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THE GENETICS OF FLORAL DIVERGENCE AND POSTZYGOTIC BARRIERS BETWEEN OUTCROSSING AND SELFING POPULATIONS OF *ARENARIA UNIFLORA* (CARYOPHYLLACEAE)

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Abstract.—The genetic architecture of floral traits involved in the evolution of self-pollination provides a window into past processes of mating system divergence. In this study, we use two generations of crosses between highly selfing and predominantly outcrossing populations of *Arenaria uniflora* (Caryophyllaceae) to determine the minimum number, average dominance relationships, and pleiotropic effects of genetic factors involved in floral divergence. Comparison of the F_1 and F_2 phenotypic means with the expectations of a completely additive model of gene action revealed a primarily additive genetic basis for floral characters associated with mating system variation. The exception was flower life span, which showed partial dominance of the outcrosser phenology. In contrast to similarly divergent species, the substantial differences in flower size between these *A. uniflora* populations appear to involve relatively few genes of large effect (minimum number of effective factors = 2.2 ± 2.8 SE). In addition, correlations among traits in the F_2 generation indicate that pleiotropy may be an important feature of the genetic architecture of floral evolution in *A. uniflora*. The evolution of selfing via major modifiers of floral morphology is consistent with other evidence for ecological selection for preemptive self-pollination in *A. uniflora*. Analyses of the genetic basis of autonomous selfing were complicated by hybrid breakdown in both F_1 and F_2 generations. Only F_1 hybrids showed reductions in female fertility, but about 30% of F_1 and F_2 hybrids exhibited partial or complete male sterility. Male sterile flowers were characterized by short stamens, reduced petals, and a lack of protandry, as well as indehiscent anthers. This morphological breakdown mimics environmental disruptions of floral development and may result from novel genic interactions in hybrids.

Key words.—*Arenaria uniflora*, autogamy, floral morphology, genetic architecture, hybrid sterility, mating system evolution, postzygotic isolation.

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The evolution of self-fertilization, which has occurred repeatedly within flowering plants (Stebbins 1970; Barrett et al. 1996), often involves correlated shifts in a common suite of floral features (Ornduff 1969; Jain 1976; Wyatt 1988). Relative to their outcrossing congeners, derived selfing taxa generally exhibit reduced allocation to corollas or other attractive structures, reduced spatial and/or temporal separation of anthers and stigmas within a flower, and reduced pollen: ovule ratios. Similar changes in this suite of floral characters characterize independently derived selfers in many lineages (e.g., *Amsinckia*, Schoen et al. 1997; *Eichhornia*, Kohn et al. 1996; *Mimulus*, Fenster and Ritland 1994a; *Linanthus*, Goodwillie 1999).

The genetic architecture of floral morphologies associated with increased self-fertilization is an important component of mating system evolution. Models that assume an automatic 3:2 transmission advantage of selfing variants predict the rapid fixation of either complete selfing or complete outcrossing, with the outcome depending only on the ancestral level of inbreeding depression (Fisher 1941; Lande and Schemske 1985; Charlesworth et al. 1990). However, because heritable changes in selfing rate must occur in the context of a developmentally integrated floral structure, these models may not be biologically realistic. The theoretical 3:2 transmission advantage of selfing alleles depends on unchanged outcross pollen donation by selfing variants, but trade-offs between self and outcross male function may be a necessary consequence feature of the genetics of floral variation. For example, a variant genotype with reduced protandry may have reduced success as an outcross pollen donor due to direct interference between stigma and anthers (e.g., Chang and Rausher 1998) and/or through pleiotropic effects on corolla size or floral longevity. Such trade-offs lessen the genetic advantages of selfing (pollen discounting; Holsinger et al. 1984). Models of mating system evolution that include pollen discounting, like those that consider the mode of self-pollination (e.g., Lloyd 1979; Lloyd and Schoen 1992; Uyenoyama et al. 1993), suggest a primary role for ecological selection in the evolution of increased selfing. However, theory also shows that a single mutation causing complete selfing can spread to fixation regardless of high inbreeding depression and pollen discounting (Lande and Schemske 1985; Charlesworth et al. 1990; Schultz and Willis 1995). Thus, both the genetic control of individual floral traits (major vs. minor genes, dominance relationships) and the genetic basis of associations among traits may provide clues to the causes of mating system divergence.

Despite its importance, we are only beginning to understand the genetic basis of shifts in selfing rate. Some biometric studies suggest polygenic control of traits associated with mating system divergence (*Mimulus*: MacNair and Cumbes 1989; Fenster and Ritland 1994b; Fenster et al. 1995; *Clarkia*: Holtsford and Ellstrand 1992; *Turnera ulmifolia*, Shore and Barrett 1990), whereas others suggest a role for major genes (Marshall and Abbott 1984; Fenster and Barrett 1994). Quantitative trait locus (QTL) mapping approaches,

which provide greater power to characterize individual gene regions contributing to divergence, have demonstrated that dramatic differences in reproductive morphology between crop plants and their wild relatives often occur through the fixation of mutations of large effect (Paterson et al. 1991; Doebley and Stec 1993; Tanksley 1993). The relative importance of major genes in adaptive evolution in natural plant populations remains controversial (Gottleib 1984; Coyne and Lande 1985; Orr and Coyne 1992), but there is evidence that major genes can also underlie interspecific floral divergence (Bradshaw et al. 1995, 1998). However, a recent QTL study of floral characters associated with mating system divergence in the *Mimulus guttatus* species complex found a highly polygenic basis for individual characters and, despite extensive pleiotropy, the potential for traits associated with self-pollination and pollinator attraction to evolve independently from one another (Fishman et al. 2002). More data on the genetic architecture of floral evolution in diverse systems is necessary to determine whether this is a general pattern.

In this study, we investigate the genetic basis of floral divergence between primarily outcrossing and highly selfing populations of the annual plant *Arenaria uniflora* (Caryophyllaceae). Previous work has implicated selection for self-fertilization by heterospecific pollen flow as a factor in the evolution of selfing in marginal populations of *A. uniflora* (Wyatt 1986; Fishman and Wyatt 1999; Fishman 2000, 2001). The genetic architecture of floral and reproductive characters in *Arenaria* may reflect that evolutionary history, and the biometric data presented here provide a first picture of the quantitative genetics of this ecologically well-characterized system. We use patterns of phenotypic variation in F_1 and F_2 hybrids between selfers and outcrossers to infer the mean dominance of alleles at loci underlying several floral divergent characters and to calculate estimates of minimum gene number for petal size and protandry. As a first pass at detecting functional relationships among traits, we calculate phenotypic and genotypic correlations among floral traits and between floral traits and autonomous self-fertilization in the segregating F_2 population.

The evolution of selfing clearly contributes to premating barriers between diverging populations, but may also accelerate the development of postzygotic isolation. We use the same set of crosses to quantify the initial crossability of selfers and outcrossers, as well as the germination, survival, vegetative growth, and male and female fertility of hybrids. We describe an unusual male sterile floral morphology seen in high frequency in hybrids, and discuss possible genetic explanations for this striking reproductive breakdown.

Materials and Methods

Study System

Arenaria uniflora (Walt.) Muhl. (Caryophyllaceae) is a winter annual plant endemic to granite outcrops in the southeastern United States. *Arenaria uniflora* is characterized by populations of large-flowered protandrous plants (outcrossers) near the center of its range and small-flowered autogamous populations (selfers) at the margins. All *A. uniflora* populations are self-compatible, but cross-pollination in the outcrossers is promoted by strong protandry and attractiveness to pollinators (generalist flies and small bees; Weaver 1970; Wyatt 1986; L. Fishman, pers. obs.). Autogamous self-pollination in *A. uniflora* appears to be a case of reproductive character displacement. Highly selfing populations are only found in areas of range overlap with congener *A. glabra*, whereas outcrossers and *A. glabra* never co-exist (Wyatt 1984, 1986). Experimental work has further demonstrated that the presence of *A. glabra* reduces outcrosser *A. uniflora* fitness and that heterospecific pollen transfer is the likely mechanism of competition, suggesting that selection for preemptive self-fertilization may have been an important factor in the evolution of autogamy in *A. uniflora* (Fishman and Wyatt 1999).

The two *Arenaria uniflora* populations used in this study represent extremes of mating system within the species, but are geographically close and appear genetically allied (Wyatt 1984, 1990; Wyatt et al. 1992). The outcrosser population (Pendergrass, GA; population 7 in Wyatt 1984) is highly protandrous, with 1-cm flowers lasting up to two weeks. Single-locus estimates of population outcrossing rate in two different years indicate a mixed mating system ($t = 0.73 \pm 0.06$ SD; Fishman 1998). Genetic substructuring and biparental inbreeding within the Pendergrass population almost certainly bias this estimate downward relative to individual outcrossing rates. Plants from the selfing population (Liberty, SC; population 8 in Wyatt 1984) are pseudo-cleistogamous and generally self-pollinate in the bud. The small (<4 mm) flowers are rarely open for more than two days, and male and female functions overlap in time and space. The Liberty selfer population does not contain enough allozyme polymorphism to estimate the population-level outcrossing rate (Wyatt et al. 1992).

See Wyatt (1986) for detailed locations and descriptions of the Liberty and Pendergrass populations. 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), we use *Arenaria* in referring to the species here.

Crossing Design and Measurement of Characters

Two generations of crosses were used to examine the genetic basis of floral traits and postmating reproductive isolation between divergent populations (Fig. 1). To control for environmental variation between years, parental control populations were grown with each hybrid generation. The initial crosses were nested within a study of inbreeding depression (Fishman 2001), which provided maternal half-sib outcrossed parental families for comparison with the between-population F_1 hybrids. The F_1 individuals and these selfer (S_1) and outcrosser (X_1) controls were then selfed to create an F_2 generation and selfer (S_2) and outcrosser (X_2) control populations. Information on the collection and culture of the original greenhouse populations is given in Fishman and Wyatt (1999).

First generation.—In spring 1996, 16 maternal plants from each population were used as pollen recipients for both between-population crosses (F_1) and within-population control crosses. We used the outcross pollination treatments from a larger inbreeding depression study as the selfer (S_1) and out-

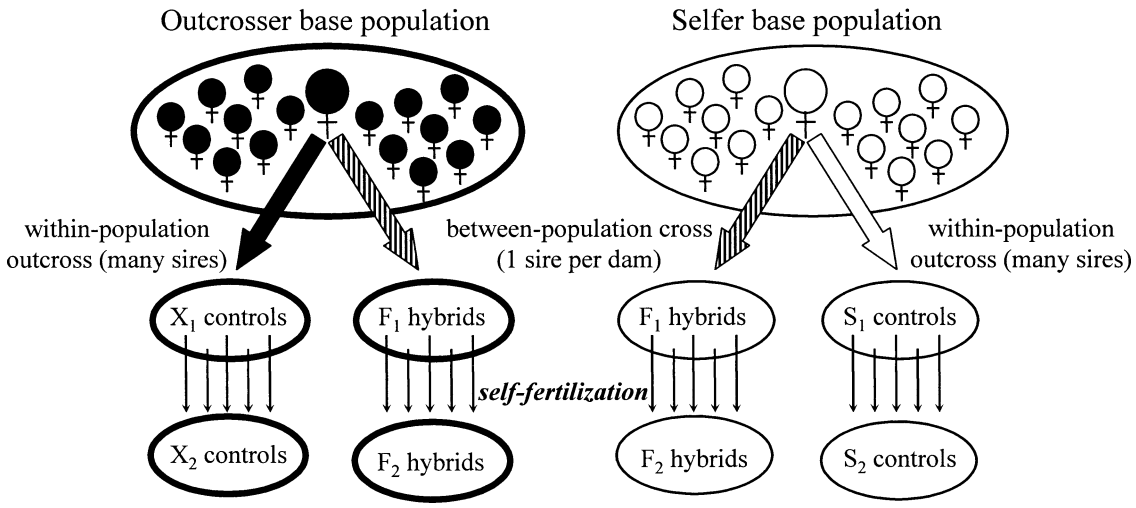

FIG. 1. Crossing design for quantitative genetic analysis of hybrids between outcrossing (Pendergrass) and selfing (Liberty) populations of *Arenaria uniflora*. Hybrids were grown with parental control lines in two common garden experiments (F_1, S_1, and X_1 in 1997 and F_2, S_2, and X_2 in 1998). Crosses were family structured, with 16 maternal plants from each parent population founding paired F_1 hybrid families and half-sib control families (S_1 or X_1). F_2 hybrids and second generation parental controls (S_2 and X_2) were generated by selfing. Not all lineages were represented in the second season (F_2; $N = 23$, S_2 and X_2; $N = 11$ each). Heavy ovals indicate experimental populations with Pendergrass (outcrosser) cytoplasmic genomes; light ovals indicate Liberty (selfer) cytoplasm.

crosser (X_1) parental controls. Because there is no outbreeding depression and low lifetime inbreeding depression in these populations (0.19 ± 0.02 in the outcrosser population, 0.05 ± 0.02 for selfers; Fishman 2001), this choice should have little effect on the results. The between-population crosses had a single pollen donor for each dam. Within-population outcrosses received mixed pollen loads (two to three donors per load) from a separate pool of about 100 anonymous donor plants. To assure no contamination by self-pollen, all recipient flowers were emasculated prior to stigma receptivity and the stigmas inspected under a dissecting scope prior to hand-pollination. Approximately five flowers on each plant received each treatment. At the end of the season, the seeds from each dam × crosstype combination were pooled and air-dried, then counted and weighed in bulk. Seed set (seeds/flower) and mean seed weight (mg) were calculated. Seed viability was assayed in Parafilm-sealed (Pechiney Plastic Packaging, Menasha, WI) petri dishes containing moist sand. Seeds were after-ripened at 30°C/15°C (12hr:12hr light: dark cycle) in an environmental control chamber for three months, then cooled to 15°C/8°C to initiate germination (Baskin and Baskin 1980). Total percent germination was recorded after one month. See Fishman (2001) for full details of pollination and seed collection protocols.

In 1997, vegetative and reproductive characters were measured on samples of plants from the F_1 generation and the S_1 and X_1 parent populations. Six seedlings were chosen from each dam × crosstype family, resulting in a maternally structured sample of 384 plants (2 populations × 2 crosstypes × 16 dams × 6 replicates). The individual seedlings were transplanted into 2.5-inch pots containing a thin layer of homogenized field soil over 1.5-inches of coarse sand and the pots were randomized into flats in the greenhouse at Princeton University. The greenhouse was kept at approximately 10°C for six weeks to ensure vernalization, and then warmed to 15–20°C to initiate shoot production and flowering. Day length was not manipulated, but supplemental light was provided during the day.

Adult characters were measured on the surviving individuals of each dam × crosstype family. The number of shoots was counted at six weeks after transplanting, prior to the appearance of flower buds. During the peak of flowering, we measured the length and width of one petal of a flower in female phase on each plant. Petal area (length × width) was used as a metric of flower size. Many hybrid plants showed some degree of male sterility, as indicated by small, indehiscent anthers on shortened filaments. We scored individuals as ''male sterile'' if more than 50% of flowers open at the survey time had at least five (of 10) deformed stamens. Most plants above this threshold were completely male sterile (all anthers deformed) in all flowers. Plants with any degree of anther deformity below this threshold were classified as ''partially male sterile.'' Flowers with deformed anthers generally had very reduced petals and were excluded from the petal size data collection. Flowers with abnormal anthers were also not used to measure autogamous seed set.

Mating behavior and reproductive performance were also measured in this season. Autonomous seed set was measured by marking unmanipulated flowers, collecting the mature fruits and calculating seeds/flower. Approximately four to five flowers per plant were marked in the F_1 and outcrosser populations, and an average of three flowers per each selfer. Maximum seed set was measured by hand-pollinating unemasculated flowers with either a mixture of F_1 and outcrosser pollen (F_1s) or within-population pollen (outcrossers). Since stamen developmental problems in the F_1 generation indicated the potential for other problems with male fertility, simultaneous pollination with donors from both sources provided the best possible opportunity for successful fertilization of the F_1 plants. A few selfer plants ($n = 10$) also received supplemental self-pollination to confirm previous work indicating that selfer reproductive success and

selfing rate are unaffected by the pollination environment (Wyatt 1990; Fishman and Wyatt 1999). Maximum seed set after supplemental pollination was used to estimate female fertility and provided a baseline for the calculation of autogamy rate. For a smaller sample of plants, we also counted the number of ovules per flower. Ovules were counted by preserving flowers in 95% ethanol, clearing the gynoecia in 1M NaOH for 10 minutes, and squashing the ovaries under a dissecting scope (Kearns and Inouye 1993).

Autogamy rate was calculated as the ratio of an individual's autogamous seed set to the mean maximum seed set of its family (for F_1 hybrids and outcrossers) or its class (for selfers). As a composite character, autogamy rate reflects both floral morphology/phenology and the potency of self-pollen. To determine whether F_1 hybrids experienced problems with female function, we also calculated mean female fertility (mean maximum seed set/mean ovule number) for F_1 and outcrosser (X_1) families. Female sterility in the F_1 population would be indicated by relatively low seed set even after supplemental hand-pollination.

Second generation.—For the 1998 season, F_2 and control parent lineages were created by hand-selfing the F_1 and outcrosser (X_1) plants and allowing autogamous seed set in the selfers (S_1). Our primary focus is on the segregation of alternative parental alleles underlying divergent floral characters, and this line-cross approach (Mather and Jinks 1982) maximizes the exposure of such variation in the segregating F_2 hybrids. This single generation of inbreeding does not risk substantial inbreeding depression, because neither parental population expresses large amounts of genetic load (Fishman 2001). We also generated a small number of backcrosses to the Pendergrass outcrosser population (B_1) by pollinating emasculated F_1 flowers with outcrosser pollen.

The seeds from each plant were collected and germinated as in the previous generation. Because of the difficulty in generating large numbers of F_2 seeds from the partially infertile F_1 plants, the seedlings of each granddam × crosstype combination were pooled for family-level analyses of adult characters. Because the second generation of crosses consisted entirely of self-pollinations, these pools share, on average, the same degree of genetic relatedness as their parents but were not actual sibships. For example, all F_2 hybrids that shared a granddam (i.e., the selfed progenies of F_1 hybrids) were pooled. We refer to these pooled groups as lineages to distinguish them from the maternal families in the previous generation. Initially, ten seedlings from each of the 64 lineages were transplanted into pots and placed in the greenhouse as in the previous year. Heavy early mortality across all treatments reduced this sample size substantially, to a mean of about three plants per lineage in 23 F_2 lineages, 11 outcrosser lineages, and 11 selfer lineages.

In 1998, we collected data on floral morphology and phenology (petal area, protandry, and flower life span) and reproductive performance in the F_2 generation and control parental populations. Petals were measured to the nearest 0.1 mm and petal area calculated as before. Floral phenology was monitored by marking flowers in the bud and noting their gender (male, male + female, female) each morning from anthesis until closing. Protandry was defined as the number of days spent in exclusively male phase. Flowers were marked at several times during the season, and average protandry and flower life span were calculated for each individual. The autogamy rate of outcrosser and F_2 hybrid plants was calculated as the ratio of the seed set of unmanipulated flowers to the mean seed set of the class after supplemental hand-pollinations, conducted as in the previous season. Because the previous season's data and earlier work (Fishman and Wyatt 1999) showed that the seed set of plants from the Liberty population is near 100% regardless of pollination treatment, we did not measure the autogamy rates of selfers this season. Ovule numbers were also counted as before. As in the first season, data were not collected from flowers with deformed anthers.

Statistical Analyses

The crossability data (seed set, seed weight, and germination after within- and between-population pollination treatments) were analyzed separately for the selfer and outcrosser maternal populations with an ANOVA model including pollination treatment (within vs. between) as the main effect and dam as a random effect. Initially, all adult characters were also analyzed with ANOVA models containing crosstype as the main effect, dam as a random effect and the crosstype × dam interaction. The two reciprocal categories of F_1 were analyzed separately, paired with their outcross maternal half-siblings. Because we observed no population-level maternal effects on the F_1 for floral and fertility characters, the two reciprocal classes were lumped together for the genetic analyses of these stages. Tests of additivity were made by comparison of the overall F_1 phenotypic mean with the average of the two parental controls (Lynch and Walsh 1998). Analyses of variance and calculation of means were performed in the standard least squares ANOVA platform in JMP 3.0.2 (SAS Institute 1994).

The F_2 and backcross floral character phenotypes were separately tested for fit to a purely additive model of gene action by comparison with the second season parental average. In addition, a minimum estimate of the number of genetic factors (n_e) affecting petal size was calculated using the Castle-Wright estimator (Castle 1921; Wright 1968; Lynch and Walsh 1998): $n_e = [\bar{z}(P_1) - \bar{z}(P_2)]2 - \mathrm{Var}[\bar{z}(P_1)] - \mathrm{Var}[\bar{z}(P_2)]/8\ \mathrm{Var}(S)$, where $\bar{z}(Pi)$ and $\mathrm{Var}[\bar{z}(Pi)]$ are the observed mean and sampling variance for the *i*th parental population and Var(S) is the segregational variance. The segregational variance was calculated as the F_2 phenotypic variance minus the F_1 phenotypic variance, which assumes equality of environmental variance across generations. The variance of the n_e estimate was calculated following Lynch and Walsh (1998). This biometrical method of gene number estimation assumes additivity of gene action and unlinked loci. Analyses of the F_1 and F_2 generations indicated that the first assumption was met for this character, but the estimate will be downwardly biased by linkage between loci affecting petal size. However, n_e provides a general indication of the potential role of major genes in the evolution of this character and can be compared with estimates for similar characters in other species.

Phenotypic correlations among floral traits and between these traits and autogamy rate were calculated for the hybrid

TABLE 1. Interfertility of Liberty selfer and Pendergrass outcrosser populations. Means (SE) for seed characters after within- and between-population pollinations of selfer and outcrosser maternal plants (n = 16 dams per population) are shown. The statistical significance of outbreeding depression (lower hybrid seed performance) is indicated adjacent to the between-population cross means. Each maternal population was analyzed separately by ANOVA, with crosstype as the main effect and maternal individual as a random effect. The relatively low seed set in both types of cross involving selfer dams is probably an artifact of emasculating the selfer flowers as small buds, because autonomous seed set was higher (Fishman and Wyatt 1999).

	Character		
Crosstype (dam × sire)	Seed set	Seed weight (mg)	Germination rate
Selfer × selfer	20.8 (1.2)	0.051 (0.02)	0.91 (0.02)
Selfer × outcrosser F_1	18.3 (1.3)†	0.051 (0.03)ns	0.76 (0.06)*
Outcrosser × selfer F_1	25.3 (1.6)ns	0.049 (0.02)*	0.76 (0.06)†
Outcrosser × outcrosser	26.5 (2.0)	0.056 (0.04)	0.88 (0.02)

† $P < 0.10$; * $P < 0.05$; ns, not significant.

and outcrosser populations. For these analyses, Pearson correlation coefficients were calculated in the program JMP 3.0.2 (SAS Institute 1994).

RESULTS

Postmating Reproductive Barriers

The Liberty selfer and Pendergrass outcrosser populations were largely compatible at the level of viable seed production. However, both maternal populations showed slight but significant outbreeding depression of seed characters after interpopulation pollination (Table 1). Between-population crossing resulted in lower seed set in selfers (12% decrease, $P = 0.054$) and reduced seed weight in outcrossers (12.5% decrease, $P = 0.013$). Both classes of hybrid seeds demonstrated germinability about 17% lower than seeds from within-population pollinations, although this difference was only marginally significant in the outcrosser dams. Increased opportunities for pollen competition in the within-population crosses (which had several sires) could also contribute to this pattern, but the effects are likely to be small relative to between-population incompatibility. Across pollination treatments, maternal identity had a significant effect on seed weight in both populations ($P < 0.05$ and $P < 0.001$, for selfers and outcrossers respectively) and on seed set only in the selfers ($P < 0.01$).

After transplanting, interpopulation F_1 hybrids grew vigorously and survived to flowering with the same frequency as plants from the two parental populations ($P = 0.13$, overall mean survival = 0.85). The early vegetative morphology (shoot number) of F_1 hybrids was intermediate between the two parental types and showed no maternal family effects or dam × crosstype interactions. However, F_1 plants tended toward the vegetative morphology of their maternal population at the early rosette stage, with outcrosser-dam hybrids producing 50% more shoots than those with selfer seed parents. Both hybrid classes were significantly different from the two parental populations and the mean shoot number of all F_1 individuals fell very near the expectation under additivity (Fig. 2a). Differences between the selfer and outcrosser populations in the timing of germination and early seedling growth (influenced by maternal contributions to seed provisioning) or in maternally inherited cytoplasmic factors probably contributed to this pattern.

Postzygotic reproductive isolation between the two populations appeared most strongly during floral development (Table 2). More than one-third of the F_1 individuals were at least partially male sterile, with indehiscent anthers on short filaments (partial sterility = 21.6%, complete sterility = 13.5%, $n = 171$). Anther sterility was accompanied by early stigma maturity and reductions in the size of some or all petals. Male sterility was equally severe in the two reciprocal classes of F_1 hybrids and much rarer in both parental populations. Only two outcrossers and no selfers in the parental control populations showed any male sterility (Table 2), although plants from both parental populations do produce individual male sterile flowers occasionally (L. Fishman, pers. obs.). The F_1 families differed widely in their expression of male sterility (Pearson chi-square, $P < 0.0001$), with some showing no problems with stamen development and others producing only abnormal flowers. Male sterility occurred at similar frequencies in the F_2 generation (partial sterility = 14.9%, complete sterility = 10%). Levels of male sterility were not significantly different among F_2 lineages, but low sample size within lineages limits the power of this test (likelihood-ratio chi square, $P = 0.09$). No correlation was observed between the frequencies of male sterility in related F_1/F_2 pairs, but fully male sterile F_1 hybrids did not contribute to the F_2 generation, reducing the strength of this test.

The female function of F_1 hybrids was also compromised. Although the mean ovule numbers of hybrids and outcrossers were not significantly different (Table 2), the maximum per flower female fitness of F_1 hybrids was much reduced relative to this parental population. On average, supplemental hand-pollination filled only 43% of F_1 ovules, as compared to 123% of outcrosser ovules, a highly significant difference ($P < 0.0001$). Because the ovule numbers and seed set data were collected from different flowers and mean ovule numbers were used for the calculation of female fertility, values greater than 100% were possible. In contrast, there was no evidence of F_2 breakdown of female fertility (Table 2). Ovule numbers in the F_2 hybrid and paired parental populations were slightly higher than the F1 season values, but again did not differ from one another (Table 2, $P > 0.9$). The autogamous seed set of selfers (S_2) and the supplemented seed set of F_2 and outcrosser (X_2) plants was relatively low this season, probably due to poor greenhouse conditions at the time of flowering, but hybrids were not disproportionately affected. No family-level association between male and female fertility was detected in either hybrid population.

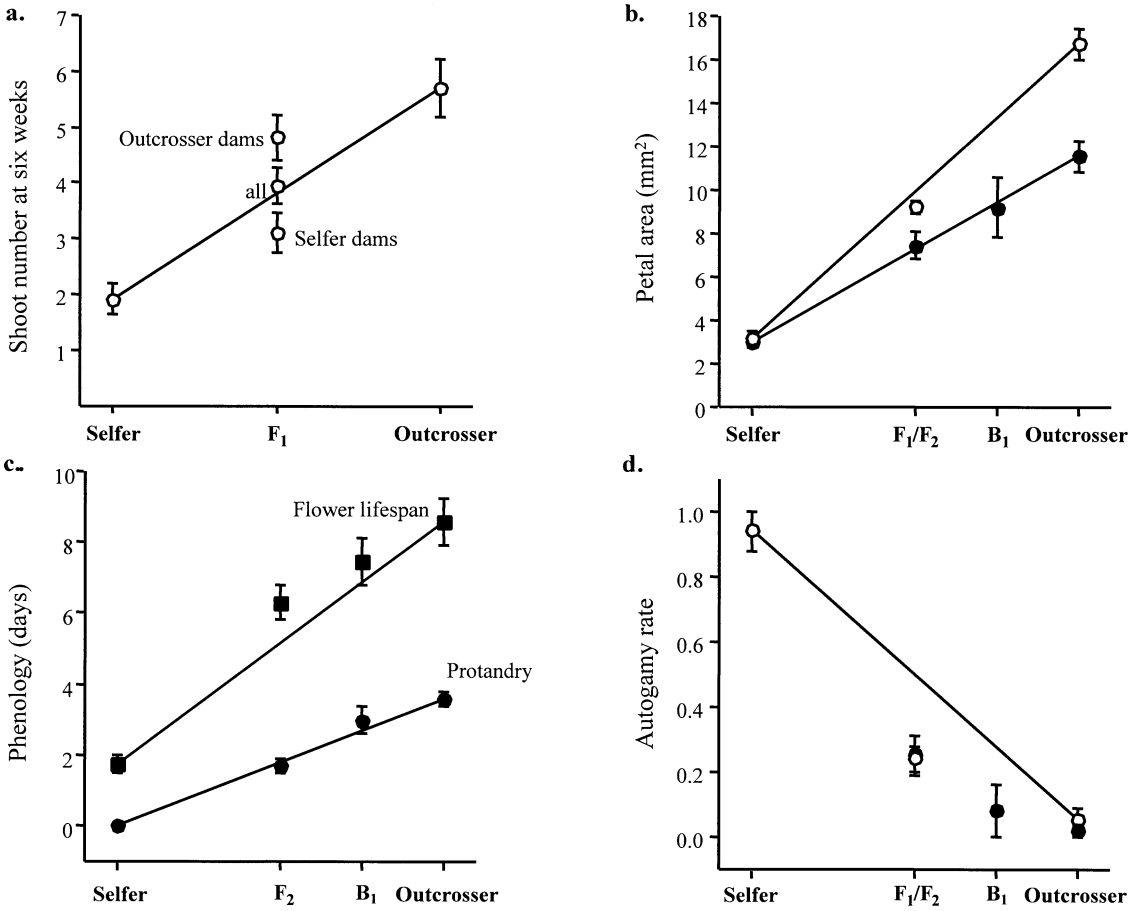

FIG. 2. Phenotypic means (± 2 SE) of hybrids and parental controls. Unfilled symbols indicate F_1 hybrids and S_1 and X_1 parents, filled symbols indicate F_2 hybrids and other populations grown in the second common garden (S_2, X_2, and B_1). For each character, the line between parental population means shows the expectation of a completely additive model of inheritance. (a) Shoot number at six weeks after germination. ANOVA revealed a maternal population effect, so the means of the reciprocal F_1 classes are both shown. (b) Petal size. Flowers of all populations were larger in the 1996 season, probably due to better growth conditions in the greenhouse that year. (c) Protandry (days open prior to stigma receptivity) and flower life span (days open). (d) Autogamy rate (seed set after autonomous selfing/ seed set after supplemental pollination).

Genetics of Floral Traits and Autogamy Rate-Dominance Relationships

The interpopulation hybrids indicate a primarily additive or recessive genetic basis for individual floral characters associated with mating system divergence in *A. uniflora*. Phenotypic means and standard deviations for individual floral characters are given in Table 3. Because of large environmental differences between the years, the F_1 and F_2 populations were compared only with parental populations grown in the same season. Mean petal area in both hybrid generations was not significantly different from the expectation under additivity, falling near the mean of the two parental classes (Fig. 2b). The F_1 families with outcrosser dams were significantly different from one another (ANOVA, $P < 0.005$), whereas those with selfer dams were indistinguishable. However, F_1 and within-population crosses sharing a dam did not exhibit significant common maternal or genetic effects on petal size. Protandry in the F_2 generation also fit a completely additive model of gene action, but flower life span did not, significantly tending in the direction of the outcrosser parent population (Fig. 2c). On average, outcrosser genes affecting floral longevity appear to be partially dominant.

Autogamy rates, which are a product of floral morphology and phenology as well as pollen fertility, did not fit an additive model of gene action in either generation (Fig. 2d). The seed set data from the first season confirmed that selfers have autogamy rates near 100% (mean autogamy = 0.94 ± 0.03 SE), whereas outcrossers autonomously self-fertilize at very low frequency (mean autogamy = 0.054 ± 0.017 SE). If the autogamy rates were simply additive products of individually additive floral characters, hybrids would have a mean autogamy rate around 50%. Instead, the F_1 hybrids self-fertilized at a much lower rate (mean autogamy = 0.24 ± 0.02 SE). In the F_2 generation, autogamous seed set and supplemented seed set both increased relative to the F_1 population, producing a similarly low mean autogamy rate (0.25 ± 0.04 SE). Because flowers close after fertilization, the apparent dominance of outcrosser flower life span in the F_2 hybrids (Fig. 2c) could reflect these low autogamy rates. However, flower life span and autogamy were not significantly correlated within the F_2 population (Table 4), suggesting that the relationship is more indirect.

TABLE 2. Means (SE) for characters related to male and female function in hybrid and control parental populations. The statistical significance of outbreeding depression (lower hybrid performance) is indicated adjacent to the F_1 and F_2 means. No maternal population effects were detected in initial analyses, so each hybrid generation was analyzed as a single class relative to the parental populations.

	Character		
Generation	Ovule number	Male sterility	Female fertility
Selfer (S_1)	—	0.00 (0.00)	—
F_1	28.9 (1.3)ns	0.35 (0.04)***	0.43 (0.04)***
Outcrosser (X_1)	26.5 (2.6)	0.03 (0.02)	1.23 (0.12)
Selfer (S_2)	33.3 (1.2)	0.00 (0.00)	0.60 (0.04)
F_2	35.2 (1.2)ns	0.28 (0.04)***	0.58 (0.07)ns
Outcrosser (X_2)	34.9 (2.6)	0.02 (0.07)	0.66 (0.08)

*** $P < 0.001$; ns, not significant.

Distribution of Floral Variation in Hybrids and Estimation of n_e

Petal area, which was measured in both hybrid populations, showed a striking increase in phenotypic variation from the F_1 to F_2 generations (coefficient of variation [CV] = 21.1 and 37.7, respectively). The F_2 hybrids were also far more variable than X_2 outcrosser controls, which experienced the same degree of inbreeding and were grown simultaneously (CV = 17.8). Despite the small sample size ($N = 87$), the range of petal sizes in the segregating F_2 population overlapped both parental distributions (Fig. 3). Petal size appears to be under primarily additive genetic control, so the standard Castle-Wright equation can be used to estimate the effective number of genetic factors (n_e) influencing this trait. The estimate of n_e for petal size was 2.0 ± 2.8 SD. Because relatively high mean performance in the first experimental season inflates the F_1 variance, this n_e is based on a fairly conservative estimate of the segregational variance. Despite increased opportunities for the segregation of genetic variation, the CV for petal size in the controls decreased from year 1 to year 2 in the selfers (from 34.0 to 25.2) and was constant in the outcrossers (16.4 vs. 17.7) suggesting that any violation of the assumption of equal environmental variance actually biases n_e upward. On the other hand, linkage and the segregation of within-population variation almost certainly bias n_e downward relative to the actual number of loci contributing to interpopulation divergence. As a crude estimate of minimum gene number, however, this low n_e suggests that divergence in flower size in *Arenaria uniflora* reflects the action of relatively few major genes rather than a large number of loci of small effect.

Correlations among Traits Associated with Mating System

The across-population association of traits (small petals, low protandry, short flower life span vs. large petals, high protandry, long flower life span) persisted in the segregating F_2 generation. Protandry and petal size phenotypes were correlated in the F_2 generation (Pearson's $r = 0.50$, $P < 0.0001$; Table 4). These characters were not significantly correlated in the outcrosser (X_2) population ($r = 0.29$, $P = 0.11$), and the selfers lacked variation in protandry. Protandry (days in male phase) and flower life span (total days open) showed a similar pattern of relationship, with a strongly positive phenotypic correlation in the F_2 population ($r = 0.62$, $P < 0.0001$) and none in either parental class. Both F_2 and outcrosser populations exhibited significant positive phenotypic correlations between petal size and flower life span (F_2: $r = 0.42$, $P < 0.0001$; outcrossers: $r = 0.41$, $P = 0.03$). Estimated genotypic correlations (across lineages) for pairs of floral characters were even stronger in the F_2 generation (e.g., $r = 0.80$, $P < 0.0001$ for petal size and flower life span).

In the F_2 generation, individual autogamy rates were negatively phenotypically correlated with petal size ($r = -0.25$, $P < 0.05$) and protandry ($r = -0.23$, $P = 0.07$), but uncorrelated with flower life span ($r = -0.15$, $P = 0.28$). The X_2 controls showed no significant correlation of autogamy rate with any floral characters. Phenotypic correlations between floral morphology and autogamy rate in the F_2 population may partially reflect shared individual environments, but the observation of significant associations only in this generation also strongly suggests the segregation of parental genes with pleiotropic effects or the action of linked genes. In contrast, the phenotypic correlation between petal area and autogamy rate in the F_1 generation was significantly positive ($r = 0.27$, $P < 0.001$, $n = 149$). Although visibly male sterile flowers were excluded, this correlation may be due to more subtle breakdowns of multiple aspects of floral development in some individuals.

Significant correlations between autogamy and floral characters in the F_2 hybrids (Table 4) confirm a fundamental

TABLE 3. Means ± 1 SD of floral characters in the hybrid and control parental populations. Sample sizes are in parentheses.

	Character			
Generation	Petal area (sq. mm)	Protandry (days)	Flower life span (days)	Autogamy rate
Selfer (S_1)	3.15 ± 1.03 (49)	—	—	0.94 ± 0.26 (69)
F_1	9.19 ± 1.95 (167)	—	—	0.24 ± 0.25 (152)
Outcrosser (X_1)	16.71 ± 3.04 (72)	—	—	0.05 ± 0.13 (60)
Selfer (S_2)	2.95 ± 0.73 (33)	0.00 ± 0.00 (24)	1.73 ± 0.65 (24)	—
F_2	7.42 ± 2.80 (86)	1.69 ± 0.80 (69)	6.29 ± 1.96 (69)	0.25 ± 0.27 (63)
Outcrosser (X_2)	11.53 ± 2.05 (34)	3.57 ± 0.58 (31)	8.56 ± 1.87 (31)	0.02 ± 0.03 (30)

TABLE 4. Phenotypic correlations among floral characters in outcrosser control population (X_2; above diagonal) and segregating F_2 generation (below diagonal). Sample sizes are in parentheses.

	Character			
Character	Petal area	Protandry	Flower life span	Autogamy
Petal area		0.29 ns (31)	0.41* (28)	0.17 ns (30)
Protandry	0.50*** (69)		0.19 ns (28)	0.08 ns (30)
Flower lifespan	0.42*** (69)	0.62*** (69)		0.04 ns (30)
Autogamy	−0.25* (62)	−0.23† (58)	−0.15 ns (58)	

†$P < 0.10$; * $P < 0.05$; *** $P < 0.001$; ns, not significant.

relationship between floral morphology and autogamous self-pollination, but interpretation is complicated by hybrid breakdown of male and female function (Table 2). Autogamy rates were calculated relative to supplemented seed set, and morphologically male sterile flowers were not included in the data collection, but more subtle effects on fertility could influence the success of self-pollination. The pollen viability of all hybrids may have been compromised, as florally normal F_1 plants also had very low outcross male fitness in experimental arrays in the field (Fishman 2000). In addition to hybrid infertility, the low autogamy rates of hybrids could result from nonadditive effects of floral morphology on self-pollination (Fig. 2). For example, two days of protandry may have the same effect on the probability of autonomous self-pollination as a five-day gap between male and female function. Such threshold effects would produce outcrosser dominance in autogamy rate even if genes for morphological and phenological characters act additively.

DISCUSSION

The Genetic Architecture of Mating System Divergence

The evolution of selfing in *Arenaria uniflora* appears to have involved the substitution of primarily additive or recessive alleles at a relatively small number of loci controlling multiple floral features. Petal size and protandry, which are negatively correlated with selfing rate both across populations (Wyatt 1986) and in the segregating F_2 hybrids, fit completely additive models of gene action (Figs. 2b and 2c). For floral longevity, which is correlated with both other characters, alleles from the selfer population appear partially recessive on average (Fig. 2c). These dominance relationships are generally consistent with other studies of the genetic architecture of mating system divergence (e.g., MacNair and Cumbes 1989; Shore and Barrett 1990; Fenster and Barrett 1994). Although favorable recessive mutations are doomed for extinction in a completely outcrossing population (Haldane's sieve: Haldane 1927), self-fertilization exposes recessive alleles, including those with beneficial effects. Because *A. uniflora* outcrossers exhibit low-moderate levels of selfing under normal conditions and grow in ephemeral habitats that may also promote biparental inbreeding, even completely recessive selfing modifiers could readily spread to fixation if advantageous.

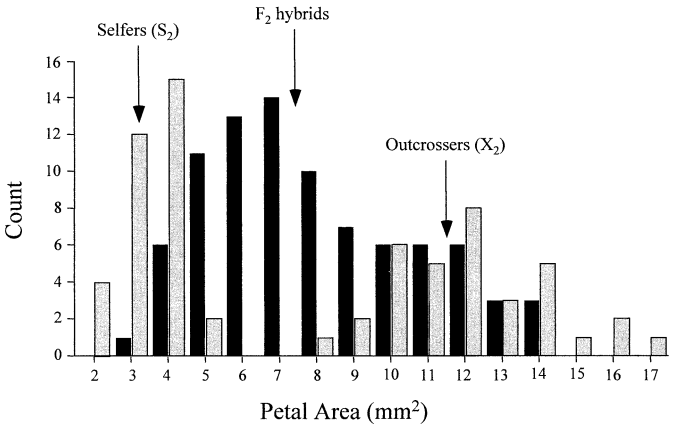

FIG. 3. Distribution of petal size in F_2 hybrids (dark bars) and control parental populations (S_2 and X_2; light bars). Arrows indicate experimental population means.

As few as two chromosomal regions may influence individual floral characters associated with mating system in *A. uniflora*. All three floral characters exhibited broadly unimodal distributions in the segregating F_2 generation, suggesting that single major genes were not responsible for the divergence between populations. However, the F_2 phenotypic distributions overlapped with both parental populations, suggesting the segregation of a relatively small number of genes (Fig. 3). For petal size, this segregational variance produced an estimated minimum n_e of 2.2 ± 2.8 SE. Similarly low values of two to three effective factors result for protandry and floral longevity (segregational variance for these was calculated from the control parental populations rather than F_1 values, which were not measured for these traits).

Any n_e estimates must be treated with caution for several reasons. Linkage between loci and inequality of gene effects will bias n_e downward even in the best of circumstances (Zeng et al. 1990). After only a single generation of recombination, the low estimates of n_e may reflect the cosegregation of parental alleles at a much larger number of linked loci with effects on floral characters. Linkage disequilibrium is a problem for both biometric and QTL studies of genetic architecture, and can only be completely resolved by identifying the nucleotide changes responsible for an estimated QTL. The petal size data conform to other assumptions of the minimum n_e calculations, but selfing of the genetically variable F_1 individuals could have somewhat amplified the F_2 variance. (However, higher homozygosity in the X_2 relative to the X_1 outcrossers does not result in much exposure of within-population variation [CV = 17.8 and 16.4, respectively]). The release of novel epistatic variation in hybrids, including the floral breakdown associated with male sterility in both F_1 and F_2 hybrids, may have also affected the calculations of segregational variance.

Given these caveats, it is still interesting to note that the minimum estimates of gene number differentiating these *A. uniflora* populations are smaller than most calculated for similarly divergent taxa. Fenster and Ritland (1994b) observed no increase in variance in F_2 relative to F_1 hybrids and estimated a minimum of at least 10 factors involved in the divergence of each floral trait between selfer and outcrosser pairs of *Mimulus* taxa. MacNair and Cumbes (1989) reported three to seven factors differentiating *Mimulus guttatus* and *M. cupriphilus* for floral traits, and a minimum of five factors were implicated in stigma-anther separation changes within *Turnera ulmifolia* var. *angustifolia* (Shore and Barrett 1990). A more powerful QTL mapping study of mating system divergence in the *Mimulus guttatus* species complex found a minimum of 11–15 QTLs affecting each floral character (Fishman et al. 2002), suggesting that the evolution of selfing in *M. nasutus* involved many loci throughout the genome. The low estimates of gene number for *Arenaria* relative to these other systems may partly reflect the statistical issues noted above, but may also be a product of the particular evolutionary history of mating system divergence in this species.

Estimates of n_e lower than the chromosome number in *Arenaria* ($n = 7$) falsify both an infinitesimal model of flower size evolution and single-step models in which selfing can evolve regardless of inbreeding depression and pollen discounting (Lande and Schemske 1985; Charlesworth et al. 1990; Schultz and Willis 1995). An oligogenic basis for floral divergence in *Arenaria* is theoretically consistent with either automatic selection (Fisher 1941) or ecological selection for selfing. However, for selfing to evolve via an automatic transmission advantage (Fisher 1941; Lande and Schemske 1985), variants with increased selfing rates must also have high outcross male fitness (e.g., lose protandry while maintaining large petals and staying functionally male/hermaphrodite long enough to attract pollinators and export pollen). In contrast, the correlations among traits observed in this study suggest that ancestral selfer genotypes may have produced phenotypes with inherent trade-offs in male function. Protandry, flower life span and petal size, which are negatively correlated with self-pollination across populations (Wyatt 1986), are significantly correlated with one another in the F_2 hybrids (Table 4). These strong phenotypic correlations are due at least in part to linkage or pleiotropy of genes underlying floral divergence, since strong environmental correlations were not observed in the more genetically uniform control populations. True pleiotropy is difficult to distinguish from linkage between genes with effects on single characters with biometric analyses, but a pleiotropic basis for floral traits is particularly consistent with other data on the adaptive significance of selfing in *A. uniflora*.

Previous work implicates female fitness losses due to pollen transfer from congener *A. glabra* as a potential selective factor in the evolution of autogamy in *Arenaria uniflora* (Fishman and Wyatt 1999). Under such ecological selection, major genes promoting autogamy could spread even if they had negative pleiotropic effects on outcross male fitness. Large reductions in petal size, which reduce attractiveness to pollinators and thus eliminate the transmission advantages of self-fertilization (Holsinger et al. 1984), would only be favored under such conditions. In fact, competition through heterospecific pollen transfer from *A. glabra* should generate selection not just for self-pollination, but also for preemptive self-fertilization (Fishman and Wyatt 1999). Unlike ecological conditions that promote delayed selfing as reproductive assurance against pollen limitation (Lloyd 1979), this mode of selection would actually favor variants with reduced petal size and flower life span, as well as reduced protandry, resulting in a shared genetic basis for these characters. The results presented here are suggestive of the rapid fixation of a few major modifier alleles with effects on multiple floral traits, but further work will be necessary to determine whether the genetic architecture of floral divergence in *Arenaria* reflects strong selection for prior selfing or a more gradual process of floral modification after the evolution of high self-fertilization. Quantitative trait locus mapping, which allows estimation of the effects of individual genetic regions and has proven useful in identifying (e.g., Bradshaw et al. 1995) or ruling out (e.g., Fishman et al. 2002) major QTLs contributing to adaptive divergence, may allow discrimination of these alternatives.

The Evolution of Selfing and the Development of Postmating Reproductive Barriers

Hybrids between selfer and outcrosser *Arenaria uniflora* revealed a surprising degree of postmating reproductive isolation. At the level of F_1 fruit production, the two populations studied here are highly cross-compatible relative to 34 other population pairs examined by Wyatt (1990), including pairs of outcrossers, and they cluster together in allozyme analyzes (genetic similarity = 0.93; Wyatt et al. 1992). They experienced only slight reductions in seed performance after cross-pollination (Table 1) and there was no evidence of hybrid inviability. However, hybrid breakdown of both male and female function indicates the rapid development of genetic incompatibility between these prezygotically isolated populations. The levels of hybrid infertility reported here are higher than those reported for many interspecific or even intergeneric crosses in flowering plants (Arnold 1997). What genetic mechanisms could cause the observed pattern of reproductive breakdown?

The female fertility (seed production after supplemental pollination) of F_1 hybrids was significantly reduced relative to parental controls, despite no decrease in ovule number. Fertility recovery in F_2 hybrids is consistent with the fixation of alternative chromosomal arrangements in these selfer and outcrosser populations. Certain kinds of chromosomal rearrangements can result in the production of up to 50% aneuploid gametes when made heterozygous in F_1 hybrids (White 1969; Walsh 1982). Because the initial fixation of alternative arrangements with negative heterozygous effects requires strong genetic drift to overcome that selective disadvantage, chromosomal explanations for hybrid sterility have been theoretically disfavored (Walsh 1982). However, both selfer and outcrosser populations of *A. uniflora* may have effective population sizes low enough to allow the fixation of alternative arrangements causing substantial heterozygote infertility. Obligate selfing in the Liberty population certainly creates conditions in which drift dominates population dy-

namics and outcrossers may also experience severe population bottlenecks during establishment on new granite outcrops or in dry years. Alternatively, negative interactions between alleles from different populations (see below) could contribute to the low female fitness of F_1 plants observed in this study. Further genetic studies will allow the differentiation of these possibilities and examination of the relationship between male and female fertility in hybrids.

Male sterility, which was observed in approximately one-third of both F_1 and F_2 hybrids, was characterized by partial or complete deformation of petals and precocious stigma maturation as well as stunted anther development. The male sterile morphology was expressed differentially across F_1 families and varied in expression among flowers on a plant and even among anthers of a flower. Novel allelic interactions or the breakup of coadapted gene complexes can lead to reduced fitness or transgressive phenotypes in hybrids (Lynch 1991). However, the pattern of male sterility observed does not readily fit with the expectations of simple models of epistatic breakdown in hybrids (Turelli and Orr 2000). Because the stunted appearance of sterile anthers resembles the anther reductions in females of gynodioecious members of the Caryophyllaceae (e.g., Desfeux et al. 1996), one attractive explanation is a mismatch between cytoplasmic male sterility (CMS) and nuclear restorer genotypes in hybrids (e.g., Michaelis 1954; Levy 1991). However, because the reciprocal classes of F_1 hybrids were equally affected, this mechanism would require that the selfer and outcrosser populations have separate and incompatible CMS-restorer systems.

Nuclear-nuclear epistatic interactions are a more likely explanation for the male sterility and associated floral deformity of *Arenaria* hybrids. If populations fix novel alleles at interacting loci, some multi-locus genotypes present in hybrids will never have been tested by selection and may exhibit reduced fitness (Dobzhansky 1937). The novel alleles may be fixed by either drift or selection and, depending on their dominance interactions, can produce negative phenotypic effects in F_1 and/or F_2 hybrids (Turelli and Orr 2000). Such epistatic interactions are thought to be primary contributors to postzygotic isolation and speciation in animals (for review, see Coyne and Orr 1998) and a few plant systems (e.g., Fishman and Willis 2001), and may readily develop among isolated populations of a single species (e.g., Christie and MacNair 1987). For epistatic breakdown to contribute to male sterility and associated floral deformation in these *Arenaria* hybrids, the novel alleles would need to be at least partially dominant in their negative interactions. Also, because F_1 families varied in their sterility, interacting factors could not be fixed within populations or their effects could be dependent on the genetic or environmental background. More quantitative methods of assessing male fertility (e.g., pollen staining) may reveal additional hybrid breakdown.

Much more work will be required to identify the specific genetic mechanisms underlying the male sterile morphology in these *Arenaria* hybrids, but some additional observations raise the intriguing possibility that postzygotic barriers may be related to the genetic architecture of divergent floral evolution. First, the widespread male sterility observed in both the F_1 and F_2 generations appears to reflect the genetic perturbation of a poorly buffered developmental process. Male sterility occurs along a continuum in the hybrid populations, varying among individuals, among flowers and even among anthers within a flower. Furthermore, the characteristic male sterile morphology (stunted anthers, reduced petals, stigma maturity at anthesis) occurs as individual flowers in the parent populations and can be environmentally induced. For example, the last few flowers produced by the field-collected plants in the base outcrosser parental population were often completely male sterile and plants from another outcrosser population produced only completely male sterile flowers after being kept at low temperature and low light for two months (L. Fishman, pers. obs.). Partial male sterility also occurs in some *A. uniflora* populations in the field (Wyatt 1990). Novel genic interactions in hybrids, like such environmental shocks, may destabilize a vulnerable developmental stage with broad effects on floral morphology.

Second, it is possible that the loci involved in floral divergence between outcrossers and selfers directly contribute to the floral breakdown of hybrids. Completely male sterile flowers were always characterized by stunted stamens, reduced petals, and a mature stigma at anthesis. The latter two alterations are distinguishing features of derived selfer populations, which also have reduced (but functional) stamens. Comparative developmental studies of this same pair of selfer and outcrosser *A. uniflora* populations found that the reduced floral morphology of selfers involves an overall slowing and lengthening of organ development (Hill et al. 1992). Various organs in selfer flowers exhibit similarly reduced rates of growth and differentiation. However, the initiation of anther differentiation and pollen division occurs at the same absolute time in both morphs, resulting in smaller mature anthers and fewer pollen grains in selfers (Hill and Lord 1990). If these changes in the rate and duration of development (heterochrony: Raff and Wray 1989) are not all under completely parallel genetic control, developmental mismatches in hybrids could result in the observed male sterile morphology. If this is the case, the apparent oligogenic and pleiotropic basis of floral divergence between outcrossers and selfers may contribute to the dramatic disruption of floral morphology in some hybrids.

These new data on reproductive barriers between selfer and outcrosser populations of *A. uniflora* suggest a degree of isolation greater than that between many plant taxa given species status. Selfer populations, which are found on the western (Alabama) and northeastern (western Carolinas) edges of the *A. uniflora* range, were originally grouped together as a separate species based on their reduced floral morphology (*A. alabamensis*: Wyatt 1977). Later analyses of vegetative morphology, allozyme identity, and crossability led Wyatt (1988) to argue for multiple derivations of the selfing syndrome and the maintenance of a single species designation. Indeed, crosses between the Liberty selfer studied here and plants from an Alabama selfer population were almost entirely unsuccessful, and the few surviving hybrids exhibited transgressive segregation for floral characters (L. Fishman, unpubl. data). Together, these data suggest that *Arenaria* populations are either much more genetically divergent than previously suspected, or that both morphological divergence and postzygotic barriers are evolving very rapidly. In general, genetic distance and postzygotic reproductive isolation are

positively correlated (Coyne and Orr 1998). If the substantial hybrid sterility reported here simply reflects the gradual accumulation of incompatible gene substitutions in allopatric populations, then all other populations of *A. uniflora* should be almost entirely isolated from one another. However, if the observed hybrid sterility derives from interactions among the particular loci involved in floral differentiation, its expression may be correlated with morphological divergence rather than divergence at neutral genetic markers.

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