POLLEN DISCOUNTING AND THE SPREAD OF A SELFING VARIANT IN TRISTYLOUS *EICHHORNIA PANICULATA*: EVIDENCE FROM EXPERIMENTAL POPULATIONS

Joshua R. Kohn[1] and Spencer C. H. Barrett[2]

[1]*University of California at San Diego, Department of Biology 0116, La Jolla, California 92093*
E-mail: jkohn@ucsd.edu
[2]*Department of Botany, University of Toronto, Toronto, Ontario M5S 3B2, Canada*
E-mail: Barrett@botany.utoronto.ca

Abstract.—Floral traits that increase self-fertilization are expected to spread unless countered by the effects of inbreeding depression, pollen discounting (reduced outcross pollen success by individuals with increased rates of self-fertilization), or both. Few studies have attempted to measure pollen discounting because to do so requires estimating the male outcrossing success of plants that differ in selfing rate. In natural populations of tristylous *Eichhornia paniculata*, selfing variants of the mid-styled morph are usually absent from populations containing all three style morphs but often predominate in nontrimorphic populations. We used experimental garden populations of genetically marked plants to investigate whether the effects of population morph structure on relative gamete transmission by unmodified (M) and selfing variants (M') of the mid-styled morph could explain their observed distribution. Transmission through ovules and self and outcross pollen by plants of the M and M' morphs were compared under trimorphic, dimorphic (S morph absent), and monomorphic (L and S morphs absent) population structures. Neither population structure nor floral morphology affected female reproductive success, but both had strong effects on the relative transmission of male gametes. The frequency of self-fertilization in the M' morph was consistently higher than that of the M morph under all morph structures, and the frequency of self-fertilization by both morphs increased as morph diversity of experimental populations declined. In trimorphic populations, total transmission by the M and M' morphs did not differ. The small, nonsignificant increase in selfing by the M' relative to the M morph was balanced by decreased outcross siring success, particularly on the S morph. In populations lacking the S morph, male gamete transmission by the M' morph was approximately 1.5 times greater than that by the M morph because of both increased selfing and increased success through outcross pollen donation. Therefore, gamete transmission strongly favored the M' morph only in the absence of the S morph, a result consistent with the distribution of the M' morph in nature. This study indicates that floral traits that alter the selfing rate can have large and context-dependent influences on outcross pollen donation.

Key words.—Context-dependent transmission, *Eichhornia paniculata*, experimental populations, mating systems, pollen discounting, self-fertilization, tristyly.

Evolutionary modifications in floral morphology can affect plant fitness by altering any of three distinct features of the mating process: female reproductive success (seed set), male reproductive success through self-fertilization, and male success through outcross pollen donation. Studies of the evolutionary ecology of floral form need to take into account each of these three components of reproductive success when evaluating the costs and benefits of floral variation in hermaphroditic plants (Horovitz and Harding 1972; Horovitz 1978; Ennos 1981; Bell 1985; Devlin and Stephenson 1987; Campbell 1989; Galen 1989; Harder and Thomson 1989; Barrett and Eckert 1990; Brown 1990; Johnston 1991; Stanton et al. 1991; Kohn and Barrett 1992a). Such an assessment may be complicated, however, because the fitness consequences of a given morphology are not a static property but will likely vary with environmental and demographic contexts, including plant and pollinator densities and the morphological attributes of conspecific neighbors (Antonovics and Levin 1980; Thomson and Barrett 1981; Epperson and Clegg 1987; Gregorius et al. 1987; Holsinger 1991, 1992; Rausher et al. 1993).

Context-dependent effects on selection for or against a given morphology can affect some or all components of reproductive success. For instance, reduced spatial or temporal separation between stigmas and anthers might improve female reproductive success where pollinators are rare by increasing seed set through autogamous fertilization (Baker 1959; Lloyd 1965; Ganders 1975; Barrett 1979; Piper et al. 1984). However, if pollinators are not limiting, this alteration may

lower plant fitness in populations with high levels of inbreeding depression by increasing the frequency of self-fertilization (Charlesworth and Charlesworth 1987). Population context can also affect the selfing rate produced by a given floral morphology. In self-compatible plants the frequency of self-fertilization is likely to be influenced by both the amount of self pollen on the stigma and the amount of outcross pollen arriving from other plants. Where outcross pollen densities are low, because of low plant density or pollinator service, deposition of a given amount of self pollen may result in a higher frequency of self-fertilization than in environments where arriving outcross pollen densities are high (Bowman 1987; Barrett and Husband 1990; Holsinger 1991, 1992; Barrett et al. 1992; Becerra and Lloyd 1992).

The male outcrossing success of a particular genotype is also likely to be highly context-dependent (Ross 1977, 1990; Thomson and Barrett 1981; Epperson and Clegg 1987, MS). A morphology that increases the frequency of self-fertilization may be associated with reduced outcross pollen success, a cost known as pollen discounting (Holsinger et al. 1984; Holsinger 1991; see Nagylaki 1976; Charlesworth and Charlesworth 1979; Lloyd 1979, 1992; Wells 1979; Ritland 1991). Pollen discounting reduces the automatic selective advantage of genes for increased self-fertilization. Although pollen discounting has been a component of mating models for more than a decade, relatively few attempts to measure it have been reported (Ritland 1991; Holsinger 1992; Rausher et al. 1993). Both the magnitude of pollen discounting, measured as reduced contribution to the outcross pollen pool, and the impact that discounting has on gamete transmission can vary with population context. The relative contribution to the outcross pollen pool achieved by a given floral morphology will depend on the morphology of conspecific neighbors as well as on the behavior of pollinators. In addition, a reduction in the relative contribution to the outcross pollen pool will have a large effect on reproductive success where population outcrossing frequencies are high, but a much smaller effect where the frequency of outcrossing in the population is low. In the latter case, fewer ovules are available for fertilization by outcross pollen, so the cost of lowered representation in the outcross pollen pool is reduced. Although in most cases the reproductive consequences of floral traits will depend on the

environments in which they are measured, relatively few studies have investigated by experimental means how mating parameters can be modified by ecological or demographic conditions (Ellstrand and Foster 1983; Schoen and Clegg 1985; Kohn and Barrett 1992a; Rausher et al. 1993).

In this paper, we use experimental populations composed of genetically marked plants to evaluate the reproductive consequences of a floral modification associated with increased self-fertilization in tristylous Eichhornia paniculata (Spreng.) Solms-Laubach (Pontederiaceae). The occurrence of selfing variants of the mid-styled morph in this species is strongly associated with variation in population morph structure (Glover and Barrett 1986; Barrett and Husband 1990). Here we investigate gamete transmission by unmodified and selfing variants of the mid-styled morph (hereafter M and M' morphs) under population morph structures designed to simulate the conditions in which the selfing variants are commonly found and those in which they are rare or absent. We test the hypothesis that the pattern of occurrence of selfing variants might be explained by the effects of population morph structure on relative gamete transmission. Seed counts, coupled with the use of genetic markers, allowed us to decompose gamete transmission by the M and M' morphs into female, male selfing, and male outcrossing components and examine how population context influences each aspect of reproductive success.

The Experimental System

Eichhornia paniculata is a self-compatible, emergent aquatic, native to northeastern Brazil, Jamaica, and Cuba. It exhibits the widest range of population outcrossing rates measured in flowering plants to date ($t = 0.002-0.96$, $N = 54$ pops; Barrett and Husband 1990; reviewed in Barrett and Eckert 1990). Studies of both natural and experimental populations have shown that this variability is correlated with variation in population morph structure (Barrett and Husband 1990; Barrett et al. 1992). In much of its Brazilian range, populations are trimorphic with all three floral morphs exhibiting high outcrossing rates. In more marginal areas, populations usually lack the short-styled morph (S). Outcrossing rates in these dimorphic populations are variable with the long-styled (L) morph largely outcrossing and the mid-styled morph displaying a high degree of self-fertilization. Monomorphic

populations are usually composed of the mid-styled morph and have the lowest average outcrossing rates (Glover and Barrett 1987; Barrett and Husband 1990). In dimorphic and monomorphic populations, plants of the mid-styled morph usually display a genetic modification in which one or more of the three short-level stamens elongate so that their anthers are placed directly on or immediately adjacent to the stigma hence promoting self-pollination (Barrett 1985; Barrett and Harder 1992). These variant (M′) morphologies appear to be under the control of one or a few recessive modifier genes, with different recessives modifying stamen position in different parts of the species' geographical range (Fenster and Barrett 1994). The M′ morph has a markedly nonrandom distribution with respect to population morph structure (Husband and Barrett 1993). It is rarely found in trimorphic populations, becomes increasingly common in dimorphic populations, and is usually fixed in monomorphic populations.

The pattern of occurrence of the M′ morph raises the question, What evolutionary forces constrain its spread in trimorphic populations? At least three hypotheses, acting alone or in concert, could explain the observed rarity of the M′ morph in trimorphic populations and its spread in the absence of the S morph. (1) The recessive mutations governing the floral modifications may fail to spread in large, outcrossing populations because they are more likely to be lost through drift than to reach high enough frequencies to enable selection to act upon them (Haldane's sieve hypothesis; Haldane 1924, 1927); (2) Inbreeding depression may be high in large, outcrossing, trimorphic populations counteracting the automatic selective advantage of a gene for increased selfing (Fisher 1941; Jain 1976). Under this hypothesis, population bottlenecks, founder events, and small population sizes associated with the loss of the S morph also cause local inbreeding that purges genetic load (Lande and Schemske 1985) and favors the spread of selfing variants (inbreeding-depression hypothesis). (3) The selfing variants have no transmission advantage in trimorphic populations because of the influence of morph structure on the mating success of plants with the M′ morphology (context-dependent mating hypothesis).

Although Haldane's sieve (Turner 1981; Charlesworth 1992) is a powerful force acting against the spread of favorable recessive mutations, it is unlikely to be a general explanation for the overall rarity of the M′ morph from trimorphic populations of E. paniculata. In such populations, outcrossing frequencies average 0.83 (range 0.5–0.96, N = 27 pops; fig. 9, Barrett et al. 1992). The practice of partial self-fertilization in many trimorphic populations greatly increases the probability of recessive mutations becoming homozygous and hence being exposed to selection. If favored, these genes should spread once expressed (Charlesworth 1992).

Population outcrossing rates and levels of inbreeding depression are negatively correlated in E. paniculata. Toppings (1989; see also Barrett and Kohn 1991) measured inbreeding depression in 11 populations varying in outcrossing rate from 0.1 to 0.96. More highly outcrossed populations exhibited the strongest inbreeding depression whereas no inbreeding depression was detected in highly selfing populations. These findings are consistent with the idea that population bottlenecks that cause the loss of the S morph purge populations of sufficient amounts of genetic load to favor the spread of genes that increase the rate of self-fertilization (Barrett et al. 1989; Barrett and Charlesworth 1991). However, the alternative possibility that a reduction in genetic load followed the spread of genes that increased the rate of self-fertilization cannot be ruled out.

The goal of this study was to examine the plausibility of the context-dependent mating hypothesis, that aspects of the transmission process favor the spread of floral modifications only in the absence of the S morph. Elongation of short-level stamens in the M′ morph is thought to be favored, at least where the S morph is absent, by the automatic selective advantage of increased self-fertilization (Fisher 1941). Two forces could act in trimorphic populations to reduce or reverse this selective advantage. First, elongation of short-level stamens to the mid position could reduce the ability of plants of the M′ morph to sire seeds on the S morph. Pollen transfer in heterostylous species is more efficient between male and female organs of the same height (Darwin 1877; Ganders 1974; Barrett and Glover 1985; Piper and Charlesworth 1986; Wolfe and Barrett 1989; Lloyd and Webb 1992). Hence, elongated short-level stamens could be costly in trimorphic populations in terms of reduced outcross siring success by the M′ morph on the S morph. In the absence of the S morph, no such cost would accrue.

Second, in trimorphic populations the density

of mid-level pollen is likely to be higher than in nontrimorphic populations because both L and S morphs produce mid-level stamens. Larger amounts of mid-level pollen could reduce the amount of selfing caused by the M' morphology because more outcross pollen might be deposited on stigmas to compete with self pollen for ovules. Therefore, genes controlling the expression of the M' morphology could fail to spread in trimorphic populations both because they are costly in terms of male outcross success and because the impact of the variant's morphology on the selfing rate is reduced.

To investigate the plausibility of the context-dependent mating hypothesis, genetic transmission by plants of the M and M' morphs were compared in synthetic trimorphic, dimorphic and monomorphic populations of *E. paniculata*. We predicted that male gamete transmission would favor the M' morph over the M morph in populations lacking the S morph. In the presence of the S morph, however, both a decreased difference between the selfing rates of the M and M' morphs and reduced male outcrossing success of the M' relative to the M morph, were expected. It was predicted that these effects would reduce or reverse the selective advantage of the M' morph seen in nontrimorphic populations.

MATERIALS AND METHODS

Source of Experimental Material.—Plants used in this study were derived from seeds collected from approximately 50 open-pollinated individuals growing in population B46, located in a low-lying pasture near Quixadá, Ceará state, northeastern Brazil. This was an unusual population in that it contained all three style morphs (L, M, S morphs) and moderate frequencies of variant M' individuals. Data on population genetic parameters for the population can be found in Barrett and Husband (1990). Seeds were grown to maturity and flower buds assayed electrophoretically at one triallelic locus (*AAT*-3) and two diallelic loci (*PGI*-2) and (*AcPH*-1; for electrophoretic procedures, see Glover and Barrett 1987; Kohn and Barrett 1992a). Controlled crosses were made among 214 individuals to produce F_1 plants that were homozygous at *AAT*-3 and at one or, usually, both diallelic loci. Additional homozygous genotypes were created by crosses among F_1 plants and both F_1 and F_2 generations were used in these experiments. All plants used in these experiments were assayed electrophoreti-

cally twice to confirm their genotypes at the three marker loci.

Measurement of Floral Traits.—To quantify morphological differences between the flowers of the M and M' morphs, lengths of male and female organs were measured on two flowers of each of 15 plants. Measurements were made from the base of the perianth to the tip of each anther and to the tip of the stigma using digital calipers. Comparisons of the length of the longest and shortest stamen in each stamen level, the distance from the base of the perianth to the stigma, and stigma-anther separation (calculated as the difference between stigma height and the length of the longest short-level stamen) were made using ANOVA.

Experimental Protocols.—Experimental populations contained 36 plants each bearing a single inflorescence, placed at random in 6 × 6 array with 20 cm between plants. The total number of flowers present on plants of each morph were counted and flowers haphazardly removed from the morph(s) containing an excess until all morphs possessed equal total numbers of flowers while variation in inflorescence sizes among individuals was maintained. Populations were exposed to pollinators for a single day. Flowers of *Eichhornia paniculata* open between 9:00 and 11:00 A.M., depending on temperature, and last about 6 h. Populations were set up daily in a garden setting in Etobicoke, Ontario during August 1990, and bumblebees (*Bombus vagans* and *B. fervidus*) were the predominant floral visitors. Fruits were counted and collected after 10–12 d, at which time seeds were mature but capsules had not dehisced. Seeds from two randomly selected capsules from each plant were counted, and five seeds from each of these two fruits were assayed electrophoretically at the three marker loci as well as at a fourth polymorphic locus, *TPI*-2. The fourth locus was used to increase the accuracy of the outcrossing rate estimates. Maternal genotypes for *TPI*-2 were inferred from seed genotypes.

The six treatments LMS, LM'S, LMM', MM', M and M' were replicated three times each in a randomized design. Treatment names (boldface) reflect the morph composition of experimental populations and equal proportions of the floral types (L, M, S, and M') were used in each treatment. Thus, the LMS treatment comprised 12 plants each of the L, M, and S floral morphs, whereas the MM' treatment comprised 18 unmodified (M) and 18 variant (M') plants of the

mid-styled morph. Comparison of the reproductive success of plants of the M and M' morphs in LMS and LM'S treatments was used to assess the relative performance of the unmodified and variant morphologies in trimorphic populations. Performance of plants of the M and M' morphs in dimorphic and monomorphic populations was evaluated using the LMM' and MM' treatments, respectively. The M and M' treatments allowed an evaluation of the effects of each floral morphology in isolation. Difficulty in breeding unique marker genotypes prevented the use of an LMM'S treatment in which the performance of M and M' morphs could be compared directly in the same "trimorphic" population.

Pollinator visits were observed for the first 3 h following the onset of visitation. For each foraging bout, we recorded the morph and the number of flowers probed on each inflorescence visited in sequence. Occasionally, more than one pollinator foraged simultaneously. We followed the first arriving pollinator until it left the population and then followed the second one. We were unable to be continuously present during visitation for 4 of the 18 experimental populations (two replicates of the LM'S treatment and one each of the M and M' treatments). For these populations, we recorded the number of flowers visited during three 10-min periods, one each hour for the first 3 h of foraging.

Measurement of Mating Parameters. —In treatments that contained more than one floral morph, populations were arranged such that morphs and electrophoretic alleles at AAT-3 were in complete disequilibrium. Thus, seeds heterozygous at AAT-3 resulted from intermorph mating and the morph that sired each seed could be determined. The association of morphs and alleles at AAT-3 was varied among replicates of each treatment. In M' and M treatments, approximately equal frequencies of plants homozygous for each AAT-3 allele were used. Morph-specific maternal rates of intermorph mating (d) were measured as the proportion of seeds produced by each morph that were heterozygous at AAT-3. The multilocus maternal outcrossing rate (t) of each morph was estimated using the MLT program of Ritland (1990). For t estimation, each morph was treated as a separate population, and pollen and ovule gene frequencies were allowed to vary independently. Intramorph outcrossing rates (a) were estimated as $a = t - d$ (Kohn and Barrett 1992a).

Selfing rates $(s = 1 - t)$ were arcsine square-root transformed for ANOVA. We investigated the effects of floral morphology and population structure on male reproductive success using log-linear analysis of the number of seeds sired. In log-linear analysis of these replicated experiments, there is a difficulty in interpreting a significant χ^2 for a treatment or morph effect when there is significant variation among replicates. We evaluated morph and treatment χ^2 values using the replicate χ^2 as the denominator in a variance ratio (F test). Both numerator and denominator χ^2 values were divided by their degrees of freedom and the resulting ratio compared to the F distribution (for discussion, see Nunney 1990,).

We calculated two different estimates of pollen discounting. The first, B (suggested by K. E. Holsinger pers. comm. 1992), measures the contribution to the outcross pollen pool of the M' morph relative to the M morph under trimorphic, dimorphic and monomorphic population structures as

$$B = 1 - p_{M'}/p_M, \qquad (1)$$

where p_i is the proportion of the outcross pollen pool contributed by morph i and frequencies of the morphs under comparison are equal. For each population, outcross pollen pool frequencies at AAT-3 were calculated using the MLT program of Ritland (1990).

We also calculated the comparative pollen discounting measure D_δ proposed by Lloyd (1992). We used the formula

$$D_\delta = \frac{P_M - P_{M'}}{S_{M'} - S_M},$$

where P_i and S_i are the number of seeds sired through outcrossing and selfing, respectively, by individuals of morph i. This measure divides the (presumed) loss in outcross pollen success that accrues to the morph with the higher selfing rate by the increased pollen success through selfing. A value of one indicates that the loss in outcross pollen success exactly equals increased transmission of self pollen. A value of zero indicates increased selfing has no effect on outcross pollen transmission, whereas negative values indicate that increased selfing was accompanied by increased outcross pollen success. The number of seeds sired through selfing, S_i, was calculated as the selfing rate, s, times the number of seeds electrophoresed (120 or 180 depending on whether two or three floral morphs were present in the treatment). The number of seeds sired
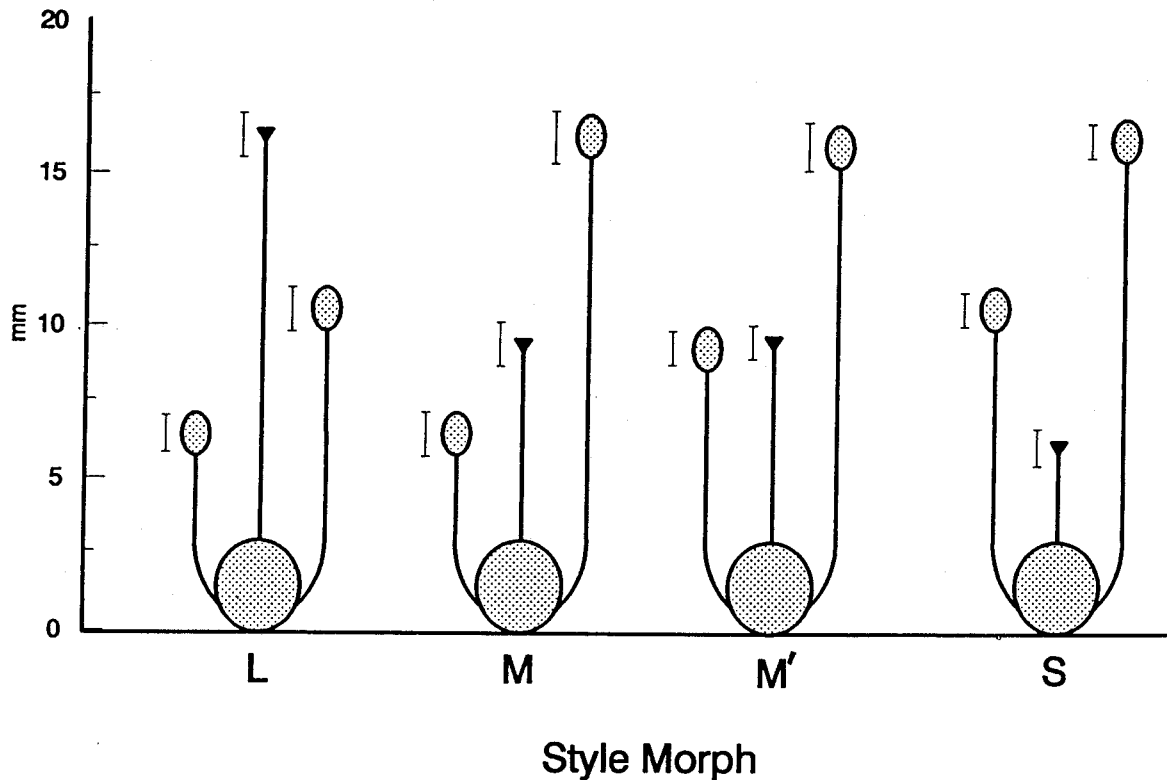
Style Morph

FIG. 1. Lengths of pistils and stamens from different floral morphs of *Eichhornia paniculata*. Bars represent ± 1 SE of the mean from 15 plants of each morph. Only the longest of the three stamens at each anther level is represented.

through outcrossing, P_i, was estimated as the number of seeds on each other morph sired by the object morph (intermorph sirings) plus a multiplied by the number of seeds sired on the object morph (120 or 180, as above), where a is the estimated rate of intramorph outcrossing. We used seed numbers rather than proportional representation in the overall outcross pollen pool to estimate D_s because the outcross pollen pool is heterogeneous among maternal morphs (see also Kohn and Barrett 1992a) and the selfing rate of morphs also differs. These facts cause estimates of male success based on representation in the (assumed homogeneous) outcross pollen pool to deviate somewhat from estimates based on actual seed paternity. For estimation of D_s, sirings on the S morph were devalued by 92/99 to reflect lower fruit set by this morph (see below).

RESULTS

Floral Measurements.—Neither stigma height nor the length of long-level stamens differed between flowers of the M and M' morph [$\bar{x}$ (SE), stigma height (mm), M = 9.4 (0.13), M' = 9.5 (0.10); long-level stamens, M = 16.2 (0.16), M'

= 15.9 (0.15); fig. 1]. These morphs differed significantly, however, in the length of both the longest and shortest of the three "short-level" stamens (longest, M = 6.4 (0.13), M' = 9.3 (0.10), $P < 0.001$; shortest, M = 4.7 (0.10), M' = 6.4 (0.22), $P < 0.001$). Increased variance in the length of the shortest short-level stamen in the M' morph reflects variation in expression of the modification (see also Seburn et al. 1990; Barrett and Harder 1992). In some plants only one stamen elongates, whereas, in others, all three stamens are affected though, in this population, only one stamen consistently reached the level of the stigma. Because of the consistent elongation of at least one of the short-level stamens in the M' morph, stigma-anther separation differed markedly between the two forms averaging 3.0 mm (SE = 0.15) in the M morph and 0.2 mm (SE = 0.09) in the M' morph ($P < 0.001$).

Pollinator Visitation.—There were no significant effects of treatment, morph, or their interaction on the number of visits to each inflorescence ($\bar{x} = 4.74$) or the number of flowers probed per inflorescence ($\bar{x} = 4.01$). However, there was significant variation among replicates within

TABLE 1. ANOVA in fruit and seed set by floral morphs in experimental populations of *Eichhornia paniculata*. In this and all subsequent ANOVA models, treatment (treat) and morph effects are fixed factors, whereas replicate (rep) is a random factor. Denominator (den) mean square for each factor as indicated. Fruit-per-flower ratios were arcsine square-root transformed prior to analysis. The average seed set of two fruits from each plant was used in the analysis of seeds per fruit. Degrees of freedom for the treatment by morph and morph by replicate interactions are reduced relative to expectations based on degrees of freedom for main effects. This reflects the fact that only a subset of morphs appears in each treatment.

		Fruits per flower			Seeds per fruit		
Source	Den	df	MS	F	df	MS	F
a = Treat	c	5	0.039	0.44	5	4300	1.64
b = Morph	e	3	0.493	8.22**	3	1005	1.11
c = Rep(treat)	e	12	0.088	1.47	12	2619	2.89*
d = Treat × morph	e	4	0.021	0.35	4	518	0.57
e = Morph × rep(treat)	f	14	0.060	3.53***	14	907	2.90***
f = Residual		609	0.017		609	313	

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

treatments for both of these measures (visits per inflorescence, $F = 101.8$, $P < 0.001$; ln flowers probed, $F = 9.41$, $P < 0.001$). Thus, visitation by pollinators was not affected by morphs or treatments, but there was significant day-to-day variation in visitation rates, presumably because of varying weather conditions.

Female Reproductive Success. — The S morph set slightly fewer fruit than did the other floral morphs which did not differ significantly (table 1). Fruit set averaged 92% for the S morph and 99% for the remaining floral morphs. Because neither the number of plants visited by bees nor the number of flowers probed differed among floral morphs, lower fruit set by the S morph may reflect less efficient pollen transfer by bumblebees from short-level stamens to stigmas of the S morph (see Harder and Barrett 1993).

Seeds per fruit averaged 85.2 (SE = 0.6, $N = 1296$) and did not differ among morphs or treatments nor was the morph by treatment interaction significant (table 1). The significant rep-

licate within treatment effect indicates differences in average seed set among replicates of the same treatment. The significant morph by replicate interaction for both fruit and seed set indicates replicate-specific differences among morphs within at least some treatments. Morph by replicate effects could result either from variation in pollinator service among morphs within replicates or differences in the vigor (probability of setting fruit or seed) of plants representing each morph on different days.

Frequency of Self-Fertilization. — The average frequency of self-fertilization in the M' morph was higher than the M morph in trimorphic (M morph in the LMS treatment vs. M' morph in the LM'S treatment), dimorphic (M and M' morphs compared within the LMM' treatment), monomorphic (MM') and isolated (M and M' treatments) population structures (table 2). The combined probability of this result is statistically significant, but differences in the selfing rates of the M and M' approached significance only with-

TABLE 2. Comparison of the frequency of self-fertilization by the M and M' morphs of *Eichhornia paniculata* under experimental trimorphic (M in LMS treatment contrasted with M' in LM'S treatment), dimorphic (LMM'), monomorphic (MM'), and isolated (M versus M') population structures. The frequency of self-fertilization in the M' morph is higher than the M morph under all conditions. Combined probability of this outcome ($\chi^2_8 = 18.94$, $P < 0.05$, Sokal and Rohlf 1981, p. 780). Differences approach statistical significance only within LMM' and M versus M' treatment comparisons. Arcsine square-root tranformation of selfing rates prior to analysis successfully removed heteroscedasticity.

	Treatment(s)			
	LMS vs. LM'S	LMM'	MM'	M vs. M'
		Mean (SE)		
M	0.39 (0.10)	0.39 (0.07)†	0.26 (0.07)	0.51 (0.05)†
M'	0.53 (0.05)	0.65 (0.06)	0.78 (0.03)	0.70 (0.04)

† $0.06 < P < 0.05$.

TABLE 3. Analysis of variance for the effect of morph structure (trimorphic, dimorphic, monomorphic, or isolated) and morph on the frequency of self-fertilization by the M and M' morphs in experimental populations of *Eichhornia paniculata*. The morph × structure interaction is evaluated only for dimorphic (LMM') and monomorphic (MM') morph structures because morphs are nested within replicates of LMS, LM'S, M, and M' treatments. Removal of the replicate effect from the model allows evaluation of the morph × structure interaction over all conditions. If the replicate effect is removed, the significance levels of structure and morph effects are inflated but their interaction remains nonsignificant ($F = 0.4$, NS; df = 3, 16).

	df	MS	F
Structure	3	0.073	6.16**
Morph	1	0.234	14.04*
Morph × structure	1	0.008	0.48
Rep(structure)	12	0.012	0.75
Residual	4	0.015	

* $P < 0.05$; ** $P < 0.01$.

in the LMM' treatment and in the comparison of the M and M' treatments. Analysis of variance indicated that population morph structure significantly affected the frequency of self-fertilization in the M and M' morphs (table 3) with the monomorphic (MM') population structure producing significantly higher selfing rates than trimorphic population structure and all others intermediate (Sheffe's test, $P < 0.05$). After removing replicate effects so that the interaction

could be evaluated across all population morph structures, the interaction of population structure and floral morphology on the frequency of self-fertilization was not significant. Hence, population morph structure affected the frequency of self-fertilization by the M and M' morphs similarly.

Male Reproductive Success. — Male reproductive success was analyzed using log-linear analysis of the number of seeds sired by the M and M' morphs in each replicate. The number of seeds sired was adjusted to account for the lower fruit production by the S morph and for small differences in the number of seeds electrophoresed from each morph in each replicate. Total male reproductive success (self plus outcross pollen success) was compared for M and M' morphs under trimorphic (LMS and LM'S treatments), dimorphic (LMM' treatment) and monomorphic (MM') population structures.

In trimorphic conditions, the number of seeds sired in each replicate by M and M' morphs was not significantly different (table 4A) averaging 163 (SE = 15) and 165 (SE = 8), respectively. In both the LMM' and MM' treatments, the M' morph sired close to 1.5 times as many seeds, on the average, as did the M morph [$\bar{x}$ (SE); LMM' treatment, M = 106 (11), M' = 169 (9); MM' treatment, M = 146 (20), M' = 214 (20)], giving highly significant morph χ^2 values (table 4A). However, these differences were not signif-

TABLE 4. Log-linear analysis of total seeds sired by M and M' floral morphs of *Eichhornia paniculata* und. experimental trimorphic (M in LMS treatment contrasted with M' in LM'S treatment), dimorphic (LMM' treatment), and monomorphic (MM' treatment) population structures. (A) Differences among morphs within population structures. The significance of the morph effect is tested using a variance ratio (*F*-test) in which the numerator is the morph χ^2 divided by its degrees of freedom and the denominator is the replicate χ^2 divided by its degrees of freedom. The nonsignificant treatment effect ($\chi^2 = 0.82$, df = 1, $F = 0.10$) has been removed from the combined analysis of dimorphic and monomorphic structures. (B) Planned contrast between trimorphic and nontrimorphic population structures. The significant *F*-test indicates trimorphic and nontrimorphic population structures differ in their effects on the siring success of M and M' morphs.

	Trimorphic			Dimorphic			Monomorphic			Dimorphic & monomorphic		
	χ^2	df	F	χ^2	df	F	χ^2	df	F	χ^2	df	F
A. Differences among morphs within population structures												
Factor												
Morph	0.18	1	0.06	44.08	1	14.27†	38.82	1	2.96	82.78	1	12.41*
Replicate	11.08	4		6.18	2		26.22	2		33.34	5	

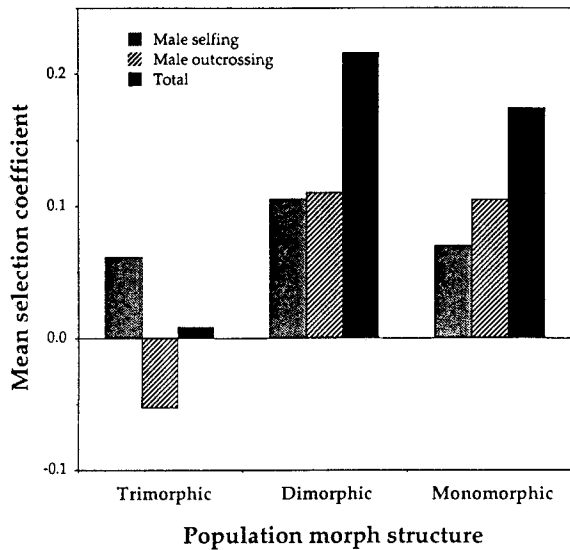|	χ^2	df	F
B. Planned contrast between trimorphic and nontrimorphic population structures			
Factor			
Structure	26.70	1	5.41*
Replicate	44.42	9	

* $P < 0.05$.
† $0.1 > P > 0.05$.

FIG. 2. Mean selection coefficient $[I = 1 - (W_M)/(W_{M'})]$ favoring the M' over the M morphology under different morph structures in experimental populations of *Eichhornia paniculata*. The total selection coefficient was decomposed into male selfing $[(S_{M'} - S_M)/W_{M'}]$ and male outcrossing $[(P_{M'} - P_M)/W_{M'}]$ sources to illustrate the strength and direction of selection on different components of reproductive success.

icant within either treatment when tested over the heterogeneity among replicates. Comparison of the siring success of M and M' morphs in **LMM'** and **MM'** treatments revealed no significant effect of population morph structure on the relative male reproductive success of the M and M' morphs. When data from these two treatments are combined, the difference in siring success between M and M' morphs is significant when tested over the variation due to replicates (table 4A).

We then tested the prediction that the presence of the S morph within experimental populations affected the relative siring success of the M and M' morphs. The error term for this comparison was the sum of the replicate χ^2 values for the analyses with the S morph present or absent. The significant effect of population morph structure (table 4B) indicates that trimorphic and nontrimorphic conditions affected the male reproductive success of the M and M' morphs differently. Specifically, The M' morph had a strong transmission advantage in the absence of the S morph, but no significant siring advantage over the M morph in the presence of the S morph (fig. 2).

Male reproductive success of the M and M' morphs was then decomposed into selfing and

outcrossing components. The number of seeds sired through outcrossing by each morph was calculated based on the number of seeds sired on other morphs and the morph-specific estimate of assortative outcrossing (Kohn and Barrett 1992a). In trimorphic populations, the M morph had slightly higher outcross male success than did the M' morph but this difference was not significant (table 5A). In the absence of the S morph, outcross male success of the M' morph exceeded that of the M morph. As in the analysis of total siring success, when trimorphic and nontrimorphic population structures are contrasted, there is a significant influence of morph structure on the relative male outcross success of the M and M' morphs (table 5B, fig. 2).

As predicted, the ability of the M' morph to sire seeds on the S morph is lower than that of the M morph (table 6). In the **LMS** treatment, the M morph sired more seeds on the S morph than does the L morph, a mating asymmetry previously reported in an independent set of three replicated trimorphic populations (Kohn and Barrett 1992a). However, in the **LM'S** treatment, the L morph sired more seeds of the S morph than did the M' morph. Of seeds produced by the S morph that resulted from intermorph mating, the fraction sired by M and M' morphs differed significantly (table 6).

Pollen Discounting. —Under trimorphic conditions, *B* was weakly positive because of slightly reduced average representation in the outcross pollen pool by the M' morph relative to the M morph (table 7). The value of D_δ under trimorphic conditions was 0.87, reflecting the fact that lost outcross pollen success by the M' morph nearly equaled increased success through self pollen. In dimorphic (**LMM'**) populations, however, *B* was negative because of the M' morph's having both a higher selfing rate and increased outcrossed pollen success. D_δ for this treatment was near negative one because increased transmission through outcross pollen by the M' morph was about equal to increased success through selfing (fig. 2). Monomorphic (**MM'**) populations exhibited large variation among replicates, but average values of *B* were less than negative 1, reflecting the more than twofold advantage in the average outcross pollen success of the M' morph relative to the M morph (table 7). The value of D_δ for the **MM'** treatment was less than negative one indicating that increased gamete transmission through outcross pollen donation by the M' morph relative to the M morph was

TABLE 5. Log-linear analysis of siring success by M and M' morphs of *Eichhornia paniculata* under experimental trimorphic (M in LMS treatment contrasted with M' in LM'S treatment), dimorphic (LMM' treatment), and monomorphic (MM' treatment) population structures. (A) Differences among morphs within different population structures. The significance of the morph effect is tested using a variance ratio (F-test) in which the numerator is the morph x^2 divided by its degrees of freedom and the denominator is the replicate x^2 divided by its degrees of freedom. The treatment effect in the comparison of dimorphic and monomorphic conditions was not significant when tested over the heterogeneity among replicates ($x^2 = 9.37$, df = 1; $F = 1.01$, NS) and has been removed from the combined analysis of dimorphic and monomorphic structures. (B) Planned contrast between trimorphic and nontrimorphic population structures.

	Trimorphic			Dimorphic			Monomorphic			Dimorphic & monomorphic		
A. Differences among morphs within different population structures												
Factor	x^2	df	F	x^2	df	F	x^2	df	F	x^2	df	F
Morph	3.60	1	0.75	20.04	1	82.30*	46.23	1	2.52	66.24	1	8.25*
Replicate	18.95	4		0.49	2		36.63	2		40.10	5	

B. Planned contrast between trimorphic and nontrimorphic population structures

Factor	x^2	df	F
Condition	44.79	1	6.82*
Replicate	59.05	9	

* $P < 0.05$.

greater than increased transmission through selfing.

To illustrate how changes in the frequency of self-fertilization and representation in the outcross pollen pool are reflected in overall reproductive success, mean selection coefficients (one minus the reproductive success of the M relative to the M' morph) were calculated for each population structure (fig. 2). Total reproductive success of each morph (W_i) was estimated as

$$W_i = 2S_i + U_i + P_i,$$

where S_i and P_i are defined as above and U_i is the number of outcrossed seeds produced on each maternal morph (t multiplied by 120 or 180, depending on whether two or three morphs were present in the treatment). Since seed set did not differ between the M and M' morphs, selection was only due to effects on the male component of fitness. For comparison, the mean selection coefficient was decomposed into male selfing [(S_M' − S_M)/W_M'] and male outcrossing [(P_M' − P_M)/W_M'] components (fig. 2). In the absence of the S morph, both increased selfing and increased male outcrossing success contributed to the strong selective advantage favoring the M' over the M morph.

Heterogeneity among Replicates. — Two striking examples of heterogeneity among replicates of the same treatment occurred during our experiments. In the third replicate of the LMS

treatment, the frequency of self-fertilization in the M morph was markedly higher than that of the other two morphs [s (SE); L, 0.145 (0.057); M, 0.578 (0.078); S, 0.209 (0.154)] and higher than the selfing rate of the M morph in other replicates of this treatment including three performed in a previous year (table 7; Kohn and Barrett 1992a). Both the number of plants visited and the number of flowers probed per visit were lowest for the third replicate of the LMS treatment (plants visited, $F = 55.9$; df = 2, 4; $P < 0.01$; flowers probed per visit, $F = 13.4$; df = 2,

TABLE 6. Intermorph siring success on the S morph in the LMS and LM'S experimental populations of *Eichhornia paniculata*. Values are the numbers of seeds of the S morph sired by each morph. The M morph has increased siring success relative to the L morph in the LMS treatment while the siring success of the M' morph is low relative to the L morph in the LM'S treatment. The percentage of intermorph sirings on the S morph by the M and M' morphs differs significantly (unpaired t test, $t_4 = 4.56$, $P < 0.02$).

	Treatment					
	LMS			LM'S		
Morph	L	M	M (%)	L	M'	M' (%)
Replicate						
1	25	54	68.3	64	41	39.0
2	28	74	72.5	62	55	47.0
3	39	52	57.1	49	34	41.0

TABLE 7. Mating parameters and siring success of M and M' morphs of *Eichhornia paniculata* under experimental trimorphic, dimorphic, and monomorphic populations structures. The frequency of self-fertilization (s), and the outcross pollen pool frequency (p) for each morph were calculated using the MLT program of Ritland (1990). Bootstrapped standard errors for s and p averaged 0.06 (range 0.037–0.095) and 0.05 (range 0.035–0.072), respectively. S, P, and T represent the number of seed assayed electrophoretically that were sired by each morph through selfing, outcross pollen donation, and their sum (total seeds sired), respectively (see Materials and Methods). Two measures of pollen discounting are presented: $B = 1 - p_M'/p_M$ represents the reduction (or increase) in outcross pollen representation by the M' morph relative to the M morph. $D_s = (P_M - P_M')/(S_M' - S_M)$ represents the change in success through outcross pollen relative to the change in success through self pollen. For the trimorphic comparison (LMS versus LM'S), B and D_s are calculated only for the mean since morphs were nested within treatments.

Treatment	Rep	Morph	s	p	S	P	T	B	D_s
LMS	1	M	0.306	0.356	36.7	97.2	133.9		
LMS	2	M	0.271	0.493	32.5	142.2	177.4		
LMS	3	M	0.587	0.477	70.4	108.4	178.8		
Mean		M	0.388	0.442	46.6	115.9	162.5		
LM'S	1	M'	0.595	0.294	71.4	86.4	157.8		
LM'S	2	M'	0.424	0.484	50.9	128.9	179.7		
LM'S	3	M'	0.579	0.378	69.5	87.1	156.6		
Mean		M'	0.532	0.385	63.9	100.8	164.7	0.11	0.87
LMM'	1	M	0.368	0.270	44.2	56.9	101.1		
LMM'	1	M'	0.771	0.471	92.5	93.6	186.1	-0.74	-0.76
LMM'	2	M	0.522	0.296	62.6	63.4	126.0		
LMM'	2	M'	0.581	0.405	69.7	89.3	159.0	-0.37	-3.64
LMM'	3	M	0.277	0.228	33.2	56.2	89.4		
LMM'	3	M'	0.604	0.402	72.5	88.6	161.1	-0.76	-0.82
Mean		M	0.389	0.265	46.7	58.8	105.5		
		M'	0.652	0.426	78.2	90.5	168.7	-0.61	-1.01
MM'	1	M	0.714	0.532	128.5	55.4	183.9		
MM'	1	M'	0.709	0.468	127.6	48.2	175.8	0.12	-8.00*
MM'	2	M	0.491	0.219	88.4	30.6	119.0		
MM'	2	M'	0.813	0.781	146.3	94.7	241.0	-2.56	-1.11
MM'	3	M	0.674	0.112	121.3	13.0	134.3		
MM'	3	M'	0.815	0.888	146.7	78.8	225.5	-6.93	-3.60
Mean		M	0.626	0.288	112.7	33.0	145.6		
		M'	0.779	0.712	140.2	73.9	214.1	-1.47	-1.49

* M morph exceeded M' morph in both self and outcross pollen success in this replicate.

4; $P < 0.05$), but visitation to morphs within replicates did not differ significantly (neither the morph nor morph by replicate interactions were significant). If variation in pollinator visitation was the cause of the fluctuation in frequency of self-fertilization, the floral morphs differed in their response.

In the first replicate of the MM' treatment, the morphs were very similar in both the frequency of self-fertilization and male reproductive success, whereas they differed markedly in both of these parameters in the other two replicates (table 7). Data on visitation rates is of little help in explaining this variation. We were not continuously present to observe visitation during the first replicate of this treatment but recorded 11 visits to M inflorescences and 20 to M' inflorescences during three 10-min observation periods indicating that, if anything, flowers of the M' morph were visited more frequently in this replicate. In the second and third replicates, the numbers of visits to M and M' morphs did not differ significantly (second replicate, M = 127, M' = 136; third, M = 75, M' = 78) but transmission substantially favored the M' morph.

DISCUSSION

In our experimental populations, floral morph structure did not affect seed set but had profound effects on relative male gamete transmission by unmodified and selfing variants of the M morph. Under trimorphic conditions, transmission of male gametes by the M and M' morphs was not significantly different. The M' morph had somewhat elevated levels of self-fertilization com-

red with the M morph, but this advantage was canceled out by a reduced ability to sire seeds through outcrossing. Apparently as a consequence of the elongation of short-level stamens, the M' morph was less successful than the M morph at siring seeds of the S morph. In the absence of the S morph, however, both the frequency of self-fertilization and the male outcrossing success of the M' morph were higher than the M morph. Thus, gamete transmission strongly favored the M' morph only in experimental populations in which the S morph was absent. This pattern of transmission is consistent with the occurrence of the M' morph in natural populations; the variant phenotype is rarely found in trimorphic populations but becomes prevalent in populations that lack the S morph.

Pollen Discounting. — In the absence of the S morph, male gamete transmission by the M' morph exceeded that of the M morph. This occurred both because the frequency of self-fertilization was elevated and because of increased outcross pollen success. These two changes in mating parameters resulted in negative estimates of pollen discounting in our experimental populations. Negative pollen discounting increases the level of inbreeding depression necessary to prevent the spread of genes that increase selfing above the 0.5 level common to models of mating-system evolution (Lande and Schemske 1985; Charlesworth and Charlesworth 1990). Inspection of figure 2 indicates that, in the absence of the S morph, the floral modification affected the male outcross component of gamete transmission as strongly (LMM' treatment) or more strongly (MM' treatment) than it affected the male selfing component. If these same transmission dynamics occurred in nature, the M' morph could increase in frequency relative to the M morph in dimorphic (LMM') populations even in the face of inbreeding depression values near one. This holds because increased male outcross success would balance lost transmission caused by the death of self-fertilized seeds. In monomorphic (MM') populations, the frequency of the M' morph would increase regardless of the value of inbreeding depression because the benefits in terms of increased outcross male success would outweigh losses resulting from the zero fitness of selfed seed. However, in trimorphic populations, even very low levels of inbreeding depression would prevent the invasion of the M' morph.

Pollen discounting is often assumed to be negligible in theoretical models of mating-system evolution. This assumption has been made both to obtain boundary conditions for other selective forces such as inbreeding depression (e. g., Charlesworth and Charlesworth 1979, 1987; Lloyd 1979) and because it is often assumed that the effects of discounting will be slight compared to those of inbreeding depression. This assumption may at first seem reasonable because it would appear to require only a very small fraction of the pollen produced by most flowers to fertilize their own ovules (Cruden 1977). This view, however, fails to take into account how changes necessary to bring about increased self-fertilization affect other aspects of gamete transfer and may have resulted in an overemphasis on the value of inbreeding depression as the single critical parameter for understanding the dynamics of mating-system evolution.

The way genes act to alter the frequency of self-fertilization will be of critical importance for determining the level of pollen discounting. If genes that change the frequency of self-fertilization alter only the reaction between self pollen and the pistil, with no effects on floral morphology (reviewed in Nettancourt 1977; Shore and Barrett 1986), then little change in outcross pollen donation would be expected. However, if increased selfing is brought about by changes in floral architecture, such as reduced stigma-anther separation (e.g., Breese 1959; Piper et al. 1984; Ganders et al. 1985; Shore and Barrett 1990), other aspects of gamete transmission are likely to be affected. In particular, bringing anthers closer to the stigma may also bring them nearer to the position within the flower that stigmas occur on other plants. This modification could increase the proficiency of pollen donation to other plants while also increasing self pollen deposition, as our data from experimental populations lacking the S morph suggest. Theories on the evolution of herkogamy (stigma-anther separation) have sometimes suggested a balance between selection for increased outcross pollen transfer and selection for decreased self-fertilization implying that in this context the concept of negative pollen discounting has been appreciated (Darwin 1862; Müller 1883; Kerner von Marilaun 1902; Brantjes and Bos 1980; but see Webb and Lloyd [1986] for an opposing view on the effect that reduced stigma-anther separation might have on pollen donation). However, models of mating-system evolution generally have not considered the possibility that genes that in-

crease the frequency of self-fertilization might also increase outcross pollen transfer.

Other floral modifications that increase selfing have contrasting effects on outcross pollen success. Ritland (1991) took advantage of linkage disequilibrium of marker loci in a hybrid zone between the small-flowered *Mimulus nasutus* and the large-flowered *M. guttatus* to estimate pollen discounting. The small-flowered species exhibited a higher frequency of self-fertilization but contributed little to the outcross pollen pool leading to large positive values of discounting. Reduction in flower size is often a correlate of increased rates of self-fertilization (Lloyd 1965; Ornduff 1969; Wyatt 1988) and may commonly reduce outcross pollen donation. Another related floral alteration associated with increased selfing, which may have strong negative effects on outcross pollen donation, is reduction in floral attractiveness to pollinators. Holsinger (1992) found high levels of pollen discounting in the non-radiate morph of *Senecio vulgaris*. The non-radiate morph has a higher selfing rate but attracts fewer pollinators than the radiate morph (Marshall and Abbott 1982, 1984; Abbott and Irwin 1988).

Work with experimental populations containing the white and pigmented floral morphs of *Ipomoea purpurea* is similar to our own in exemplifying the dependence of both outcrossing rates and male outcrossing success on population morph structure (Schoen and Clegg 1985; Epperson and Clegg 1987; Rausher et al. 1993). When white-flowered morphs are at higher frequency than pigmented morphs in experimental populations, the frequency of self-fertilization and the male outcrossing success of both morphs are equal. When morphs are at equal frequency, selfing rates are similar but white-flowered plants have greater male outcrossing success. When white-flowered plants are in the minority, they are undervisited by pollinators, which apparently leads to increased self-fertilization. Under white-minority conditions however, the white-flowered morph exhibits a small male outcrossing advantage over the pigmented morph, an advantage limited to sirings on other white flowers. The cause of increased male success of white-flowered plants when at low or equal frequency has not been elucidated.

In *Eichhornia paniculata,* increased selfing arises by alterations in the relative positions of reproductive parts rather than by changes in flower size or attractiveness (Morgan and Barrett 1990). The consequences for pollen donation are strong and in the opposite direction from those documented in *Mimulus* and *Senecio*. Under nontrimorphic conditions, increased selfing in *E. paniculata* is associated with strongly increased male outcrossing success. The effects on outcross pollen donation seen in this study and those reviewed above clearly caution against ignoring the potential significance of pollen discounting in both theoretical models and empirical studies of mating-system evolution.

Neither of the measures of discounting used here is entirely satisfactory for evaluating fitness differences between floral morphs. The measure B (K. E. Holsinger pers. comm. 1992), evaluates relative pollen pool frequencies without reference to either the magnitude of the differences between morphs in the selfing rate or the magnitude of fitness differences gained through outcrossing. The fitness gained through outcrossing will be a function of the population outcrossing rate and thus measures of pollen pool frequency alone cannot evaluate the magnitude of these differences. The second measure, D_s (Lloyd 1992), evaluates the difference in fitness though outcrossing relative to the difference through selfing. This comparative measure perhaps better characterizes the verbal definition of discounting as the cost, in terms of (decreased) outcross pollen success, that accrues to a floral phenotype with increased levels of self-fertilization (Holsinger et al. 1984). However, values of this parameter do not reflect the magnitude of the absolute fitness differences between the morphs. For instance, when the difference between the selfing rates of two morphs are small, values of D_s are likely to be large, even where the difference in absolute fitness of the two morphs is very small (table 7, see replicate 1 of the MM′ treatment for example). Thus, D_s is informative as to the *direction* of selection acting at the gamete transmission stage when changes in both self and outcross pollen success occur but does not indicate the *magnitude* of selection favoring one morph or the other. This requires calculation of the total reproductive success of each morph.

Floral Morphology and Male Fitness. —Why should the male outcrossing success of the M′ morph be greater than that of the M morph in populations lacking the S morph? Our previous work with experimental populations has shown a general male reproductive advantage accrues to morphs possessing longer-level stamens (i. e., M and S morphs, fig. 1). Evidence for the benefit

of longer-level stamens comes from the mating asymmetries found in experimental trimorphic populations and from manipulative experiments on floral morphology. In trimorphic (LMS) populations, the L morph is less likely to sire seeds on the S morph than is the M morph and the L morph is also less likely to sire seeds on the M morph than is the S morph (Kohn and Barrett 1992a). The L morph lacks the long-level anthers present in the other two morphs. In an experiment in which the contribution of particular stamen levels to male reproductive success was assessed, we demonstrated that long-level anthers of the S morph were largely responsible for its advantage over the L morph in siring seeds on the M morph (Kohn and Barrett 1992b). Longer-level stamens in tristylous species of *Eichhornia* and in related *Pontederia cordata* dehisce earlier than shorter-level stamens and may also contact pollinators more efficiently thus transferring more pollen to stigmas (Kohn and Barrett 1992b; Harder and Barrett 1993).

Elongation of one or more short-level stamens in the M′ morph not only increases selfing but also appears to allow this morph to increase its outcross siring success. Stamen elongation may improve the ability of the M′ morph to sire seeds on both the M and L morphs. In the LMM′ treatment, the M′ morph sired more seeds on the L morph than did the M morph in all three replicates although this difference was not statistically significant when tested over heterogeneity among replicates ($F = 3.2$; df = 1, 2; NS). In the same treatment, the number of seeds sired by the M′ morph on the M morph exceeded those sired by the M morph on the M′ morph ($F = 66.9$; df = 1, 2; $P < 0.05$). In the MM′ treatment, the M′ morph sired 2.9 times more seeds on the M morph than the converse but the difference was not statistically significant due to large variation among replicates ($F = 2.74$; df = 1, 2; NS). Thus stamen elongation appeared to improve outcross pollen donation onto mid- and perhaps also long-level stigmas.

Pollen-pistil interactions in *E. paniculata* may also play a role in explaining the large effects on the male outcross component of fitness caused by such a seemingly small morphological modification involving increased stamen length. In self-incompatible heterostylous species, pollen from contrasting anther levels differs in size, tapetal sculpturing, and incompatibility type (reviewed in Dulberger 1992; Richards and Barrett 1992). In self-compatible *E. paniculata,* pollen

heteromorphism is much reduced but nevertheless pollen from different anther levels has significantly different mean diameters. In particular, short-level pollen is considerably smaller than the other two pollen types whose size distributions overlap broadly (Barrett 1985). Pollen from the same level as the stigma (legitimate pollen) maintains a siring advantage over other pollen types when placed simultaneously on stigmas (Cruzan and Barrett 1993). Pollen size is correlated with pollen performance such that, of the two types of illegitimate pollen for any stigma level, the one closest in size to legitimate pollen has greater siring ability than the other illegitimate type. Genes that increase short-level stamen length in the M′ morph could affect the pollen produced, making it behave more like pollen from mid-level anthers. This could contribute to both lower siring success by the M′ morph on the S morph and an increased ability to sire outcrossed seeds on both the L and M morphs. Further experimentation is required to determine the relative roles of pollination and post-pollination phenomena on pollen transmission by the M′ morph.

Ecological Realism. — How might the transmission dynamics of the floral morphs in natural populations differ from those measured in our experimental populations? These experiments were performed using bumblebees as surrogates for the long-tongued bees that commonly pollinate *E. paniculata* in Brazil (*Ancyloscelis* and *Florilegus* spp.; Husband and Barrett 1992). Although the frequencies of outcrossing and disassortative mating in experimental and natural trimorphic populations are quite similar (fig 9; Barrett, Kohn and Cruzan 1992), other aspects of the mating process influencing male reproductive success may be affected by the use of exotic pollinators. Because of this caveat, these experiments should properly be seen as an investigation of some potential influences of floral morphology and population morph structure on genetic transmission rather than as evidence for selective mechanisms that occur in nature. The fit between our experimental results and the observed distribution of the M′ morph in natural populations, however, suggests that the effects observed in this study are worthy of future investigation in natural populations.

A flaw in our experimental design that could not be avoided, given the difficulty in breeding for unique marker genotypes, was that we did not compete the M and M′ morphs directly in

trimorphic populations (no LMM'S treatment). Given the extreme sensitivity of relative transmission to population morph structure it is difficult to know whether predictions based on comparison of LMS and LM'S treatments would have been upheld if both M and M' morphs were present in the same replicates. Some of the lost male fitness that the M' morph experienced in the presence of the S morph might have been recovered in increased transmission of outcross pollen from the M' to the M morph. Nevertheless, presence of the S morph in experimental populations would appear to substantially reduce the transmission advantage of the M' morph.

Context-Dependent Selfing. — The frequency of self-fertilization exhibited by the M and M' morphs increased as morph diversity of experimental populations was reduced. This correlation underscores the fact that it is the interaction of floral morphology with population structure, rather than morphology alone, which determines the frequency of self-fertilization in self-compatible species. Although floral morphology may control the amount of self pollen deposited, the fraction of the total stigmatic pollen load that this represents is determined by how much pollen arrives from other plants (Holsinger 1991, 1992). Where outcross pollen deposition rates are high, a given amount of self pollen on the stigma will cause less selfing than where outcross pollen deposition rates are low. Therefore, the success of a variant morphology that increases self-pollen deposition is likely to largely depend upon the outcross pollen transmission dynamics of the population in which it arises.

The amount of outcross pollen arriving may be due not only to population density and rates of pollinator visitation, but also to the floral morphology of other plants in the population. In these experiments, plant density was held constant and rates of pollinator visitation did not vary in any consistent way among treatments. With respect to the M and M' morphs, however, the amount of mid-level pollen produced in experimental populations differed substantially between treatments. When both L and S morphs are present, production of mid-level pollen is high because both of these morphs have mid-level stamens. Mid-level pollen is the type that is most efficiently transferred to stigmas of the M morph in tristylous species (Barrett and Glover 1985; Lloyd and Webb 1992) and also, in *E. paniculata*, has the highest siring ability on stigmas of the M morph (Cruzan and Barrett 1993). Both in-

creased rates of deposition and performance of mid-level pollen may lower rates of self-fertilization in tristylous populations. However, in the absence of the L and S morphs, the frequency of self-fertilization increases, presumably reflecting the lower availability of mid-level pollen.

In the M' morph used in our experiments, an otherwise short-level anther is placed either directly on its own stigma or held within a millimeter of it. Even so, this morphology fails to produce a high level of selfing under trimorphic conditions. This is striking testimony to the importance of population context in determining the mating system. Under conditions of high rates of deposition of outcross pollen, morphologies that increase self-pollen deposition may fail to spread even when inbreeding depression is slight (Holsinger 1991). In natural trimorphic populations, the M' morphology could have little effect on rates of self-fertilization while being costly in terms of lost ability to sire seeds through outcrossing, particularly seeds of the S morph.

We predicted that because of the high density of outcross mid-level pollen in trimorphic populations, the difference between the frequency of selfing in the M' relative to the M morph would be reduced. Although the difference in selfing of the M and M' morphs was lowest in the comparison under trimorphic conditions (see table 2), the morph-by-treatment interaction did not approach statistical significance. In light of the variation among replicates, we lacked the statistical power to detect such an effect, even if one occurs. Data on the frequency of self-fertilization from a trimorphic population from northeastern Brazil (population B58, Belo Jardim, Pernambuco), in which both M and M' morphs occurred suggest that such an effect may be important in nature. In this large, dense population, outcrossing rates of the M and M' morphs were not significantly different nor was there evidence of significant self-fertilization by either morph [s (SE), N families; M = 0.0 (0.04), 19; M'= 0.05 (0.05), 19; M. R. Dudash and S. C. H. Barrett unpubl. data). Thus, placement of an anther directly on the stigma may cause little selfing under conditions of sufficiently dense outcross pollen. Such effects are likely to be particularly pronounced in species with short-lived flowers where pollinator activities are usually high and the delivery of outcross pollen reliable (Primack 1985).

Lloyd (1979, 1992) and Lloyd and Schoen (1992) distinguish three modes of selfing based on whether self pollen arrives before (prior self-

ing), at the same time (competing selfing), or after (delayed selfing) outcross pollen. These authors show that the way selection acts differs among modes. For instance, delayed selfing is always favored while selection on competing and prior selfing is subject to the standard selective forces such as the level of inbreeding depression. Population context could affect not only the rate of self-fertilization but also the mode. Where anthers contact stigmas within the flower, as is often the case in the M' morph, the mode of selfing may vary from prior to competing depending upon the timing of outcross pollen arrival and its density. The schedule of outcross pollen arrival may vary with plant density, floral morphology, community composition, or any ecological factor that affects pollinator behavior.

We cannot determine the level of prior versus competing selfing in these experiments, but delayed selfing is unlikely to have played a role because increased selfing was not associated with elevated seed set. It would appear that ovules used for selfing came at the expense of ovules that would otherwise have been outcrossed and "seed discounting" (Lloyd 1992) equaled one. Complete seed discounting is implicit in all models of mating-system evolution in which changes in the selfing rate are unaccompanied by differences in seed set.

Our ability to study the effects of floral variation on all aspects of gamete transmission is in its infancy. With the exception of flower color polymorphisms (Stanton et al. 1986; Brown and Clegg 1984) studies of the effects of floral variation on gamete transmission in natural populations have been limited by the lack of sufficient genetic markers to reliably infer the male component of fitness. Progress in studies of natural populations is being made by analysis of components of the pollination processes (Campbell 1989, 1991; Galen 1989; Schemske and Horvitz 1989; Young and Stanton 1990; Campbell et al. 1991) and by statistical genetic inferences of male reproductive success (Meagher 1986, 1991; Devlin et al. 1992). However, experimental populations of genetically marked plants continue to provide a worthwhile avenue of research (Schoen and Clegg 1985; Stanton et al. 1989, 1991; Rausher et al. 1993) because they allow more reliable estimation of components of reproductive success and provide opportunities to manipulate and replicate population-level ecological factors. In the experimental populations explored here, floral morphology, population

morph structure, and their interaction all had strong effects on the transmission of male gametes through selfing and outcrossing. Our results emphasize the important influence that population-level ecological factors can have on transmission dynamics. Taken together with findings from other studies (e.g., Ritland 1991; Holsinger 1992; Rausher et al. 1993) our results suggest that floral traits that increase the frequency of self-fertilization can have large positive or negative effects on outcross pollen transmission. Such effects should not be ignored in either theoretical models or empirical studies of mating-system evolution.

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Corresponding Editor: D. Campbell