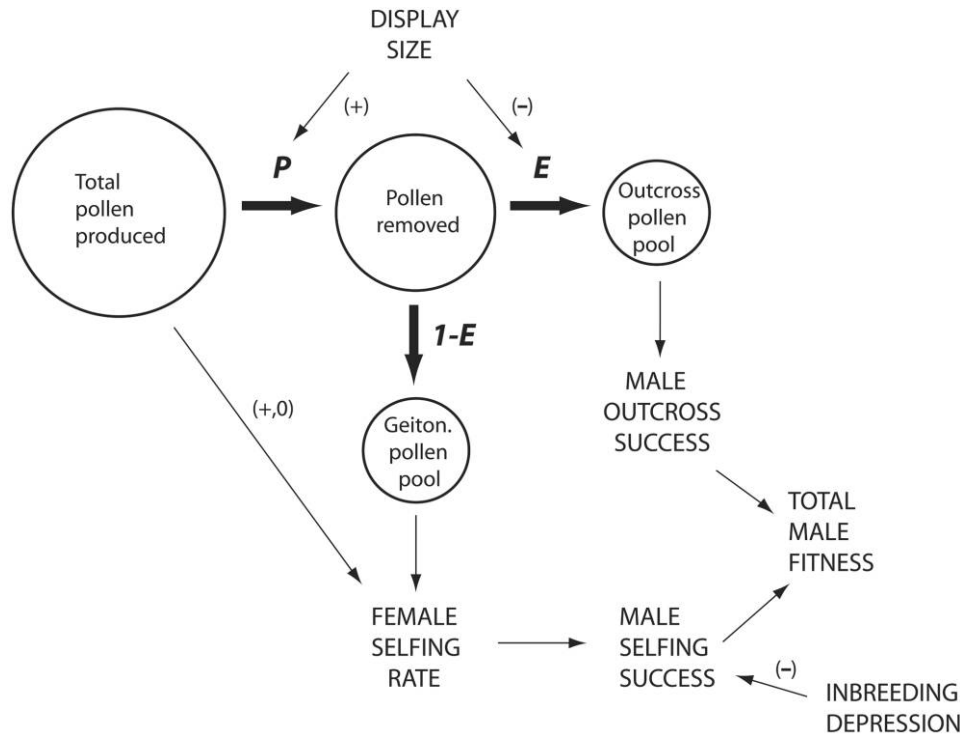
Figure 1: Expected effects of display size on total male fitness, mediated by effects on sizes of the outcross and geitonogamous pollen pools. Circles represent pollen pools. Thick arrows represent contributions from one pollen pool to another. Thin arrows indicate effects. Symbols in parentheses indicate sign of expected effects. Arrows without signs represent positive effects. P is proportion of total pollen produced by a flower that is removed by pollinators. E is the proportion of pollen removed by pollinators that is contributed to the outcross pollen pool.

can result in reduced male outcross success in plants with larger displays, which has been observed in several investigations (de Jong et al. 1993; Harder and Barrett 1995; Rademaker and de Jong 1998).

Because the total pollen from a flower available for geitonogamy is just $P \times (1 - E)$, increasing display size, which increases both P and $1 - E$, will lead to increased geitonogamy and thus increased male selfing success. By contrast, the contribution to the outcross pollen pool is just $P \times E$. Because E is expected to decrease with increasing display size, the net effect of display size on male outcross success may be positive or negative. Thus, the overall effect of display size on total male fitness, which is the sum of male selfing success (discounted by inbreeding depression; fig. 1) and male outcross success, can be either positive or negative, depending on the relative magnitudes of the effects of display size on P and E.

Little empirical information exists regarding the effects of display size on the relative magnitudes of outcross male success and geitonogamy (selfing success) in the same plant. Harder and Barrett (1995) demonstrated that increased floral display size in *Eichhornia paniculata* both increases female selfing rate and decreases outcross male

success, but they did not report whether the this trade-off favors large or small displays. Rademaker and de Jong (1998), using artificial dye as a pollen analog, showed that in one species, larger displays exhibited greater geitonogamy but no detectable decrease in pollen export, while in two other species the effects of the two processes approximately canceled each other. We are aware of no investigation demonstrating that the balance of geitonogamy and pollen export favors smaller displays; we are also unaware of any theoretical argument demonstrating that this is not possible.

We report here an experiment designed to determine the degree to which the advantage of increased geitonogamous selfing in large displays is offset by decreased pollen export. The study organism is the common morning glory *Ipomoea purpurea*. In this self-compatible annual species, individual flowers are open for only one morning, but flowering occurs over a period of several months (R. E. Miller and M. D. Rausher, unpublished data). Thus, on any given day, only a small fraction of the total flowers produced during a season are open simultaneously. The observation of relatively few flowers being open on any particular day is consistent with what we demonstrate here:

in larger displays, decreased per-flower pollen export counteracts the advantages associated with increased geitonogamy to the extent that we detected significant selection favoring smaller floral display size.

Methods

Study Organism

The common morning glory *Ipomoea purpurea* (L.) Roth (Convolvulaceae) is an annual vine common throughout the southeastern United States. It is a member of section *Pharbitis*, subgenus *Quamoclit* (sensu Miller et al. 1999). *Ipomoea purpurea* is a self-compatible species with hermaphroditic flowers. Anthesis begins at dawn, and flowers close in mid- to late morning. At dawn anthers are below the stigma. During the morning, filaments elongate to the point where anthers touch the stigma, resulting in the potential for autogamous selfing. *Ipomoea purpurea* has a mixed mating system with population-average outcrossing rates ranging from 0.44 to 0.97 in different studies (Brown and Clegg 1984; Schoen and Clegg 1985; Epperson and Clegg 1987; Rausher and Fry 1993). Several species of *Bombus* are the primary pollinators (Rausher and Fry 1993). On a particular day, flower number per plant typically ranges from 0 to 10 flowers in natural populations of *I. purpurea*, but plants with more than 20 flowers are not uncommon (R. E. Miller and M. D. Rausher, unpublished data).

Experimental Design

Plants for this study were drawn from an experimental pool of plants representing six inbred lines (selfed for 13 generations) originally collected from a single population in Durham County, North Carolina. These particular lines were chosen from a larger collection because of their unique genotypes at four electrophoretic loci (Esterase-1, Esterase-2, MUP, and PGM; Fry and Rausher 1997; Chang and Rausher 1998).

Seeds of inbred lines were planted in an experimental garden with no other morning glories in the vicinity. The layout included 10 genotypes, with seven replicates of each genotype planted in seven rows (1.5 m between rows) and genotypes randomized within each row (1 m between plants). Plants were fertilized and watered to encourage flower production. Each plant was allowed to grow up a 2-m stake. The vines of these plants were constantly tended to avoid intertwining of individuals.

From this larger selection of genotypes and replicates, six genotypes (inbred lines) were selected from two replicates per inbred line based on individuals that consistently produced an adequate number of flowers to establish six floral display size treatments. Plants from each of the six inbred lines were randomly assigned to one treatment each day so that each treatment was represented by each genotype over the entire experiment (six days in September 1998). Specifically, the displays included 2, 8, 14, 20, 26, or 32 flowers. Flower buds were removed the previous day to establish these displays. In addition, we removed all buds from the other, unused plants in the array.

Each array was left for 1 day. Plants were visited by bumblebees (*Bombus* spp.). Bee visitation was high, and most flowers in the experimental array were visited by a bee within a few hours after dawn (J. A. Lau and R. E. Miller, personal observations). Flowers were tagged, and capsules were collected when mature. During the entire experiment, 1,224 flowers were monitored; of these, 153 aborted (12.5%). From the remaining capsules, one seed per capsule was scored for genotype at each of the four electrophoretic markers. We sampled only one seed per capsule to maximize the probability that siring events are independent, a requirement of our statistical analysis.

Likelihood Analyses of Mating System Parameters and Male Fitness

The focus of this study was to estimate female selfing rate *s* (the proportion of seeds that a plant produces through self-fertilization), male selfing success (the number of ovules fertilized by selfing, which is equal to *s* times the number of seeds produced), and relative male outcross success *x* (the proportion of all outcrossed seeds sired by plants of a particular display size through outcrossing; Chang and Rausher 1998). Ideally, estimates of inbreeding depression should be incorporated into the estimation of *s* and male selfing success. However, inbreeding depression was not estimated in this experiment, and it is not clear how previous estimates of inbreeding depression can be incorporated into this analysis in a statistically appropriate manner. Consequently, for this analysis, we assume no inbreeding depression, and in "Discussion," we consider how the presence of inbreeding depression would modify our conclusions.

For determining the net effects of these fitness components, the appropriate measure of outcross success is the total number of successful pollen grains over the lifetime of the plant, or over the flowering season if the plant is an annual. However, discussions of the evolution of display size typically assume that the total number of flowers produced by a plant over a season or a lifetime is constrained by available resources and that larger floral displays can be achieved only by reducing the number of days over which flowering occurs (Schoen and Dubuc 1990; de Jong et al. 1992; Fishbein and Venable 1996). In this context, per-flower male outcross success, x/f flower

number, is equivalent to per-plant outcross success and is more easily measured in experiments. We therefore focus on estimating the per-flower male selfing success, per-flower male outcross success, and per-flower total male fitness in our analysis.

To estimate female selfing rate and per-flower outcross success, we employed a likelihood analysis based conceptually on the partitioning of pollen into geitonogamous and outcross pollen pools portrayed in figure 1. The geitonogamous pollen pool is all pollen deposited on pollinators that does not remain on the pollinators when they leave the plant. The outcross pollen pool consists of all pollen remaining on the pollinators when they leave the plant, which may include pollen previously picked up from other plants.

In the likelihood model, female selfing results from both autogamous and geitonogamous selfing. We represent the female selfing rate, that is, the probability that an ovule is fertilized by pollen from the same plant, as s_i, where the subscript i denotes a particular display size. The probability that an ovule is fertilized by pollen from plants of display size j is then the product of the probability that the ovule is not selfed, $1 - s_i$, and the proportion of pollen in the outcross pollen pool contributed by plants of display size j, which we designate x_j. Consequently, the probability of observing that an ovule on a plant of display size i is sired by pollen from a plant of display size j is given by

$$P_{ij} = \begin{cases} s_i + (1 - s_i)x_j, & i = j, \\ (1 - s_i)x_j, & i \neq j. \end{cases} \quad (1)$$

In this formulation, pollinators are allowed to revisit and deposit pollen on plants that they have previously visited. Because the outcross pollen pool contains pollen from this plant, revisitation may technically result in selfing. However, we consider this selfing component to be largely an artifact of the small arrays used in this experiment, in which return to a previously visited plant occurs with reasonable frequency. In large populations typical of this species, return visits are expected to be negligible, and this component of selfing would be essentially zero. Therefore, we take s_i to estimate the true female selfing rate. A benefit of this formulation is that, as long as the composition of the outcross pollen pool is roughly stable, pollen from every flower in the experiment, regardless of the display size of the plant on which it grows, has equal access as outcross pollen to all other flowers in the experiment.

With these probabilities, the likelihood of the data for treatment (display size) i, L_i, was estimated as

$$L_i = k \prod_j P_{ij}^{a_{ij}}, \quad (2)$$

and the overall probability of the data was

$$L = \prod_i L_i, \quad (3)$$

where a_{ij} is the number of seeds produced by maternal plants of treatment i that were sired by plants of treatment j and k is a combinatorial constant (Edwards 1972). Maximum likelihood estimates of the parameters s_i and x_j were obtained simultaneously with Mathematica software (ver. 5; Wolfram 1991). In some cases, a maximum was not found, and therefore a separate program written in APL (Gilman and Rose 1976) was used to search the (s_i, x_j) parameter space for the parameter combination that maximized the likelihood. Analyses were performed on data pooled across days and across inbred lines because of the balanced nature of the data and because preliminary analyses revealed no heterogeneity in the number of capsules matured across days or lines.

Specific hypotheses regarding the relationships between display size and female selfing rate, male outcross success, and total male fitness were tested in standard fashion by comparing the log likelihood of unconstrained and constrained models, where the constraint corresponded to the specific hypothesis being tested (table 1; Edwards 1972). In all cases, the test statistic was $\Lambda = 2 \times (\log \text{likelihood of unconstrained model} - \log \text{likelihood of constrained model})$, which is approximately χ^2 distributed with degrees of freedom equal to the number of constrained parameters (Hocking 1985).

Tests involving total male fitness assumed that the number of seeds produced per flower m was the same for all treatments. While we did not count the number of seeds produced per flower and therefore cannot test this assumption directly, we do have data on flower failure rates. A fruit was considered to have failed if it produced no seeds, either because it was not pollinated or because the fruit aborted. We tested for differences in the proportion of failed flowers between treatments, using a factorial ANOVA (PROC GLM; SAS Institute 1990), in which floral display size was treated as a covariate and marker genotype and its interaction were treated as fixed predictor variables. Day was also included as a blocking factor.

Assuming equal seed production per flower, overall selection through male function on display size results from the combined effects of display size on self- and outcross pollination success. Using a likelihood analysis, we represented total male fitness for treatment i by

$$W_i = s_i F_i m + x_i \sum_{j=1}^{n} (1 - s_j)F_j m, \quad (4a)$$

or, equivalently,

Table 1: Results from likelihood analyses examining models for selfing rate, male outcross success, and male fitness

Hypothesis	Restricted model	Unrestricted model	Λ	df	P
A. Selfing rate (s_i); best model, $s_i = 5.46f_i - 12.72f_i^2$:					
Equal selfing rates	s_i all equal (−1,317.56)	All parameters free (−1,306.99)	21.14	5	<.001
Relationship of s_i to f_i:					
Quadratic term = 0	$s_i = \beta_0 + \beta_1 f_i$ (−1,315.64)	$s_i = \beta_0 + \beta_1 f_i + \beta_2 f_i^2$ (−1,307.68)	15.92	1	<.0001
Linear term = 0	$s_i = \beta_0 + \beta_2 f_i^2$ (−1,316.93)	$s_i = \beta_0 + \beta_1 f_i + \beta_2 f_i^2$ (−1,307.68)	18.50	1	<.0001
Intercept = 0	$s_i = \beta_1 f_i + \beta_2 f_i^2$	$s_i = \beta_0 + \beta_1 f_i + \beta_2 f_i^2$	.38	1	NS
B. Male outcross success (x_i); best model, $x_i/f_i = 2.50 - 15.83f_i + 38.76f_i^2$:					
Equal outcross success	x_i all equal (−1,356.81)	All parameters free (−1,306.99)	99.64	5	<.0001
Equal per-flower outcross success	$x_i = f_i$ (−1,322.35)	All parameters free (−1,306.99)	30.72	4	<.0001
Relationship of x_i/f_i to f_i:					
Quadratic term = 0	$x_i/f_i = \beta_0 + \beta_1 f_i$ (−1,314.15)	$x_i/f_i = \beta_0 + \beta_1 f_i + \beta_2 f_i^2$ (−1,310.22)	7.86	1	<.0001
Linear term = 0	$x_i/f_i = \beta_0 + \beta_2 f_i^2$ (−1,316.15)	$x_i/f_i = \beta_0 + \beta_1 f_i + \beta_2 f_i^2$ (−1,310.22)	11.86	1	<.0001
Intercept = 0	$x_i/f_i = \beta_1 f_i + \beta_2 f_i^2$ (−1,351.1)	$x_i/f_i = \beta_0 + \beta_1 f_i + \beta_2 f_i^2$ (−1,310.22)	81.76	1	<.0001
C. Male fitness (w_i); best model, $w_i/f_i = 1.19 - .85f_i$:					
Equal male fitness	w_i all equal (−1,509.28)	All parameters free (−1,306.99)	404.58	5	<.0001
Male fitness equal flower number	$w_i = f_i$ (−1,315.22)	All parameters free (−1,306.99)	16.47	5	<.001
Relationship of w_i/f_i to f_i:					
Quadratic term = 0	$w_i/f_i = \beta_0 + \beta_1 f_i$ (−1,312.22)	$w_i/f_i = \beta_0 + \beta_1 f_i + \beta_2 f_i^2$ (−1,312.10)	.242	1	NS
Linear term = 0	$w_i/f_i = \beta_0$ (−1,315.22)	$w_i/f_i = \beta_0 + \beta_1 f_i$ (−1,312.22)	6.00	1	<.02

Note: "Best model" reports the best regression model relating these parameters to proportion of flowers in each treatment (f_i). Values in parentheses are log likelihoods of associated model. NS = not significant.

$$w_i = s_i f_i + x_i \sum_{j=1}^{n} (1 - s_j) f_j, \qquad (4b)$$

where F_i is the number of flowers per plant in treatment i, $w_i = W_i / \sum_{j=1}^{n} Fjm$, and $f_i = F_i / \sum_{j=1}^{n} Fj$. The first term on the right-hand side of equation (4) represents the number (eq. [4a]) or proportion (eq. [4b]) of seeds sired by selfing, while the second term represents the number (eq. [4a]) or proportion (eq. [4b]) of seeds sired by outcrossing. Equations (4a) and (4b) represent per-plant fitness, that is, the total number or proportion of all seeds in the experiment sired by a plant in treatment i. To obtain the total number or proportion of all seeds sired per flower, these equations are divided by F_i.

Hypotheses about mating system parameters and male fitness were tested in two stages. In the first stage, we tested for equality of these values across treatments. In these tests, the unconstrained model was that represented by equations (4a) and (4b). In the second stage, we assessed whether there was a relationship between mating system parameters and flower number and, if so, whether this relationship was linear or nonlinear by comparing the log likelihoods of models that represented the relationship as constant, linear, or quadratic. A significantly better fit of the quadratic model, compared to the linear model, was taken to indicate that the relationship was nonlinear but not that it was specifically quadratic (Snedecor and Cochran 1967).

Regression Analysis of Total Male Fitness

It is not possible to say with certainty whether a given pollination was the result of self-pollination or outcross

pollination by the other plant in the same treatment. Nevertheless, because marker genotypes were unique to each treatment, it is possible to infer with certainty which treatment sired any seed in our experiment. Consequently, we could obtain exact counts of total male siring success directly. This allowed us to perform an additional analysis of total male fitness using regression analysis. Specifically, we examined the relationship of number of seeds sired per flower to the size of the floral display. In this analysis, performed with PROC GLM of SAS (SAS Institute 1990), seeds sired per flower was the dependent variable, and display size and genotype were the independent variables. We examined models that included interactions, as well as quadratic terms, and tested the significance of each effect using Type III sums of squares.

Results

Mating System Parameters

Female selfing rates differed significantly among treatments (table 1*A*). As would be expected if larger floral displays experienced greater geitonogamous selfing, female selfing rate increased with number of flowers per plant (fig. 2; table 2). Over the range of 2–20 flowers per plant, this increase was approximately linear, while above 20 flowers per plant, selfing rate appeared to plateau. The statistical significance of this nonlinearity was confirmed by the significantly better fit of a quadratic relationship of selfing rate to flower number, compared to a linear relationship (table 1*A*).

As expected, plants with large displays also contributed more total successful outcross pollen than plants with small displays (table 2). However, on a per-flower basis, plants with few flowers (two and eight flowers per plant) were more successful at fertilizing other plants than plants with many flowers (fig. 3). Once again, this pattern is expected if greater geitonogamous selfing experienced by larger floral displays results in reduced pollen export. Like female selfing rate, per-flower male outcross success also appeared to plateau for plants with greater than 20 flowers. The statistical significance of this nonlinearity is evident from the comparison between models with and without a quadratic term (table 1*B*).

Total Male Fitness

Not surprisingly, plants with larger displays had greater total male fitness (table 2). By contrast, analysis of the relationship of per-flower total male fitness to flower number indicates a significantly negative relationship (table 1*C*; fig. 4), and there is no evidence that the relationship is nonlinear (quadratic model not significantly better than linear model; table 1*C*). The slope of the relationship of per-flower outcross success to selfing rate, calculated from the data in figures 2 and 3, is -3.99, indicating that, on a per-flower basis, as display size increases, each additional seed sired through geitonogamy is associated with a loss of 3.99 seeds sired by outcrossing. In other words, the advantage of large displays associated with increased geitonogamous selfing is more than offset by the disadvantage of decreased male outcross success.
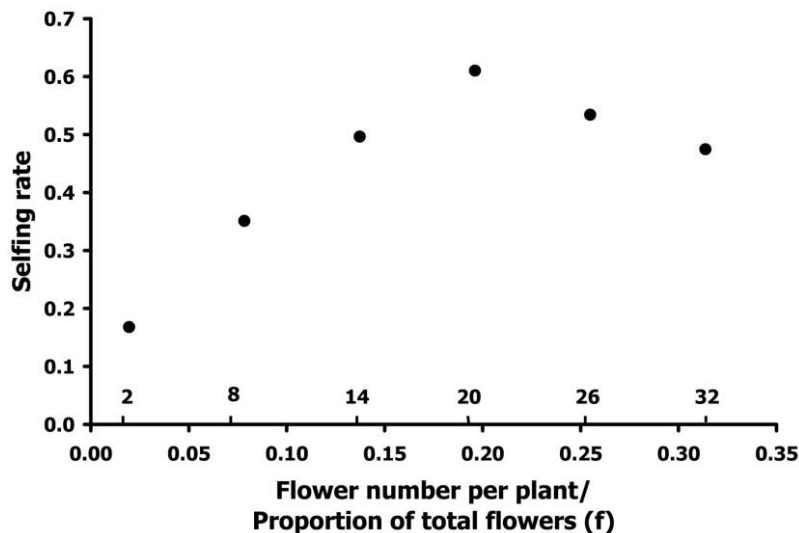

Figure 2: Relationship of female selfing rate to display size in *Ipomoea purpurea*. The *X*-axis is given in two scales: the proportion of all flowers in an array that were on both plants of a given treatment and the number of flowers per individual plant.

Table 2: Values for female selfing rate, male outcross success, and total male fitness obtained from an experiment where flower number was manipulated for an experimental array of common morning glory *Ipomoea purpurea*

	Flower number per plant					
	2	8	14	20	26	32
Proportion of flowers in experiment	.020	.078	.137	.196	.255	.314
Selfing rate[a]	.168	.351	.496	.610	.534	.475
Male outcross success[a]	.051	.120	.116	.208	.236	.269
Outcross success/flower[a]	2.616	1.526	.844	1.060	.926	.858
Total male fitness[a]	2.354	9.016	15.078	20.540	25.376	29.632
Total male fitness/flower[a]	1.467	1.108	.915	1.136	.994	.901
Total male fitness[b]	3.054	8.394	12.941	23.120	26.135	28.356
Total male fitness/flower[b]	1.527	1.049	.924	1.156	1.005	.886

Note: Male outcross success is the proportion of outcrossed seeds in the experiment that were sired by flowers from indicated treatment. Male outcross success per flower is the number of successful outcrossed seeds per flower. Total male fitness is the number of seeds sired by a plant in indicated treatment. Total male fitness/flower is the total number of seeds sired by a flower on a plant in indicated treatment.

[a] Values obtained from likelihood analysis.

[b] Values obtained from regression analysis.

This inference is supported by an additional regression analysis of per-flower total male fitness using the observed number of seeds sired for each genotype. This analysis indicated that the slope of the relationship of seeds sired per flower to flower number (as well as flower number squared) differed among genotypes (flower number × genotype interaction: $F = 9.83$, df $= 5, 18$, $P = .0001$; (flower number)^2 × genotype interaction: $F = 2.89$, df $= 5, 18$, $P = .0436$). Despite this heterogeneity, however, the average relationship was negative, with a slope of -1.508, as indicated by the flower number effect in the analysis ($F = 12.82$, df $= 1, 18$, $P = .0021$). Deviation from linearity, as tested by the significance of the main quadratic effect, was not detected (flower number^2: $F = 3.17$, df $= 1, 18$, $P = .0920$). Estimates of total male fitness from this analysis are similar to (table 2) and highly correlated with ($r = 0.99$) estimates from the likelihood analysis.

Flower Failure

The proportion of failed flowers per plant increased significantly with display size ($F = 13.26$, df $= 1, 17$, $P < .002$). This effect was minor, however, with each additional flower in a display increasing the proportion of failed flowers by 0.02 (± 0.01). The effects of genotype, genotype × display size, and day were all nonsignificant ($P > .25$). This result suggests that if there is an effect of display size on seed production per capsule, it is likely negative.

Discussion

The temporal pattern of floral presentation varies greatly among plant species. In some species, all flowers that a plant produces open over a very short period of time, whereas in other species, only a few flowers are open at any one time, while the total flowering period is relatively long (Bawa 1983). Although a number of advantages and disadvantages of mass versus extended flowering have been suggested (Bawa 1983; de Jong et al. 1992), much recent work directed at understanding the evolution of display size has focused on assessing the contribution of selection through male function in shaping display size (Burd and Callahan 2000; Harder et al. 2004). As explained by de Jong et al. (1992), the most relevant measure of male fitness in this context is number of successful pollen grains per flower, since it is generally assumed that the total number of flowers produced by a plant over a flowering season is constrained by available resources. Thus, evolution of display size represents selection among different strategies for temporally allocating these resources to flower production.

The pattern and magnitude of selection through male function on display size depends on the relationship of male fitness to display size (reviewed by Burd and Callahan [2000] and Harder et al. [2004]). This relationship has two components, corresponding to the two components of male success: self- and outcross pollination (de Jong et al. 1992). Theoretical arguments (Lloyd 1992; de Jong et al. 1993; Harder and Barrett 1996), supported by extensive empirical investigation (see de Jong et al. 1992; Snow et al. 1996; for a review, see Harder et al. 2004), including our results, indicate the general form of the first com-
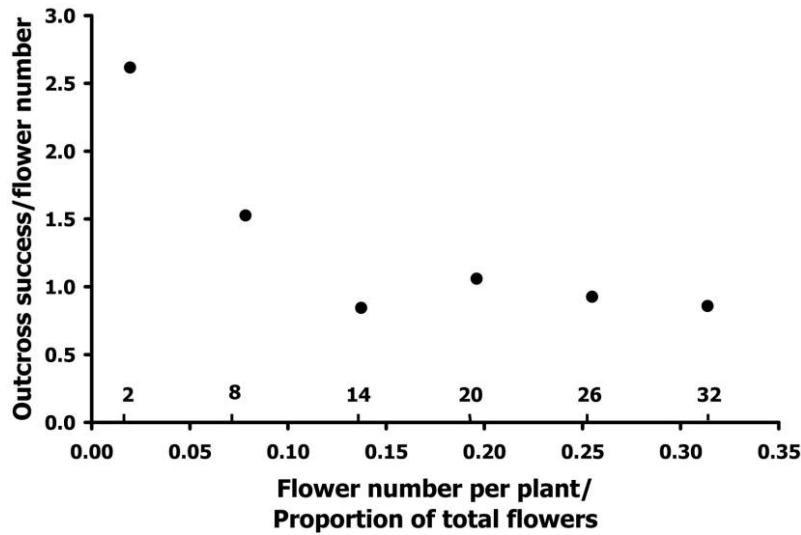
Figure 3: Relationship of male outcrossing success per flower number (i.e., number of pollen grains fertilizing ovules on other plants per flower) to display size in *Ipomoea purpurea*. X-axis as in figure 2.

ponent: increased floral display size generally is accompanied by increased geitonogamous selfing. As long as there is little or no decrease in male outcross success and little or no inbreeding depression associated with this increased selfing, selection through male function will tend to favor large displays.

The second component of male fitness is male outcross success. Theoretical expectations for the relationship of this component to display size are less clear. Models of

pollen export predict that per-flower outcross success will decrease with increasing number of visits to flowers on the same plant and thus with display size (Lloyd 1992; de Jong et al. 1993; Morris et al. 1994; Harder and Barrett 1996). This decrease is expected because pollen picked up by pollinators is lost on visits to other flowers on the same plant through selfing and grooming and is unavailable for export. However, these models do not take into account the possibility that larger displays often attract more pol-
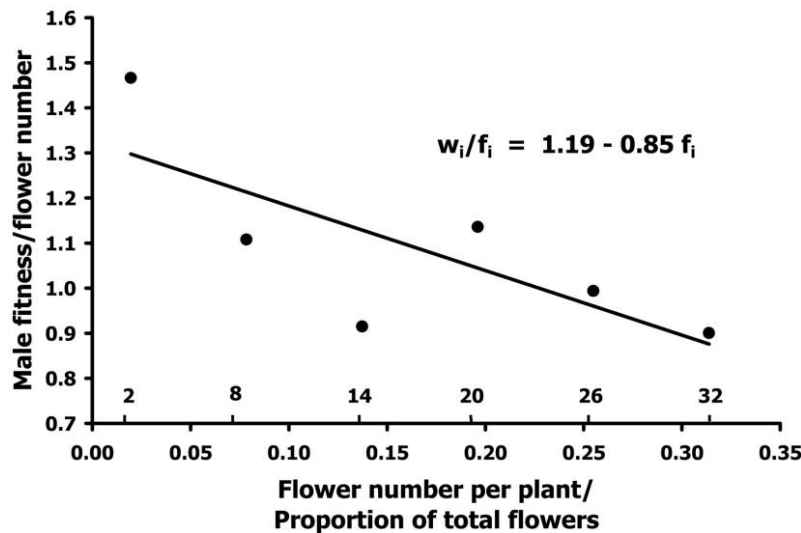

Figure 4: Relationship of total male fitness per flower number (i.e., number of pollen grains fertilizing flowers in the array per flower) to flower number from an experiment involving *Ipomoea purpurea*. The equation and trend line for the linear relationship are given. X-axis as in figure 2.

linators (Ohashi and Yahara 2001). If large displays enhance pollinator visitation enough, an increase in pollen removed by pollinators may more than compensate for the decreasing fraction of pollen exported and may result in large displays exporting more pollen per flower than small displays. Empirical support for a negative effect of display size on pollen export has been provided by several studies (Harder and Barrett 1995; Rademaker and de Jong 1998) in addition to this one, although it appears to be absent in others (Rademaker and de Jong 1998). Because increased visitation and increased geitonogamy have opposite effects on outcross success, it is unclear whether predictions can be made a priori about their net effect.

It is precisely the sign of this net effect, however, that determines whether selection through male fitness favors large or small displays. If outcross success increases with display size, then this effect will supplement the effects of increased geitonogamy in favoring large display sizes. By contrast, if outcross success declines with display size and this effect is large enough, it may counteract the effects of increased geitonogamy to favor small displays.

The effect of display size on total per-flower male fitness, as determined by a combination of these two components (self- and outcross pollination success), has been assessed in only one previous investigation. Using dye as a pollen analog, Rademaker and de Jong (1998) found that in *Cynoglossum officianale*, total per-flower male fitness increased with display size because of increased geitonogamy and no detectable decrease in pollen export. They also found that in *Echium vulgare* and *Oenothera erythrocepala*, there was little effect of display size on per-flower male fitness because an increase in geitonogamy with increasing floral display size was counteracted by a decrease in outcross success.

Although Harder and Barrett (1995) did not specifically report the relationship of per-flower total male fitness to display size, it is possible to calculate this effect from the data they presented (see the appendix). These calculations demonstrate that in *Eichhornia paniculata*, selection through male function favors large display size despite a decrease in pollen export with larger display sizes.

Our results, in combination with those of Harder and Barrett (1995) and Rademaker and de Jong (1998), suggest that while geitonogamous selfing generally increases as display size increases, the effect of display size on pollen export is variable among species. In turn, this variability appears to be responsible for differences among species in whether selection through male function favors or disfavors large display size. Specifically, for two species (*C. officianale* and *E. paniculata*), the magnitude of the effect of display size on pollen export is not large enough to offset the advantage of increased geitonogamous selfing. For two other species (*E. vulgare* and *O. erythrocepala*), decreased

pollen export just compensates for this advantage. Finally, our study provides the first example of a third outcome: selection through male function favors smaller display sizes because reduced pollen export more than counteracts the effects of increased geitonogamous selfing with larger display size.

One factor not explicitly considered in this discussion is the effect of inbreeding depression. Although increased geitonogamy increases the component of male fitness associated with selfing, the magnitude of this increase may be partially offset by inbreeding depression. In *Ipomoea purpurea*, inbreeding depression is moderate, approximately 12% and 24% for male and female fitness, respectively (Chang and Rausher 1999). The existence of this inbreeding depression does not, however, alter our conclusion that selection through male function in this species favors smaller displays. The effect of inbreeding depression simply reinforces this conclusion because plants with larger displays, and thus higher female selfing rates, experience greater fitness reduction due to increased inbreeding depression in their offspring.

By contrast, in cases such as *E. vulgare* and *O. erythrocepala*, in which increased geitonogamous selfing is just balanced by decreased pollen export, any inbreeding depression will tip the balance to favoring smaller displays. This is also possible for cases such as *C. officianale* and *E. paniculata*, in which the balance between geitonogamy and pollen export appears to favor large displays if inbreeding depression is strong enough. Unfortunately, no attempts have been made to examine whether inbreeding depression in these species would alter the expected pattern of selection through male function deduced from effects of display size on selfing rates and outcross success.

One possible limitation of our analyses is that some of the assumptions of our likelihood analysis may not be appropriate. We have no direct way to evaluate, for example, whether the composition of the outcross pollen pool with respect to pollen from different display size classes reaches a roughly stable equilibrium and is not greatly affected by the pollen picked up from the most recent plant visited, which could bias our estimates of both male outcross success and total male success. However, the similarity of our results for total male fitness in the likelihood analyses and the regression analysis, which makes no such assumption, suggests that deviations from this assumption are probably relatively minor. In particular, the main conclusion that (per-flower) total male fitness declines with increasing display size seems to be robust. Given this result and the clear result that selfing rate increases with increasing display size, it is a logical necessity that (per-flower) male outcross success must decrease with display size. Our results from the likelihood analysis are consistent with this conclusion.

Our likelihood analyses also assume that seed set per flower is equivalent across display-size classes. Unfortunately, we did not collect data on seed set per fruit; however, we did detect evidence that the proportion of failed flowers increased slightly with increasing display size ($F = 13.26$, df $= 1, 33$, $P < .002$). We would expect that mean seeds produced per capsule would be inversely correlated with proportion of failed flowers, which would mean that in our experiment, seed set per flower decreases slightly with increasing display size. To the extent that this is true, this result also reinforces our conclusion because decreased number of seeds per flower in larger displays would reduce the total male fitness of plants with large displays relative to plants with small displays.

Although the number of species examined is still small, the results of these studies suggest that it is primarily variation in effects of display size on pollen export, rather than that in effects on geitonogamous selfing, that determines whether selection through male function favors large or small display sizes. While the causes of this type of variation in the magnitude of pollen export are not yet understood, we suspect that they are due largely to differences among species in the mechanics of pollination and in pollinator behavior.

APPENDIX

Calculating Effect of Display Size on Total Male Fitness in *Eichhornia paniculata*

Harder and Barrett (1995) demonstrate that increased floral display size in *Eichhornia paniculata* results in both increased geitonogamy and decreased male outcross success. Although they do not report the net effect of display size on per-flower total male fitness in *E. paniculata*, it is possible to calculate this effect from their data. To do so, we use estimates of selfing rate s_i for four different display sizes obtained from their figure 2*A*. In addition, we estimate relative per-flower outcrossing success of their different display-size treatments from their reported relationship of selfing rate to o_i, the proportion of outcross seeds in their experiment that were sired by all plants with display size *i*:

$$o_i = 0.573 - 0.254 s_i. \tag{A1}$$

Harder and Barrett's o_i is equivalent to our x_i, except that it is normalized for only two treatments. To renormalize for four treatments, we calculate o_i', the proportion of outcrossed seeds sired by treatment *i* when all four display size treatments are present, as

$$o_i' = o_i / \Sigma o_i. \tag{A2}$$

Because, in Harder and Barrett's experiments, the total number of flowers per treatment is the same, s_i and o_i' represent the per-flower number of ovules sired by selfing and outcrossing, respectively. As expected, the slope of the relationship between these two quantities is negative (fig. A1), indicating a trade-off between success through geitonogamous selfing and success through male outcrossing. Moreover, the slope of this line is -0.124, indicating that as selfing rate increases, there is a net increase in total male success. This is also evident in the relationship of total per-flower male fitness to display size, which for treatment *i* is estimated by

$$W_i = s_i + o_i' \sum_{j=1}^{n} (1 - s_j). \tag{A3}$$

Despite decreasing per-flower pollen export with increasing display size, total per-flower male fitness increases with display size (fig. A2). Thus, in this species, selection through male function appears to favor larger displays, although this conclusion should be viewed as tentative because we cannot assess the significance of the positive relationship in figure A2.
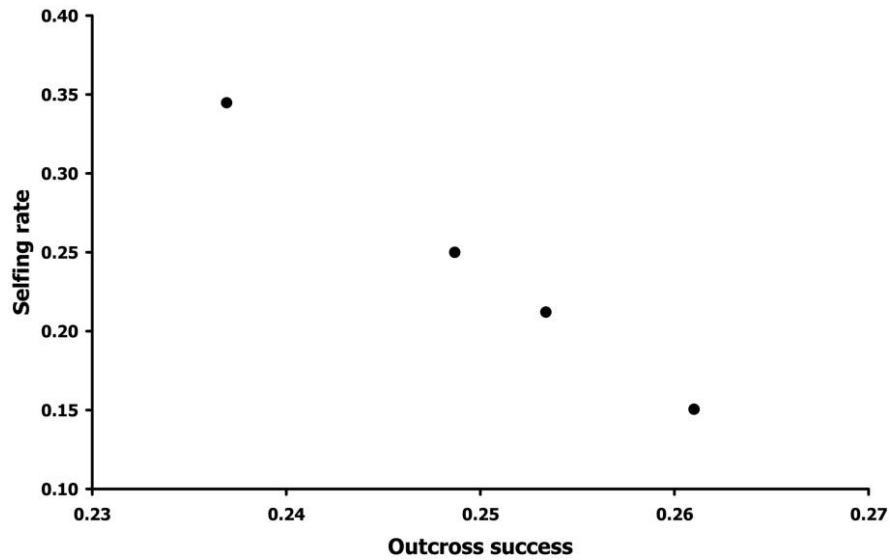
Figure A1: Relationship of outcrossing success to selfing rate in *Eichhornia paniculata*, based on results presented by Harder and Barrett (1995).
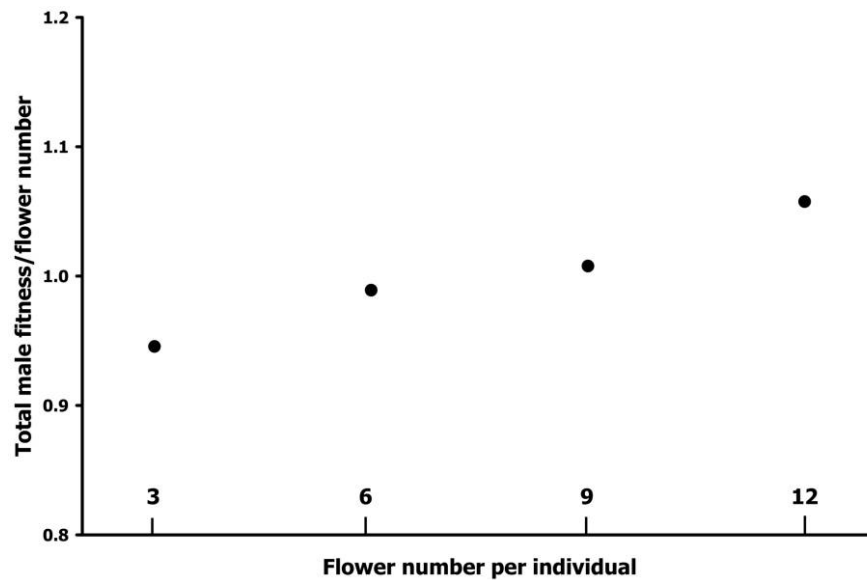

Figure A2: Relationship of total male fitness per-flower to display size in *Eichhornia paniculata*, based on results presented by Harder and Barrett (1995).

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