POLLEN DISCOUNTING AND THE EVOLUTION OF SELFING IN *ARENARIA UNIFLORA* (CARYOPHYLLACEAE)

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Abstract.—Although most models of mating system evolution assign a central role to the male transmission advantage of selfing genotypes, empirical data on the male fitness consequences of increased self-pollination are still uncommon. Here, I use measures of pollen import and export by focal plants in genotyped arrays to investigate the effects of floral morphology and pollination environment on self and outcross male function. Plants from an autogamous population of *Arenaria uniflora* (Caryophyllaceae) exhibit complete pollen discounting relative to closely related outcrossers, as do morphologically intermediate F_1 hybrids between the two populations. However, the low cumulative male fitness of hybrids probably results from reduced pollen number or competitive ability, rather than a nonlinear relationship with floral morphology. When surrounded by selfers, plants from the outcrosser population self-fertilize at nearly the same rate as selfers (>80%), but have much lower self male fitness due to reduced fruit set. Because outcross siring success is also extremely low (<8%) in this treatment, these mate-limited outcrossers are at male fitness disadvantage to both pseudocleistogamous selfers and nonlimited outcrossers. The relative male fitness of plants with different mating systems appears dependent on the ecological context, as well as on morphological trade-offs.

Key words.—*Arenaria uniflora,* autogamy, male fitness, pollen discounting, self-fertilization.

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Given the prevalence of inbreeding depression, evolutionary biologists have long sought to explain the evolution of increased rates of self-fertilization in flowering plants (Darwin 1876; Stebbins 1957; Jain 1976; Barrett et al. 1996). Fisher (1941) first recognized that additional transmission via male function could provide selfing genes with an automatic selective advantage. At the theoretical extreme, a modifier producing complete self-fertilization with no loss of outcross male fitness enjoys a 50% transmission advantage over outcrossing genotypes and, all else being equal, will rapidly spread to fixation. Such automatic selection for selfing became a central element of most models of mating system evolution, leading to the general prediction that selfing will evolve whenever inbreeding depression is less than 0.5 (Maynard Smith 1977; Charlesworth 1980; Lande and Schemske 1985). However, constant genetic selection for selfing rests on the assumption that increased self-pollination involves no concomitant loss in outcross male function. Mating-system models that incorporate decreased outcross male fitness upon selfing or ''pollen discounting'' have encouraged critical examination of this assumption (Nagylaki 1976; Lloyd 1979, 1992; Holsinger et al. 1984; Holsinger 1991; Harder and Wilson 1998), but only a handful of empirical studies have measured the male fitness consequences of increased self-pollination (Ritland 1991; Holsinger 1992; Rausher et al. 1993; Kohn and Barrett 1994; Holsinger and Thomson 1995; Chang and Rausher 1998).

The genetics of mating system modifiers, the mode of self-pollination (Lloyd 1979, 1992; Schoen et al. 1996), and the local pollination environment may all affect the relationship between self and outcross male fitness. Highly selfing populations or species generally display correlated suites of characters promoting self-fertilization, many of which preclude or decrease opportunities for pollen donation (Jain 1976; Wyatt 1988). Although not always mediated by direct trade-offs

in pollen grain fate (as the term would imply), pollen discounting in such populations can be very high (Ritland 1991; Holsinger 1992). Character shifts that increase the selfing rate (decreased petal size, loss of protandry, etc.) may necessarily reduce pollen export, or be developmentally linked to other changes that do. Even in cases where the switch to selfing has a simple genetic basis and no obvious pleiotropic effects on pollen export (e.g., the loss of heterostyly), outcross male fitness depends on the identity of neighboring competitors and mates (Kohn and Barrett 1994). Thus, an ''automatic'' selective advantage cannot be viewed as a constant property of all selfers, but must be considered in the context of individual morphology and the pollination environment.

In this study, I use the mating system polymorphism of *Arenaria uniflora* (Caryophyllaceae) to examine male fitness trade-offs associated with increased rates of self-fertilization. Populations of *A. uniflora* exhibit widely divergent mating systems, with predominantly outcrossing populations near the center of the species range and highly autogamous populations at the margins (Wyatt 1984, 1986). Although the selfing syndrome in *Arenaria* appears to have evolved at least partly in response to local pollinator-mediated selection (Fishman and Wyatt 1999), a genetic transmission advantage could also have played a role in the spread of selfing variants. To empirically examine the male fitness consequences of selfing, I trace pollen import and export by genotyped focal plants in naturally pollinated arrays. This design allows me to estimate the pollen discounting associated with the shift to complete selfing by comparing the cumulative male fitness of plants with contrasting mating systems. Because complete selfing may evolve through incremental changes in floral morphology and phenology, I also ask whether plants with intermediate floral morphologies (F_1 hybrids) experience parallel trade-offs in male fitness. This is a first step towards disentangling the male fitness effects of the many correlated characters that make up the extant selfing syndrome. Because male success should vary with effective pollen transfer, I

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examine the effects of individual variation in selfing rate and the local pollination environment on the male fitness components of outcrossers. Conditions creating pollen limitation of female fitness (here, low mate availability) may also constrain outcross male fitness, increasing the transmission advantage of selfing variants.

MATERIALS AND METHODS

Study Populations

Arenaria uniflora (Walt.) Muhl. (Caryophyllaceae) is a winter annual plant endemic to granitic outcrops in the southeastern United States. *Arenaria uniflora* is characterized by large-flowered protandrous populations (outcrossers) near the center of its range and small-flowered pseudocleistogamous plants (selfers) in marginal populations. All *A. uniflora* populations are self-compatible, but cross-pollination in the outcrossers is promoted by strong protandry and pollinator attraction. Outcrosser *A. uniflora* flowers produce nectar and are visited by generalist flies (primarily Syrphidae) and small bees (primarily Halictidae), as well as honeybees (Weaver 1970; Wyatt 1986; Fishman, pers. obs.).

The evolution of autonomous self-pollination in *A. uniflora* appears to be a case of reproductive character displacement (Fishman and Wyatt 1999). Selfer populations are only found in areas of range overlap with the congener *A. glabra,* while outcrossers and *A. glabra* never co-occur. Wyatt (1984, 1986) proposed that competition for pollinators between ancestral outcrossers and the showier *A. glabra* favored selfing variants in areas of sympatry. Recent experimental work points to the negative effects of interspecific pollen transfer, rather than competition for pollinator service, as the likely mode of pollinator-mediated selection for selfing (Fishman and Wyatt 1999). Outcrosser *A. uniflora* show no premating barriers to hybridization with *A. glabra,* but the few hybrid seeds formed do not germinate. As a result, outcrossers suffer substantial reductions in female fitness in the presence of *A. glabra,* whereas selfers are unaffected. This suggests that selection for preemptive self-fertilization may have been an important factor in the evolution of autogamy in marginal *A. uniflora* populations.

The two *Arenaria uniflora* populations used in this study represent extremes of mating system within the species. The outcrosser population (Pendergrass, GA; Wyatt's (1986) population 7) is highly protandrous, with the 1 cm diameter flowers lasting up to two weeks. Autonomous seed set in the greenhouse is low (<10%). Single locus estimates of population outcrossing rate in two different years suggest a mixed mating system ($t = 0.73 \pm 0.06$ SD; Fishman, unpubl. data). Biparental inbreeding in the genetically structured Pendergrass population probably biases this estimate downward. Plants from the selfer population (Liberty, SC; Wyatt's [1986] population 8) are pseudocleistogamous, generally self-pollinating in the bud. The small (<4 mm) flowers are rarely open for more than two days, and the stigma and anthers mature simultaneously. Supplemental pollination does not increase seed set. The Liberty selfer population does not contain enough allozyme polymorphism to estimate its population-level outcrossing rate (Wyatt *et al.* 1992).

Despite their striking mating system divergence, the Liberty and Pendergrass populations appear geographically and genetically allied. Allozyme and crossability data indicate that the Liberty selfer population is more closely related to the Pendergrass outcrossers than to more distant *A. uniflora* populations, including other selfers (Wyatt 1990; Wyatt et al. 1992). The two populations also have similar ovule numbers and vegetative morphology, although outcrosser plants are larger overall. For details of population locations and descriptions, see Wyatt (1986). Taxonomic note: both *Arenaria glabra* and *A. uniflora* have been placed in the genus *Minuartia* (McNeill 1962; McCormick et al. 1971). However, because this assignment is controversial (Wyatt 1977) and continuity with previous work on the group is desirable, I use *Arenaria* in referring to these species.

Experimental Design

The individual pollen import and export rates of focal plants were measured in genotyped experimental arrays. The general design consisted of a focal plant homozygous for one allele at a marker locus surrounded by four plants fixed for a second allele. This small-array design was chosen to allow detection of focal plant pollen donation to background individuals, which should be inversely proportional to the total array size. Four types of arrays were constructed: focal selfer, focal outcrosser, focal F_1 hybrid, and low frequency outcrosser (Fig. 1). The first three types of arrays measured focal plant male performance against a background of outcrosser plants. The low frequency outcrosser array examined outcrosser performance against a background of selfer plants, which quantifies the potential for outcrossing genotypes to reinvade selfing populations and also creates conditions of mate and pollinator limitation.

A single allozyme locus was used to trace paternity in the experimental arrays. Prior to placement in the field, plants were genotyped at the nuclear aspartate amino-transferase (*Aat*) allozyme locus and homozygous individuals were selected for inclusion in the experiment. Plants were genotyped with cellulose acetate electrophoresis (Titan III gels; Helena Laboratories, Beaumont, TX), which allows rapid assaying of many individuals with only small amounts of tissue. The running conditions and staining protocols followed those recommended for *Aat* in the cellulose acetate electrophoresis manual (Hebert and Beaton 1996). A previous survey had reported that the Pendergrass outcrossers had two common alleles at this locus, whereas the Liberty selfers were fixed for an alternative allele (Wyatt et al. 1992). In this study, the Liberty allele (c) identified by Wyatt et al. (1992) proved indistinguishable from the fastest-migrating allele in the Pendergrass outcrossers. A preliminary survey of field-collected plants indicated common fast (F: 0.45) and slow alleles (S: 0.47) and a rare intermediate allele (I: 0.08) in the Pendergrass population ($N = 800$), with the selfers fixed for the fastest migrating (F) allele ($N = 50$).

The background plants for all arrays were transplanted from the field and genotyped as dormant rosettes several months prior to placement in the experimental arrays. To ensure comparability with F_1 hybrid plants that were necessarily bred and raised in the greenhouse, all other focal plants were also chosen from second-generation greenhouse
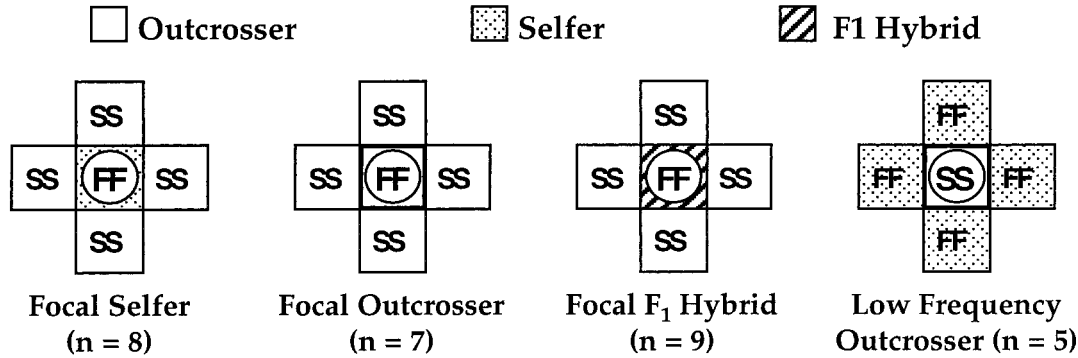
FIG. 1.   Design of experimental arrays for measuring pollen import and export. Focal plants homozygous for one allele at the *Aat* allozyme locus were surrounded by four recipient/donor plants homozygous for the alternative allele (some SI plants were used as SS background plants). Because the Liberty selfer population is fixed for the F allele at the *Aat* locus, selfers could not be tested against a background of selfers. Additionally, focal individuals could not be randomized with respect to *Aat* genotype. However, the possibility of allelic transmission bias is reduced by the parallel construction of the three array types with outcrosser backgrounds.

populations. The outcrossers were the outcrossed progeny of plants from the Pendergrass population and the selfers were selfed progeny of field-collected Liberty plants. The F_1 hybrids were derived from crosses between selfers and outcrossers in both directions and were intermediate in flower size and degree of protandry (Fishman 1998). Although the F_1 generation also had intermediate levels of self-pollination in the greenhouse (0.24 ± 0.25 SD), this mean autogamy rate was well below the midparent value. Morphological male sterility (stunted petals and anthers) was common in both classes of hybrids and may have reduced the probability of self-fertilization. However, the flowers used in the estimation of autogamy rate did not exhibit arrested anther development or other obvious evidence of male sterility, suggesting that nonadditivity of the genetic control of selfing and pollen sterility may be responsible. Only morphologically normal individuals were chosen for use in the male fitness experiment.

Focal and background plants were raised to flowering in a cool greenhouse at the University of Georgia Botany Department in Athens, Georgia. In April 1997, I selected plants of appropriate genotypes and counted their flowers and buds. I assembled replicates of the four types of arrays, making an effort to equalize the total background number of flowers across arrays. Low frequency outcrosser arrays ($n = 5$) were constructed of SS homozygote outcrossers surrounded by four FF selfers. The other three types of arrays contained FF focal plants (selfers [$n = 8$], outcrosser [$n = 7$], and hybrids [$n = 9$]) surrounded by SS or SI outcrossers (Fig. 1). Unequal numbers of replicates resulted from constraints on genotype availability and predation by rabbits after placement in the field.

The arrays were placed about 10 m apart at random positions in a mown field adjacent to the University of Georgia botany greenhouse. The flowering plant community at this site supported similar pollinators (syrphid and andremonid flies and small bees) as a nearby (<5 miles) granite outcrop with abundant *A. uniflora*. Natural pollination of flowering arrays was allowed for three weeks, during which time pollinators were observed to visit the experimental plants. After three weeks, the persistent sepals of all incipient fruits were marked with a permanent marker and the plants were moved back into the greenhouse. The mature seeds of marked fruits were collected prior to capsule dehiscence. Full-sib families of the outcrosser and F_1 hybrid focal individuals were also grown in the greenhouse (Fishman 1998). The petal size of outcrosser and F_1 focal individuals and the autogamy rates of their families were measured in the greenhouse for comparison with male function in the field.

Assignment of seed paternity

Paternity was assigned by randomly choosing a seedling from each fruit and scoring its *Aat* genotype with the same protocol used for the parental plants. Seeds were germinated on moist sand in 24-well microtiter plates, with seeds from individual fruits in separate wells. At the two to six leaf stage, individual seedlings were sampled from each well and scored at the *Aat* locus. To check for multiple paternity, a second seedling was sampled from a subset of the fruits. From focal plants, 177 fruits were sampled and 31% (55) were resampled. A total of 501 fruits were sampled from background plants and about 25% (117) were resampled. The paternity of genotyped seedlings should be a good estimator of pollen movement among selfers and outcrossers. Previous greenhouse experiments have indicated that crossability between the two populations is high (>95%, Wyatt 1990; ~ 85%, Fishman 1998). The only evidence for incompatibility was a slight decrease in the seed set of selfers after hand-pollination with outcrosser pollen. The reciprocal cross had seed set indistinguishable from controls and the germination rates of both classes of F_1 hybrid seeds were not significantly different from the progeny of within-population crosses (Fishman 1998). Furthermore, inbreeding depression of seed production or germination should not affect estimates of pollen movement, because neither population demonstrates inbreeding depression of these characters (Fishman 1998).

In this design, heterozygous seeds can only result from pollen movement (rare I alleles were treated as S's for the assignment of heterozygosity). On focal plants, the proportion of heterozygous fruits equals the rate of pollen import from surrounding plants (*t*). The proportion of heterozygous fruits on recipient plants equals the focal individual's pollen export rate (*p*). Fruits sampled more than once were assigned

TABLE 1. One-way analyses of variance for selfing rate, siring success, and cumulative male fitness. The four array types (focal selfer, focal outcrosser, focal hybrid and low frequency outcrosser) are shown in Figure 1.

Source	df	Selfing rate			Siring success			Cumulative male fitness		
		SS	F	P	SS	F	P	SS	F	P
Array type	3	1.59	14.61	<0.0001	3.84	22.30	<0.0001	2.90	7.35	0.0011
Error	25	0.91			1.43			3.29		

the average of the two seeds (e.g., if one seed was heterozygous and the other homozygous, the fruit was assigned a heterozygosity of 0.5). On average, 22.3 ($\pm$4.2 SD) seeds were genotyped from each array to estimate pollen export by the focal plant and 8.2 ($\pm$2.6 SD) seeds were genotyped to estimate import. Pollen movement between arrays may have led to some misassignment of paternity. However, pollen transfer involving the majority of nonfocal plants (SS outcrossers) would not affect the estimation of focal plant import and export rates.

Analysis of individual selfing rates and siring succes

The selfing rate and outcross siring success of each focal individual were calculated from pollen import and export rates. The selfing rate of each focal plant was calculated as $1 - t$, or its proportion of homozygous seeds. Outcross siring success was standardized as $4p$, since four recipient plants were available for pollen donation. This calculation assumes that the amount of pollen contributed by the focal plant to any individual recipient is inversely proportional to array size (e.g., an obligate outcrosser surrounded by four obligate outcrossers would sire one-fourth of the seeds on each surrounding plant for an outcross siring success of 1.0). It also assumes that all background plants produce equal numbers of fruits, which was true within the context of this experiment.

Individual selfing rates and siring rates were translated into self and outcross male fitness components, which were summed to generate cumulative male fitness. Because the fruit set of focal plants varied among array types ($P = 0.02$), self male fitness was standardized by multiplying each focal individual's selfing rate by the ratio of its fruit set to the mean fruit set of the focal selfers (0.614). The fruit set of background plants did not vary significantly among array types, so outcross male fitness was not standardized. In the field, plants from the Liberty selfer population generally produce fewer flowers and fruits than Pendergrass outcrossers over the whole season (Wyatt 1984; Fishman and Wyatt 1999). However, the selfers in this experiment produced numbers of flowers and fruits equivalent to the outcrossers, as might be assumed for ancestral selfing variants.

Statistical analyses were performed using the standard least-squares analysis of variance platform in JMP 3.0.1 (SAS Institute 1994). I used one-way ANOVA to test for differences in selfing rate, outcross siring success, self male fitness, and cumulative male fitness among the array types. In preliminary analyses, the proportion of focal flowers as included as a covariate to control for differences among arrays in focal plant flower number, but this factor was never significant ($P > 0.6$) and was excluded from the final analyses. The siring rate and cumulative male fitness data could not be made to conform to ANOVA assumptions of residual normality, even

with arcsine square-root transformation. For simplicity, untransformed data are presented here. Non-parametric analyses (Kruskal-Wallis test) and Welches-ANOVA (which accounts for unequal variances) of all measures of male performance produced results quantitatively similar to the ANOVA, indicating that the conclusions presented here are not contingent on the method of analysis. Tukey-Kramer HSD tests were used to explicitly compare the mean performances of pairs of array types (SAS Institute 1994).

Assessment of pollen discounting

In this study, I assessed pollen discounting in two ways. First, I used the comparisons of cumulative male fitness to determine whether pollen discounting occurs with shifts in mean selfing rate between the array types, particularly between selfers and outcrossers. The presence or absence of pollen discounting generates clear predictions about the relative male fitness of selfing and outcrossing individuals. In the absence of pollen discounting, the cumulative male fitness of selfers should be twice that of outcrossers, because they should have the opportunity to sire all seeds on themselves as well as many of those on surrounding plants. In contrast, when pollen discounting produces a 1:1 tradeoff between self and outcross male function, the cumulative male fitness of selfers and outcrossers should be equivalent.

In addition to this comparison across focal classes/populations, I examined the relationship between individual self and outcross male fitness within each array type. For this analysis, I calculated Pearson's correlation coefficients between siring success and self male fitness for each array type separately. A negative correlation between outcross and self male fitness would indicate pollen discounting due to individual variation or environmental differences between replicates.

RESULTS

Selfing Rates and Self Male Fitness

Individual selfing rates calculated from the pollen import data differed strongly (Table 1), and were consistent with previous estimates of mating system in the selfers and outcrossers (Fig. 2). Selfer plants sired more than 88% of their own seeds even when surrounded by outcrosser pollen donors. Outcrossers exhibited mixed mating behavior when surrounded by other outcrossers (mean selfing rate = 0.29 $\pm$ 0.07). Focal selfer and focal outcrosser plants had nearly identical levels of fruit set (mean fruits/flower = 0.59 $\pm$ 0.07SE and 0.61 $\pm$ 0.06 SE for outcrossers and selfers, respectively). The F_1 hybrids had a mean selfing rate of 0.56, intermediate between the two parental types. The fruitset of hybrids was lower than either parent, resulting in significantly
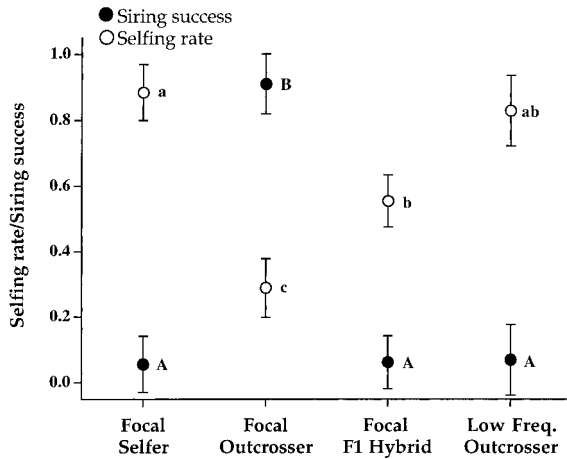
FIG. 2. Selfing rate and outcross siring success (mean ± 1 SE) of focal plants in the four array types. The selfing rate was calculated as $1 - t$, where t is the proportion of focal plant seeds that were heterozygous. Outcross siring success was calculated as $4 p$, where p is the proportion of heterozygous seeds produced by background plants. Means with different letters (uppercase and lowercase for siring success and selfing rate, respectively) are significantly different from one another (Tukey-Kramer HSD, α = 0.05).

FIG. 3. Cumulative male fitness (mean ± 1 SE) of focal plants in the four array types. Because focal plant fruitset varied significantly among array types, self male fitness was standardized by multiplying the selfing rate of each focal plant by its fruitset relative to the mean of the focal selfer treatment (0.614), which had the highest mean fruitset. The fruitset of background plants did not differ significantly, so outcross male fitness was not adjusted. Means with different letters are significantly different from one another (Tukey-Kramer HSD, α = 0.05). In the absence of pollen discounting, the cumulative male fitness of selfers would be twice that of outcrossers.

lower self male fitness. Outcrossers surrounded by selfers (low frequency outcrossers) self-fertilized 83% of their seeds, indicating that selfing rate is dependent on the local pollination environment. However, low frequency outcrossers had much lower fruitset than focal outcrossers and selfers (mean seeds/fruit = 0.35 ± 0.08). This apparent pollen limitation of fruit production resulted in self male fitness significantly lower than focal selfers (Tukey-Kramer HSD, $P < 0.05$). The mean selfing rate of low frequency outcrossers could not be differentiated from focal selfers and hybrids, but all other pairs were significantly different ($P < 0.05$; Fig. 2).

Outcross Siring Success

Pollen export rates and outcross siring success differed strongly among the four types of focal plants (Table 1, Fig. 2). On average, focal outcrossers sired 22.8% of the seeds produced by a given neighbor, resulting in high mean outcross male fitness (0.91 ± 0.09 SE). The other three array types had extremely low levels of pollen donation to neighbors (<2%), which translated into outcross male fitness less than 0.08. These three treatments were significantly different from the focal outcrossers in siring success (Tukey-Kramer HSD, $P < 0.001$), but not different from one another. The negligible outcross siring success of low frequency outcrossers suggests that reinvasion (though gene flow) of a selfer population by an outcrosser strategy is unlikely even in the absence of continued selection for selfing.

Cumulative Male Fitness and Pollen Discounting

Complete pollen discounting accompanies the evolutionary shift from outcrossing to selfing in *A. uniflora*. The pseudocleistogamous Liberty selfers have virtually no outcross male fitness, while the Pendergrass outcrossers successfully donate pollen both to themselves and to neighbors (Fig. 3). When surrounded by outcrossers, the cumulative male fitness
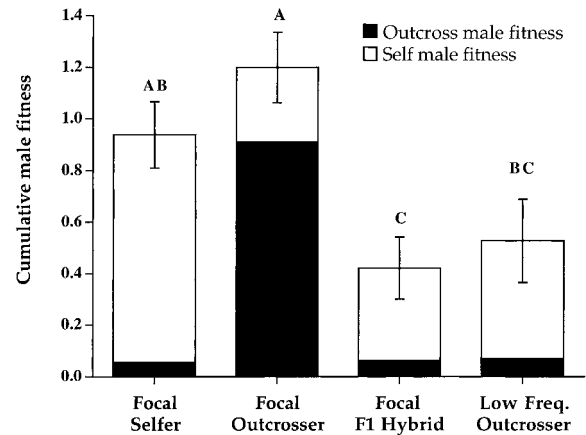
of outcrossers was as great or greater than that of selfers. Focal selfer and focal outcrosser male fitness did not differ, and the mean of selfers was not significantly different from the value of 1 predicted by complete pollen discounting. Clearly, selfers do not enjoy the two-fold transmission advantage predicted in the absence of pollen discounting. Indeed, autogamous selfers appear to be at a slight (nonsignificant) male fitness disadvantage when surrounded by outcrossers (i.e., their morphology reduces pollen export more effectively than it precludes pollen import). F_1 hybrids were at significant male fitness disadvantage to both parental populations (Fig. 3). This could represent reduced outcross male function in plants of intermediate floral morphology or may simply reflect problems with F_1 pollen production or pollen viability (see Discussion). Pollen limitation in the hybrids and low frequency outcrossers makes direct comparisons with the focal outcrossers tentative without larger sample sizes and better estimates of relative seed production. If the self-pollination by low frequency outcrossers was primarily autonomous, their loss of male fitness was probably much greater than indicated by the measured reduction in fruit set. Even so, low frequency outcrossers had cumulative male fitness significantly lower than focal outcrossers (Tukey-Kramer HSD, $P < 0.05$), and marginally lower than focal selfers ($P = 0.058$). It is clear that increased selfing by lone outcrossers does not compensate for the male fitness losses imposed by the lack of appropriate mates and/or pollinators (Fig. 3).

Several of the focal outcrossers displayed cumulative male fitness values much greater than 1, with one robust individual achieving total male fitness of 2.5, a consequence of moderate selfing in addition to high outcross pollen donation. At first glance, it might appear that the mixed mating system of the Pendergrass population provides a male transmission advantage. However, this excess male fitness must come at the

expense of the surrounding plants (also Pendergrass outcrossers), because the number of ovules within an array is a fixed resource. These mixed-mating plants might have a male fitness advantage against a background of obligate outcrossers, but a lack of pollen discounting cannot be a general characteristic of a population-wide mixed mating strategy. Instead, this apparent excess male success may result from the central placement of the focal plants, since they produced no more flowers than background individuals on average. The relatively high mean and variance of focal outcrossers could also be due to the small number of plants assayed here.

I did not observe pollen discounting at the level of individual functional trade-offs, as would have been indicated by a negative correlation between self and outcross male fitness within an array type. No significant correlations between self and outcross male fitness were found within any of the array types, and in the focal outcrossers the trend was towards a positive relationship (Pearson's $r = 0.18$, $P = 0.67$). In addition, no relationship was observed between floral character values (petal size and greenhouse autogamy rate) and the individual selfing or siring success of focal hybrids or outcrossers. However, a larger study might detect either positive correlations or trade-offs in male fitness within the outcrossers, since even these small samples displayed a wide range of mating behavior.

DISCUSSION

Trade-offs in male function often accompany the evolutionary shift from predominant outcrossing to routine selfing. Highly selfing populations generally display suites of floral characters that reduce or preclude outcross pollen donation (Jain 1976; Wyatt 1988) and result in complete pollen discounting (Ritland 1991; Holsinger 1992). In contrast, moderately or facultatively selfing plants often show little evidence of pollen discounting against backgrounds of outcrossers (Rausher et al. 1993; Kohn and Barrett 1994; Holsinger and Thomson 1994). The *Arenaria uniflora* populations in this study demonstrate the former pattern. The Liberty selfers maintained high selfing rates ($>85\%$) even when surrounded by outcrosser pollen donors, and selfer gametes made up less than 7% of the successful outcross pollen pool. The morphological changes associated with automatic self-pollination apparently reduce pollen export, resulting in total male fitness no higher than plants from the predominantly outcrossing Pendergrass population.

Genotypic variants with increased selfing rates can fall into three general phenotypic categories. In the first, self-pollination entails no reductions in siring success, so that cumulative male fitness rises with increased self-fertilization. Selfing genotypes of this sort are assumed by many models of mating system evolution (e.g., Maynard Smith 1977; Lande and Schemske 1985), and will rapidly spread to fixation if high inbreeding depression does not counteract the male fitness advantage (Fisher 1941). In the second, genetic changes increasing the self-pollination rate have pleiotropic or direct negative effects on outcross male fitness (e.g., Nagylaki 1976; Lloyd 1979; Holsinger et al. 1984). Such trade-offs may or may not involve actual limits on pollen availability, but are equivalent to pollen discounting (Holsinger

et al. 1984). With trade-offs, the specifics of pollen transfer dynamics become important factors in mating system evolution (Holsinger 1991, 1992; Harder and Wilson 1998). When the trade-off is complete, cumulative male fitness remains constant across all morphologies and increased selfing confers no immediate selective advantage. A third possibility involves variants with both increased self *and* outcross male fitness, as may occur under some pollination conditions in *Eichornia paniculata* (Kohn and Barrett 1994). Because such genotypes should be universally favored, they are not considered further here.

Autonomous self-pollination may evolve via a series of phenotypes with inherent male fitness trade-offs or through automatic selection on morphologies without trade-offs and later frequency-dependent selection against traits promoting pollinator attraction and pollen export. While the lack of discounting in some moderately selfing variants suggests that some morphologies can ''have it both ways'' with regards to male fitness (Rausher et al. 1993; Kohn and Barrett 1994), these may or may not correspond to intermediate stages in the evolution of complete selfing. An understanding of the genetic basis and reproductive consequences of variation in individual components of floral morphology is necessary. In *A. uniflora,* analyses of a selfer $\times$ outcrosser F_2 generation indicate that floral traits associated with the shift to autonomous selfing do not to vary independently (Fishman 1998). Even if reductions in protandry alone would not produce male fitness trade-offs, strong genetic correlations between protandry and petal size mean that increases in autonomous self-pollination may necessarily reduce pollinator attraction and outcross pollen donation. Furthermore, changes in even a single floral trait affecting self-pollination may produce pollen discounting. Under some conditions, Chang and Rausher (1998) found that reduced outcross pollen success accompanied increased selfing in artificially selected lines of *Ipomoea* with reduced stigma-anther separation.

The *A. uniflora* F_1 hybrids, which were intermediate in both petal size and autonomous selfing rate between the parental populations (Fishman 1998), were included in this study to examine trade-offs in male fitness associated with novel trait combinations. This is a first step towards deconstructing the completely discounted selfer morphology. Whereas hybrids sired about 50% of their own seeds, they contributed very little ($\sim6\%$) to the successful outcross pollen pool and had low cumulative fitness relative to both parental types (Fig. 3). Ritland (1991) found similar ''super-discounting'' of male fitness in a natural hybrid zone between *Mimulus* taxa with contrasting mating systems. Because pollen import by the hybrid plants in this study was relatively high, poor insect visitation cannot entirely account for this pattern. If hybrids produced amounts of pollen intermediate between the widely divergent parental populations, a nonlinear relationship between pollen grain number and outcross success could result in low hybrid pollen export. However, male fitness gain curves are generally linear or decelerating rather than accelerating (Emms et al. 1997, and references therein).

More probably, the hybrid origin of the focal F_1 plants may have made them poor models for naturally intermediate floral phenotypes. Although the selfer and outcrosser populations used in this study are highly cross-compatible at the

level of F_1 seed production (Wyatt 1990, Fishman 1998), hybrids were often partially sterile. Petal and anther developmental problems producing male sterility affected over one-third of plants in the F_1 generation and appear to reflect nuclear genomic incompatibility between the parental populations. Although the F_1 individuals used in this experiment were morphologically normal (and thus underestimate the true male fitness consequences of hybridity), their low outcross male fitness probably reflects related outbreeding depression of pollen number and fertility. Greenhouse hand-pollinations also indicated moderate female sterility in the F_1 hybrids, which may account for the relatively low hybrid fruitset observed in this experiment. Ongoing studies of the evolutionary relationships among selfer and outcrosser *A. uniflora* and the fitness consequences of intermating between populations should help clarify the evolutionary history of routine self-fertilization in this system. Given partial hybrid sterility, marker-assisted quantitative genetic approaches may be necessary to fully isolate components of floral morphology associated with selfing and determine their individual and joint effects on self and outcross male fitness.

The pollination environment may also strongly influence the male fitness consequences of increased self-pollination. The relative male fitness of a selfing variant depends not only on its absolute success as a pollen donor but on the performance of the background population. Whenever ecological conditions reduce maximum outcross male fitness below the potential self male fitness (i.e., whenever there is pollen limitation of female fitness), selfing genotypes will have higher cumulative male fitness relative to the background population. In such a pollination environment, phenotypic trade-offs in male success that would otherwise result in complete pollen discounting are less important, and an obligately selfing genotype will have the highest male fitness. The low frequency outcrosser results from this study illustrate the context-dependence of pollen discounting. Surrounded by selfers, the focal plants in this treatment had reduced fruitset in addition to extremely low outcross male fitness. Although their mean selfing rate was similar to the Liberty selfers, their self male fitness was much lower. Cumulative male fitness in this treatment was also significantly lower than the morphologically identical focal outcrossers. In such a pollination environment, variants resembling the pseudocleistogamous Liberty selfers would have a male fitness advantage in addition to the benefits of female reproductive assurance. Although this double benefit is numerically contained in some models of mating system evolution (Lloyd and Schoen 1992), it is in contrast to the general assumption that automatic selection (male fitness advantage) and reproductive assurance (female fitness advantage) are ecologically exclusive modes of selection for self-pollination (e.g., Schoen et al. 1996).

In *Arenaria uniflora,* localized ecological selection through competition for pollinators and interspecific pollen transfer may have been an important factor in the evolution of selfing (Fishman and Wyatt 1999). Selfers only occur in areas of range overlap with congener *A. glabra.* Field and greenhouse experiments found that outcrossers suffer female fitness losses of 20–35% in the presence of *A. glabra,* primarily due to heterospecific pollen transfer, ovule usurpation, and the production of inviable hybrid seeds (Fishman and Wyatt 1999).

If competition with *A. glabra* also reduced the outcross male fitness of ancestral *A.uniflora* outcrossers, selfing variants could have had an additional reproductive advantage. Competition for pollinator service has been shown to affect outcross male fitness and gene flow in plants dependent on similar generalist insect visitors (Campbell 1985). Moderate pollinator-mediated selection through female and male fitness could have allowed the spread of even completely discounted selfer variants, because inbreeding depression in outcrosser *A. uniflora* is low (0.19 ± 0.02 SE; Fishman 1998).

Although the potential transmission advantages of self-fertilization are indisputable (Fisher 1941; Nagylaki 1976; Wells 1979), the actual role of male fitness differences in the evolution of selfing is still poorly understood. Models of mating system evolution that incorporate pollen discounting (Lloyd 1979, 1992, Holsinger 1992; Harder and Wilson 1998) have encouraged the measurement of male fitness components in variable taxa. However, drawing connections between the fitness parameters in these models and the behavior of extant or ancestral mating system variants remains difficult. By attempting to deconstruct the floral trait associations of an autonomous selfer, this study is a first step towards understanding the underlying genetic relationship between selfing rate and outcross male fitness in *A. uniflora.* It also emphasizes that the relative male fitness of different floral morphologies will depend on the local pollination environment, as a few other studies have also found (Kohn and Barrett 1994; Chang and Rausher 1998). Given the importance of differential male fitness, the apparent sensitivity of pollen discounting to both ecological conditions and the particular floral morphology of selfing variants presents a strong empirical challenge to the study of mating system evolution.

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