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Lila Fishman; Robert Wyatt

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POLLINATOR-MEDIATED COMPETITION, REPRODUCTIVE CHARACTER DISPLACEMENT, AND THE EVOLUTION OF SELFING IN *ARENARIA UNIFLORA* (CARYOPHYLLACEAE)

LILA FISHMAN^{1,2} AND ROBERT WYATT³

¹Department of Ecology and Evolutionary Biology, Princeton University, Princeton, New Jersey 08544

³Highlands Biological Station, P.O. Box 580, Highlands, North Carolina 28741

Abstract.—Ecological factors that reduce the effectiveness of cross-pollination are likely to play a role in the frequent evolution of routine self-fertilization in flowering plants. However, we lack empirical evidence linking the reproductive assurance value of selfing in poor pollination environments to evolutionary shifts in mating system. Here, we investigated the adaptive significance of prior selfing in the polymorphic annual plant *Arenaria uniflora* (Caryophyllaceae), in which selfer populations occur only in areas of range overlap with congener *A. glabra*. To examine the hypothesis that secondary contact between the two species contributed to the evolution and maintenance of selfing, we used field competition experiments and controlled hand-pollinations to measure the female fitness consequences of pollinator-mediated interspecific interactions. Uniformly high fruit set by selfers in the naturally pollinated field arrays confirmed the reproductive assurance value of selfing, whereas substantial reductions in outcrosser fruit set (15%) and total seed production (20–35%) in the presence of *A. glabra* demonstrated that pollinator-mediated interactions can provide strong selection for self-pollination. Heterospecific pollen transfer, rather than competition for pollinator service, appears to be the primary mechanism of pollinator-mediated competition in *Arenaria*. Premating barriers to hybridization between outcrossers and *A. glabra* are extremely weak. The production of a few inviable hybrid seeds after heterospecific pollination and intermediate seed set after mixed pollinations indicates that *A. glabra* pollen can usurp *A. uniflora* ovules. Thus, any visit to *A. uniflora* by shared pollinators carries a potential female fitness cost. Moreover, patterns of fruit set and seed set in the competition arrays relative to controls were consistent with the receipt of mixed pollen loads, rather than a lack of pollinator visits. Competition through pollen transfer favors preemptive self-pollination and may be responsible for the evolution of a highly reduced floral morphology in *A. uniflora* selfers as well as their current geographical distribution.

Key words.—*Arenaria*, hybridization, mating system evolution, reproductive character displacement, self-pollination.

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Shifts in plant mating systems are often correlated with changes in habitat, life history, or other ecological parameters (Stebbins 1957; Barrett and Eckert 1990; Barrett et al. 1996). Such associations suggest that the frequently observed evolutionary transition from outcrossing to selfing may involve adaptation to local pollination environments. However, quantitative studies of mating system evolution have primarily focused on genetic factors, particularly the magnitude of inbreeding depression relative to the theoretical transmission advantage of selfing genotypes (Fisher 1941). Given the potential reproductive assurance value of selfing, measures of the prevalence and strength of ecological selection for self-pollination are a necessary complement to our knowledge of the costs of inbreeding.

The most influential model of mating system evolution (Lande and Schemske 1985) focuses on the coevolution of inbreeding depression and selfing rate below a threshold level set by the theoretical transmission advantage of selfing genes that also allow outcross pollen donation (Fisher 1941). The distribution of selfing rates across plant populations generally fits the predictions of this model (Schemske and Lande 1985; Barrett et al. 1996; but see Aide 1986), and it has inspired a rich empirical literature on the causes and consequences of inbreeding depression (for reviews, see Charlesworth and Charlesworth 1987; Husband and Schemske 1996). By assuming a null state of strong genetic selection for selfing,

such approaches obviate ecological selection as an explanatory force in mating system evolution. However, trade-offs in male fitness associated with increases in the selfing rate will reduce the genetic transmission advantages of self-fertilization (e.g., Holsinger 1992) and recent empirical work has indicated that “automatic” selection through male fitness can depend on pollination ecology (Kohn and Barrett 1994; Holsinger and Thomson 1995). If selfing variants do not have a constant selective advantage, pollinator-mediated selection becomes a potentially important explanation for the evolution of self-pollination.

The reproductive assurance value of self-fertilization under some ecological conditions is clear. Self-fertile plants appear to be preadapted for long-distance dispersal and the colonization of sites where the supply of pollinators and/or mates is uncertain (Baker 1955), producing a general correlation between selfing rate and weediness (Lloyd 1980). Associations between selfing and annual or herbaceous habit contribute to this pattern (Barrett et al. 1996). Selfing is also common in ephemeral habitats, which place a premium on rapid development and small reproductive size, as well as creating uncertainty in pollinator service (Barrett et al. 1989; Dole 1992). In general, selfing should provide reproductive benefits whenever the pollination environment (availability of mates and/or pollinators) is poor (Lloyd 1979, 1988, 1992).

Selfing may insure against variation in the quality as well as the quantity of mates. Increased self-fertility has evolved in local metal-tolerant populations of several species, apparently in response to strong selection against mating with

² Present address: Ecology and Evolution Program, 1210 University of Oregon, Eugene, Oregon 97403-1210; E-mail: lfishman@darkwing.uoregon.edu.

neighboring nontolerant populations (Antonovics 1967; LeFebvre 1970). By selfing, metal-tolerant individuals lose the benefits of outcrossing, but also avoid gene transfer from nontolerant individuals and the consequent production of unfit offspring. The evolution of self-fertilization in such diverging populations provides the best evidence for ecological selection on mating system. These cases exemplify Dobzhansky's (1937) process of speciation by reinforcement, in which ecologically dependent hybrid unfitness favors the development of pre-mating barriers to hybridization. However, the evolution of selfing as reproductive character displacement could also be an outcome of pollinator-mediated interactions between taxa that have already developed substantial postzygotic barriers in isolation.

Pollinator-mediated competition between species can operate through two different mechanisms, which Waser (1983) termed competition for pollinator service and competition through interspecific pollen transfer. In sympatric plants that share insect visitors, competition for pollinator service may reduce both female fitness and successful pollen export (Rathcke 1983; Campbell 1985a; Rathcke and Real 1993). Obligate outcrossers appear particularly vulnerable to such competition (Feinsinger and Tiebout 1991). Even in the absence of pollinator preferences, division of a limited pollinator resource may reduce reproductive success. Rates of successful pollen transfer can be extremely low even in single-species arrays (Ornduff 1980; Thomson and Thomson 1989) and effective pollen carryover is reduced by intervening visits to competitors (Campbell 1985b; Feinsinger and Busby 1987; Rathcke 1988). The local frequencies of coflowering species, and pollinator constancy will determine the strength of the competitive interaction (Levin and Anderson 1970).

The transfer of heterospecific pollen between coflowering species can negatively affect reproductive success even if pollinators themselves are not limiting. Mechanisms of post-pollination competition range from pollen allelopathy to hybridization (although hybridization does not necessarily result in reduced fitness; Arnold 1997). Pollen transfer from unrelated species has been shown to reduce seed production in several hummingbird-pollinated wildflowers (Waser and Fugate 1986; Galen and Gregory 1989). Pollen transfer between sympatric congeners is a frequent and potentially costly phenomenon. Incomplete barriers to interspecific mating may result in inhibition of legitimate pollen tubes, usurpation and abortion of ovules, and/or the production of less fit hybrid progeny. The negative effects of interspecific pollen transfer may lead to reproductive character displacement or the reinforcement of barriers to hybridization and may underlie the tremendous diversity of floral morphology in some taxa (Waser 1983; Armbruster et al. 1994). Local reproductive character displacement reduces hybridization and attendant fitness losses in *Phlox* (Levin and Schaal 1970, Levin 1985), and the high selfing rate of one species appears to limit damaging pollen flow between sympatric species of *Ipomea* (Stucky 1985). However, only a few studies have examined the costs of pollinator-mediated competition between related plant species.

In this study, we examine the strength and mode of ecological selection for autonomous self-pollination in the annual plant *Arenaria uniflora* (Caryophyllaceae). This species,

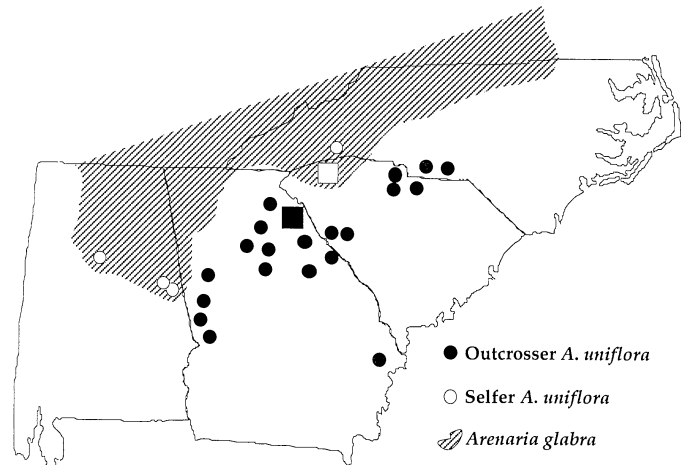


FIG. 1. Distribution of selfer and outcrosser populations of *Arenaria uniflora* in the southeastern United States, redrawn from Wyatt (1984). The square symbols identify the selfer (Liberty, SC) and outcrosser (Pendergrass, GA) populations used as sources of plants for this study. The hatched area indicates the species range of *Arenaria glabra*, as determined from populations mapped in Weaver (1970).

which is endemic to granite outcrops in the southeastern United States, consists of well-differentiated but closely related selfer and outcrosser populations (Wyatt 1984, 1986). Plants from selfer populations are pseudo-cleistogamous (i.e., they have highly reduced flowers that may not open and generally self-pollinate in the bud). The selfers are phenotypically distinct enough to have been initially treated as a separate species (*A. alabamensis*; Wyatt 1977), but appear to be closely related to nearby outcrosser populations (Wyatt et al. 1992). The selfers are restricted to the western and northeastern edges of the species range, in areas of sympatry with congener *A. glabra* (Fig. 1). *Arenaria glabra* is phylogenetically distinct from *A. uniflora* (McCormick et al. 1971; Fishman 1998), but shares a generalized floral morphology and pollinator community with the outcrossers. Wyatt (1984, 1986) hypothesized that competition for pollinators during secondary contact with *A. glabra* may have selected for self-pollination in ancestral *A. uniflora* outcrossers. However, the observed pattern of reproductive character displacement could result from either mode of pollinator-mediated selection. No natural hybrids between *A. glabra* and *A. uniflora* have been reported (Weaver 1970; McCormick et al. 1971; Wyatt 1986), but preliminary experimental crosses suggest that barriers to mating between outcrosser *A. uniflora* and *A. glabra* are incomplete. *Arenaria glabra* pollen tubes grow normally through outcrosser *A. uniflora* styles and outcrosser flowers behave as if legitimately pollinated following heterospecific hand-pollination (L. Fishman, unpubl. data). These observations suggest that heterospecific pollen transfer, as well as competition for pollinator service, could have selected for selfing in ancestral *A. uniflora* populations.

Using selfer and outcrosser plants from established populations as experimental models, we examine the female fitness consequences of pre- and postmating interactions with *A. glabra*. We use measures of fruit production in naturally pollinated experimental arrays to determine whether auton-

omous self-pollination provides reproductive insurance against interactions with *A. glabra*, as Wyatt (1984, 1986) suggested. We estimate the strength of pollinator-mediated selection for selfing from the fitness losses of outcrossers in the experimental presence of *A. glabra* and consider such selection relative to genetic factors favoring and opposing self-fertilization. We determine the nature of postmating barriers to hybridization by quantifying seed performance after heterospecific and mixed hand-pollinations. By comparing patterns of variation in female fitness components in arrays with *A. glabra* to pollen-limited and unlimited controls, we assess the relative contributions of heterospecific pollen transfer and competition for pollinators to the fitness losses in the field. Finally, we consider the relevance of pollinator-mediated interactions to both mating system evolution and the development of reproductive isolation.

MATERIALS AND METHODS

Study Populations

Arenaria uniflora (Walt.) Muhl. and *A. glabra* Michx. (Caryophyllaceae) are winter annual plants endemic to granitic outcrops in the southeastern United States. *Arenaria uniflora* ($2n = 14$) is thought to be a relictual element of the southwestern Madro-tertiary flora, whereas *A. glabra* ($2n = 20$) is a relatively recent derivative of the northern Arcto-tertiary perennial *A. groenlandica* (McCormick et al. 1971). The species ranges overlap in northeastern Alabama and the western Carolinas (Fig. 1). *Arenaria uniflora* is characterized by large-flowered protandrous populations (outcrossers) in the center of its range and small-flowered autonomously selfing populations (selfers) in areas of sympatry with *A. glabra*. *Arenaria glabra* and outcrosser *A. uniflora* flowers are nectariferous and are pollinated by generalist flies (Syrphidae, Bombyliidae) and small bees (Halictidae), as well as honeybees (Weaver 1970; Wyatt 1986; L. Fishman, pers. obs.). All annual *Arenaria* are self-compatible, with protandry and pollinator attraction promoting outcrossing in *A. glabra* and outcrosser *A. uniflora*. Because the spring growing season on granite outcrops is limited to a few months by low water availability, *Arenaria* plants at a given site generally flower synchronously.

The two *A. uniflora* populations used in this study represent extremes of mating system within the species. The outcrosser population (Pendergrass, GA; Wyatt's [1984] population 7) is strongly protandrous, with the 1-cm diameter flowers lasting up to two weeks. Single-locus estimates of population outcrossing rate in two different years suggest a mixed mating system (outcrossing rate = 0.73 ± 0.06 SD; L. Fishman, unpubl. data). Evident population genetic structure makes this a minimum estimate of individual outcrossing, but it is generally corroborated by individual pollen import rates in naturally pollinated arrays (Fishman 1998). Plants from the selfer population (Liberty, SC; Wyatt's [1984] population 8) are virtually cleistogamous, usually self-pollinating in the bud. Male and female function occur simultaneously and the small (< 4 mm) flowers are rarely open for more than a day. Supplemental pollination does not increase seed set in these selfers. The selfer population does not contain enough al-

lozyme polymorphism to estimate population-level outcrossing rate (Wyatt et al. 1992).

Despite their striking floral and mating system divergence, the Liberty and Pendergrass populations are geographically and genetically allied. The populations are only 115 km apart, although isolated on discrete granite outcrops. Allozyme and crossability data indicate that the Liberty selfer population has had more recent genetic contact with the Pendergrass outcrossers than with other *A. uniflora* populations (Wyatt 1990; Wyatt et al. 1992). The two populations also have similar ovule numbers and vegetative morphology, although outcrosser plants are larger overall. See Wyatt (1984, 1986) for details of population locations and descriptions. (Taxonomic note: Both *A. glabra* and *A. uniflora* have been placed in the genus *Minuartia* by some authors [McNeill 1962; McCormick et al. 1971]. This assignment is controversial [Wyatt 1977], however, and to provide continuity with previous work, we use *Arenaria* in referring to these species.)

General Culture

We collected dormant rosettes of outcrosser *A. uniflora* (Pendergrass, GA), selfer *A. uniflora* (Liberty, SC) and *A. glabra* (Liberty, SC), in early March of each season. Individual rosettes were transplanted into 8-cm square plastic pots containing a 1–2-cm layer of field soil over 6 cm of coarse sand. The plants were raised in an insect-free greenhouse at the University of Georgia Plant Growth Facility. The greenhouse was kept cool (15°C) and naturally lit throughout the growing season. Experimental densities (2.6 plants/100 cm²) were lower than natural densities (7/100 cm²; Wyatt 1986), and by the end of the season the experimental plants were substantially larger than most wild individuals. Greenhouse plants remained in phenological synchrony with plants in the field until late spring.

Hand-Pollinations

The effects of heterospecific pollen deposition were measured on 36 outcrosser *A. uniflora* mother plants randomly chosen from the 1996 greenhouse population. Each plant received four hand-pollination treatments: outcross, self, heterospecific (*A. glabra*), and mixed (*A. glabra* + outcross), and was also allowed to set seed autonomously. Recipient flowers were emasculated prior to anther dehiscence and hand-pollinated after the stigma lobes became receptive (four to five days after anthesis). Each outcrossed flower received pollen from two or more donor plants per pollination from a separate pool of 100 plants. Pollen from newly dehisced donor anthers was mixed on the forceps prior to application. Selfed flowers received pollen from younger flowers on the maternal plant. Flowers in the heterospecific treatment received pollen from a pool of 24 *A. glabra* plants. Flowers in the mixed treatment received a mixture of *A. glabra* and outcross *A. uniflora* pollen from the same donor pools. Pollen was not carefully titrated in this treatment, but approximately equal volumes combined on the forceps prior to application. Approximately five flowers per plant received each hand-pollination treatment. Autonomous seed set was allowed on seven to 10 unmanipulated flowers per plant. Lack of fruit set by emasculated but unpollinated control flowers con-

firmed that there was little illicit pollen movement in the greenhouse. Plants were hand-pollinated over a four-week period, with an effort made to pollinate each plant with every pollination treatment on any given day.

Preliminary crosses had indicated that outcrosser *A. uniflora* flowers often form empty capsules upon receipt of *A. glabra* pollen, resulting in little or no fruit set reduction in the greenhouse environment. Fruit set after heterospecific pollination ranged from 50% to 100% of conspecific fruit set. In addition, some unmanipulated outcrosser flowers form uninflated fruits containing one or two selfed seeds. To avoid undercounting few-seeded fruits, we harvested all marked flowers after three weeks or just prior to capsule dehiscence. The collections from each treatment-by-dam combination were pooled. Thus, the seed set values represent the number of seeds per flower and may incorporate some variation in capsule production. After air-drying, seeds were counted and weighed in bulk. Capsule contents were counted as seeds if the seed coat appeared filled (i.e., if they rolled between finger and table). Germination was assayed in parafilm-sealed petri dishes containing moist sand. Seeds were after-ripened at 30°C/15°C (12h/12h cycle) in an environmental control chamber for three months, then cooled to 15°C/8°C to initiate germination (Baskin and Baskin 1987). Total percent germination was recorded after one month.

Analysis of variance (ANOVA) was used to determine whether pollen source affected seed set, with maternal identity as a random block effect. Seed weight was analyzed with a similar ANCOVA, with the number of seeds per fruit added as a covariate. Comparisons of pollination treatments were made with linear contrasts of the model least squares means in the program JMP 3.0.2 (SAS Institute 1994).

Field Competition Experiments

Pollinator-mediated effects of *A. glabra* on *A. uniflora* female fitness were measured at the Rock and Shoals granite outcrop near Athens, Georgia, in 1995 and 1996. The outcrosser *A. uniflora* plants that naturally occur at this outcrop closely resemble the Pendergrass outcrosser population (32 km away) and the site supports typical granite outcrop plant and pollinator communities. Several weeks prior to flowering, experimental arrays composed of outcrosser *A. uniflora*, selfer *A. uniflora*, and/or *A. glabra* were placed on the outcrop in areas of natural *A. uniflora* growth. Arrays were assigned arbitrary positions in these areas, with at least 10 m between replicates. The arrays were set 4 cm into the ground to facilitate water uptake and to minimize height differences with surrounding natural *A. uniflora* plants. In 1995, the outcrop experienced a severe drought and the arrays were watered three times per week.

1995 Season.—In 1995, the fruit set of selfer and outcrosser *A. uniflora* plants was measured in experimental arrays containing *A. glabra* plants (+*glabra*) and in control arrays without *A. glabra* (Fig. 2a). Plants were arranged checkerboard fashion within each 6 × 6 array, such that control arrays ($n = 4$) contained 18 selfers and 18 outcrossers, whereas +*glabra* arrays ($n = 6$) contained 12 plants each of selfers, outcrossers, and *A. glabra*. The persistent sepals of all selfer and outcrosser flowers were marked with a permanent marker

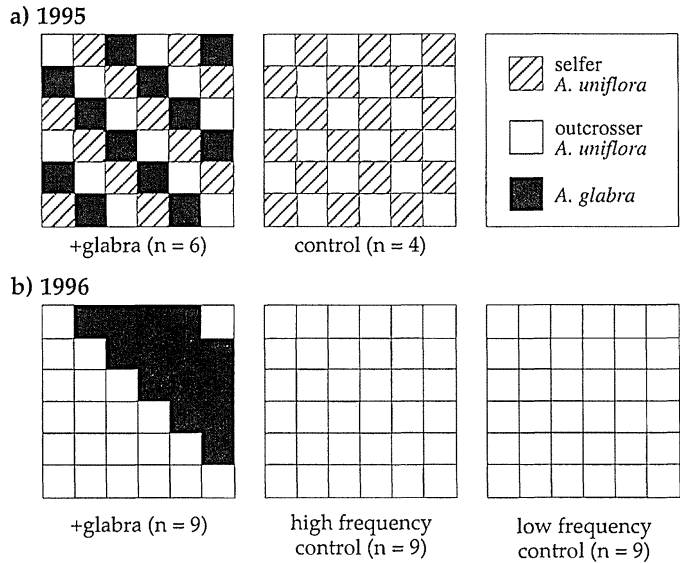


FIG. 2. Experimental design of field competition experiments. Each season, replicate arrays were assembled and placed at random locations at least 10 m apart at the Rock and Shoals granite outcrop near Athens, Georgia. (A) In 1995, the fruit set of selfer and outcrosser *Arenaria uniflora* plants was measured in competition arrays with *A. glabra* and in control arrays. (B) In 1996, both the fruit set and seed set of outcrosser *A. uniflora* plants were measured in competition arrays with *A. glabra* and in two types of control arrays. The high-frequency control maintained total display size relative to the competition treatment; the low-frequency control maintained *A. uniflora* display size.

during the six-week flowering season. At the end of the season, marked capsules were counted in the field. Fruit set was calculated as the ratio of marked capsules to marked flowers.

The fruit set data were analyzed using a nested mixed-model ANOVA with competition treatment and source population as fixed effects and array (a random block effect) nested within treatment. The fruit set ratios were arcsine-square-root transformed for analysis to normalize the residuals. Differences between the populations and between treatments within each population were analyzed with linear contrasts of the effect least squares means (LSMs). The analyses were performed in the standard least squares ANOVA platform in JMP 3.0.2 (SAS Institute 1994).

1996 Season.—To more closely examine pollinator-mediated competition with *A. glabra*, the 1996 experiment focused on outcrossers and measured both fruit set and seed set. Greenhouse crosses had shown that pollen transfer from *A. glabra* to outcrossers can lead to the production of empty or few-seeded fruits. In the field, transfers of heterospecific pollen should result in both reduced seed set and increased variability in seed set among an individual's fruits. By measuring both fruit set and seed set, this experiment estimated the total effect of *A. glabra* on outcrosser female fitness. Selfers were not included in this design because they showed no effect of the +*glabra* treatment in 1995 and because their elimination allowed increased replication and time for the collection of seed set data.

Three types of experimental arrays were constructed (Fig. 2b). Each contained a block of 14 outcrosser plants on which data were collected, plus an adjacent block of either *A. glabra*

plants (+glabra), outcrossers (high-frequency control), or empty pots (low-frequency control). The low-frequency treatment was added for two reasons. It decouples the effects of *A. glabra* presence from the effects of a lower local frequency of legitimate mates, which were confounded in the 1995 design. By potentially affecting pollinator visitation (but not pollen load composition), the low-frequency array also allows discrimination of the effects of heterospecific pollen transfer from those of decreased pollinator service. The competition treatments were placed in a block adjacent to the outcrossers to better reproduce likely distributions in natural populations and provide a more conservative test of pollinator-mediated competition than the intermixed arrays used in 1995.

Nine replicates of each treatment were placed at the Rock and Shoals outcrop as in the previous season. The sepals of approximately 45 newly opened flowers on each outcrosser plant were marked over a three-week period during the peak of the flowering season. Two weeks after the last marking date, the experimental plants were moved to the greenhouse. We counted all marked capsules and collected and counted mature seeds from up to 12 undehiscent capsules on each plant (mean $n = 10.7 \pm 1.5$ SD).

Fruit set (fruit per flower) was calculated for each outcrosser plant, and seed set (seeds per fruit) was counted for each sampled fruit. The fruit set ratios were arcsine-square-root transformed and analyzed with a mixed-model ANOVA with treatment as a fixed factor and replicate array nested within treatment. The seed set mean and coefficient of variation for each outcrosser plant was calculated and analyzed with a similar model with the addition of plant as a random variable nested within array. The coefficients of variation (CV) were arcsine-square-root transformed for analysis. Within each analysis, comparisons of treatments were made with linear contrasts of the main effect least squares means. All analyses were performed using the standard least squares ANOVA platform in JMP 3.0.2 (SAS Institute 1994).

RESULTS

Hand-Pollinations

Seed Set.—*Arenaria glabra* pollen was able to compete for, and in some cases fertilize, outcrosser *A. uniflora* ovules in greenhouse crosses. However, seed set after heterospecific pollen transfer was very low relative to conspecific self and outcross hand-pollinations (2.5 vs. 23.8 and 26.1, respectively; Fig. 3a). Flowers that received mixed heterospecific/conspecific pollen loads had intermediate levels of seed set (16.3 seeds/fruit), indicating that *A. glabra* pollen interfered with fertilization by *A. uniflora* pollen. The differences in seed set between each of these treatments (heterospecific, mixed, and conspecific) were highly significant ($P < 0.0001$). Autonomous seed set was also very low (11% of hand-selfed) and was not significantly different from seed set after *A. glabra* pollination. Seed set after self hand-pollination was lower than after outcrossing ($P = 0.045$), but the comparison does not meet significance criteria when corrected for multiple tests (sequential Bonferroni; rice 1989). Overall, pollen source had a highly significant effect on seed production ($P < 0.0001$), as did maternal plant identity ($P < 0.01$).

Fruits from heterospecific pollinations contained many flat

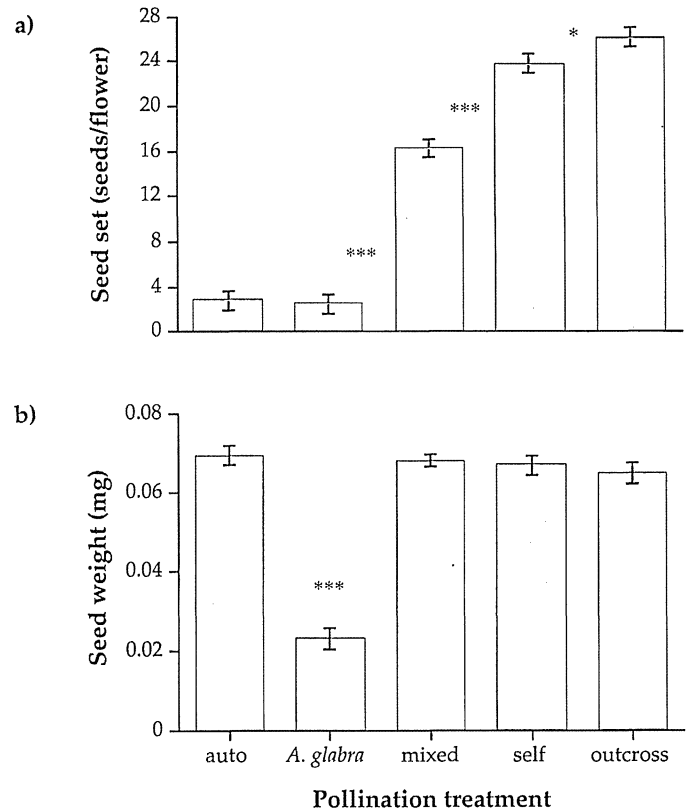


FIG. 3. Seed set and seed weight following autonomous self-pollination (auto) and heterospecific (*Arenaria glabra*), heterospecific + conspecific (mixed), and conspecific (self and outcross) hand-pollinations of outcrosser *A. uniflora* maternal plants ($n = 36$). (A) Seed set means are the treatment least squares means (LSMs) from ANOVA with maternal identity as a random block effect. (B) Seed weight means are treatment LSMs from ANCOVA with maternal identity as a random block effect and seed set as a covariate. Bars extend ± 1 SE. Asterisks indicate the significance of linear contrasts of adjacent LSMs (***) $P < 0.0001$; * $P < 0.05$).

or shriveled seedlike objects of various sizes as well as ones inflated enough to be counted as seeds. Such objects were not as prevalent in the few-seeded autogamous fruits, suggesting that they were aborted embryos, rather than unfertilized ovules.

Seed Weight.—Seeds formed after pollination with *A. glabra* were only one-third as heavy as the seeds from the other pollination treatments (Fig. 3b), which produced a highly significant effect of pollen source in the ANCOVA ($P < 0.0001$). These other treatments were not significantly different from one another when seed set was included as a covariate in the model. However, there was a significant negative correlation between seed set and seed weight ($P < 0.005$), presumably due to resource or packaging trade-offs within fruits. Maternal identity was a significant factor in determining seed weight beyond its effects on seed set ($P < 0.0001$).

Germination.—A small number of seeds from the heterospecific pollination treatment did germinate (17 of 426), but the seedlings did not appear intermediate in morphology. We used cellulose acetate electrophoresis to assess the paternity of these putative “hybrid” seedlings genotyped the seedlings

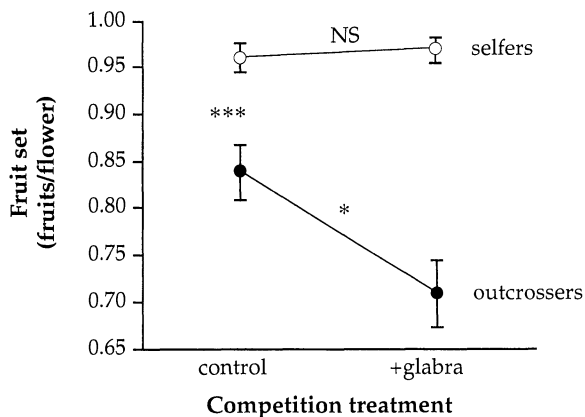


FIG. 4. Fruit set of selfer and outcrosser *Arenaria uniflora* in the 1995 competition experiment. Means are back-transformed population treatment interaction least squares means from ANOVA (Table 1). Bars extend ± 1 SE. Asterisks indicate the significance of tests from ANOVA and a posteriori linear contrasts (***) $P < 0.001$; * $P < 0.05$).

at a diagnostic allozyme locus (alcohol dehydrogenase, *Adh*). The Pendergrass *A. uniflora* plants used in this experiment were fixed for a single *Adh* allele, whereas *A. glabra* expresses several other alleles (L. Fishman unpubl. data). Hybrids should be heterozygous. Contrary to expectation, all putative hybrid seedlings were homozygous for the *A. uniflora* allele and presumably resulted from contamination of the pollination forceps or inadequate emasculation. Germinating seeds were removed from the seed production dataset. Because these seedlings represent only 4% of the putative hybrid seeds, and the germination rate of conspecific treatment seeds was greater than 0.80, we estimate that over 95% of seeds from the heterospecific treatment were true hybrids (and inviable). Thus, the effects of this contamination on the interpretation of the hand-pollination data should be negligible and bias the hybrid seed weights only slightly upward.

Germination of seeds from other treatments was high (mean = 0.84 ± 0.16 SD) and unaffected by treatment. The continuum of aborted seeds observed in fruits from heterospecific pollinations suggests that most barriers to hybridization between *A. uniflora* and *A. glabra* occur after fertilization. Those products of fertilization counted and weighed as hybrid seeds may simply have been later aborts or may have included some fully developed inviable seeds. Because seeds were too small to weigh individually with the balances available, these possibilities cannot be distinguished.

Field Competition Experiment, 1995

Outcrosser *A. uniflora* plants are more vulnerable than selfers to pollinator-mediated competition (Fig. 4). Experimental proximity to *A. glabra* reduced outcrosser fruit set by 15% relative to controls (0.69 vs. 0.81, $P < 0.05$), whereas selfer fruit set was uniformly high in the two treatments (0.93 vs. 0.94, $P > 0.5$). This difference in the response to competition with *A. glabra* resulted in a marginally significant population \times treatment interaction in the ANOVA ($P < 0.054$; Table 1).

Selfers had significantly higher fruit set than outcrossers

TABLE 1. Nested mixed-model ANOVA on individual fruit set in the 1995 field competition experiment. Population (selfer vs. outcrosser) and treatment (+glabra vs. control) are fixed effects and array(treatment) is a random block effect nested within treatment. Fruit set ratios were arcsine-square-root transformed prior to analysis to normalize the residuals.

| Source | df | MS | F | P |
|--------------------------------------|-----|---------|---------|--------|
| Population | 1 | 6.65904 | 59.3479 | 0.0001 |
| Treatment | 1 | 0.31147 | 1.8545 | 0.2112 |
| Array(treatment) | 8 | 0.16151 | 1.4888 | 0.2933 |
| Population \times treatment | 1 | 0.58117 | 5.1796 | 0.0535 |
| Population \times array(treatment) | 8 | 0.10848 | 3.0083 | 0.0030 |
| Error | 264 | 0.03606 | | |

overall ($P < 0.0001$; Table 1) and were unaffected by environmental variation among replicate arrays. There was no overall effect of array on fruit set, but a significant array \times population interaction ($P < 0.005$) reflects consistent differences among arrays in outcrosser, but not in selfer, fruit production. Some of this variation in fruit set is probably related to abiotic conditions affecting overall plant performance, because there were also highly significant differences between arrays in outcrosser flower production, a measure of plant size (linear contrasts, $P < 0.001$). However, there was no consistent relationship between the flower production and fruit set of outcrossers across arrays. Uncontrolled variation in the pollinator environment due to background *A. uniflora* density, the relative size of *A. uniflora* and *A. glabra* floral displays within arrays, or the vagaries of pollinator behavior may also have contributed to the array effects on fruit set in the outcrossers.

Field Competition Experiment, 1996

Although competition for pollinator service should primarily result in unvisited flowers and reduced fruit set, heterospecific pollen transfer should also reduce seed set and increase variability in seed set in plants receiving mixed pollen loads. The 1996 competition experiment was designed to measure the effect of proximity to *A. glabra* on outcrosser fruit set and seed set and to enable detection of both modes of pollinator-mediated competition. As in the previous season, array was a highly significant factor in all of the analyses of outcrosser reproductive success ($P < 0.0001$). Array effects are included in the analysis of the treatment effects, the calculation of least squares means, and the construction of contrasts, but are not presented individually below.

Fruit Set.—As in 1995, the presence of *A. glabra* dramatically reduced outcrosser *A. uniflora* fruit set relative to the high-frequency controls (0.72 vs. 0.84; Fig. 5a). This 14% decrease was similar in magnitude to the previous season, as were overall levels of fruit set. The low-frequency treatment had intermediate fruit set (0.78), suggesting that the reduced display size of these arrays may have led to lower levels of pollinator visitation. The differences among treatments are all highly significant (linear contrasts, $P < 0.0001$), leading to a highly significant treatment effect in the ANOVA.

Mean Seed Set.—Competition treatment also strongly affected the seed set of outcrosser plants ($P < 0.0001$; Fig.

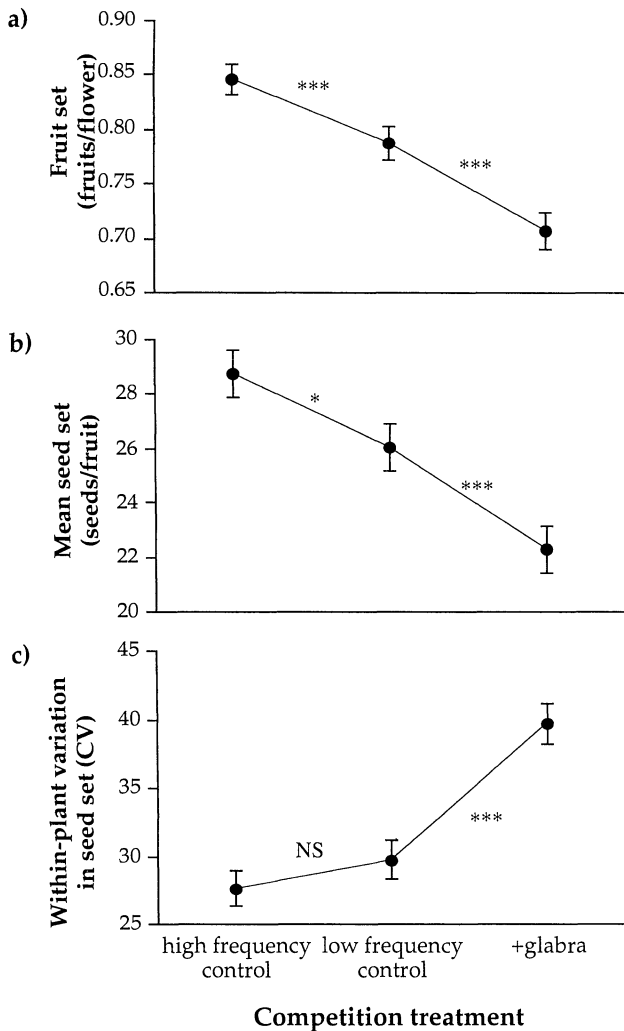


FIG. 5. Female fitness components of outcrosser *Arenaria uniflora* plants in the 1996 competition experiment. Bars extend ± 1 SE. Asterisks indicate the significance of linear contrasts of adjacent means (***) $P < 0.001$; * $P < 0.05$). (A) Fruit set values are back-transformed least squares means (LSMs) from ANOVA on arcsine-square root transformed data. (B) Mean seed set values are treatment effect LSMs from ANOVA. (C) Seed set coefficient of variation (CV) values are back-transformed LSMs from ANOVA on arcsine-square-root transformed data.

5b). Plants in the +glabra treatment had significantly reduced seed set relative to both the low-frequency and high-frequency controls (22.4 vs. 26.1 and 28.2, respectively; $P < 0.001$), but the two controls were only different at the $P < 0.05$ level. The similarity of seed set in the control treatments (relative to the fruit set difference) suggests that interactions with *A. glabra* have a negative effect above and beyond changes in pollinator visitation also experienced by the low-frequency arrays.

Within-Plant Variation in Seed Set.—The coefficient of variation of seed set, which reflects variability in the number of seeds per fruit within individual plants, was also strongly affected by competition treatment ($P < 0.0001$; Fig. 5c). Plants in the +glabra treatment were significantly more variable than those in either control ($P < 0.001$), and the controls

were not different from one another ($P > 0.4$). Again, this suggests that pollinator-mediated interactions affecting seed set were qualitatively different than those reducing fruit set. Variability in seed set within plants in +glabra arrays could result from increased variation in the pollen load composition of insects visiting both species.

DISCUSSION

Costs of Pollinator-Mediated Competition

Autogamous selfing assures reproduction whenever the pollination environment is limiting (Darwin 1876). However, it is less clear whether pollinator-mediated selection has been a major factor in the repeated evolution of routine self-pollination from outcrossing within plant lineages (Barrett et al. 1996). Theoretical approaches that incorporate constant genetic selection for selfing generally assume no external selection and vary only the costs of inbreeding (e.g., Lande and Schemske 1985). This removes the mating system from the context of the flower and the individual from the context of the environment. Empirical measures of the costs of outcrossing are a necessary complement to estimates of the costs of inbreeding and allow the application of recent models of mating system evolution that include ecological selection (e.g., Lloyd 1988, 1992).

Our experimental results are consistent with the hypothesis that pollinator-mediated interactions between *A. uniflora* and *A. glabra* in areas of sympatry selected for selfing in ancestral outcrosser populations. Outcrosser *A. uniflora* plants suffered substantial female fitness losses in the presence of *A. glabra*. The fitness costs were consistent across years and included reductions in both fruit set (fruits per flower) and seed set (seeds per fruit) relative to controls. Selfers were completely unaffected by the competition treatment and had higher fruit set overall. Autonomous selfing, which decouples reproduction from the pollination environment, appears to shelter selfers from negative interactions with *A. glabra*.

All else being equal, autogamous variants should be favored in a hypothetical ancestral *A. uniflora* population in competition with *A. glabra*. Of course, all else is not equal. The products of self-fertilization frequently suffer inbreeding depression—reductions in fitness relative to outcrossed progeny. In plants, inbreeding depression (d) primarily results from the exposure of deleterious partially recessive alleles in inbred individuals (Charlesworth et al. 1990; Husband and Schemske 1996). Inbreeding depression can coevolve with selfing rate as routine inbreeding exposes and purges such alleles (Lande and Schemske 1985; Charlesworth et al. 1990) and varies with mutation rate, population history, and the test environment (for review, see Husband and Schemske 1996). Reduced quality of selfed progeny due to inbreeding depression can offset the relative increase in seed production that autonomous selfing provides.

Lloyd's (1988, 1992) models of the evolution of different modes of selfing provide a quantitative framework for assessing the evolutionary fate of autogamous variants in an outcrosser population. When pollen discounting is complete (i.e., when there is an absolute trade-off between self-fertilization and outcross male success), these models collapse to a direct comparison of the ecological costs of outcrossing and the

genetic cost of selfing. Cleistogamous selfing is favored whenever $q > e$, where q is the relative fitness of selfed progeny (or $1 - \delta$) and e is the effectiveness of outcross pollination (Lloyd 1988, p. 244). In this study, we can estimate e under conditions of pollinator-mediated competition by dividing outcrosser fecundity in the presence of *A. glabra* by the control fecundity ($e = 0.68$ or 0.79 depending on which control treatment is used as a standard). This is a conservative estimate of the effectiveness of outcross reproduction in the presence of *A. glabra*, because it assumes that competition does not alter the selfing rate of the mixed-mating Pendergrass plants. Inbreeding depression (δ) has also been measured in these two study populations (Fishman 1998). Lifetime inbreeding depression in the Pendergrass outcrosser population varied widely among families, with a population mean of less than 20% ($\delta = 0.19 \pm 0.02$ SE). In an ancestral population of *A. uniflora* resembling the Pendergrass outcrossers ($q = 0.81$), competitive interactions as strong as those observed in this study ($e > 0.68$) would favor the spread of even completely cleistogamous selfers. Although these experimental measures of relative fitness cannot duplicate conditions in ancestral populations, net selection for autogamy in *A. uniflora* in areas of sympatry with *A. glabra* is a strong possibility.

Mechanisms of Pollinator-Mediated Competition

Pollinator-mediated interactions with *A. glabra* substantially reduced outcrosser *A. uniflora* fitness, which is consistent with Wyatt's (1984, 1986) suggestion that competition for pollinator service between the two species precipitated the evolution of autogamy in some populations of *A. uniflora*. However, our greenhouse and field results suggest that heterospecific pollen transfer may be the primary mechanism of pollinator-mediated competition in *Arenaria*. The controlled hand-pollinations demonstrate the potentially high cost of hybridization; receipt of pure *A. glabra* pollen loads resulted in inviable hybrid seeds and complete reproductive failure. In addition, intermediate seed set following mixed hand-pollinations indicate direct competition between conspecific and heterospecific pollen for outcrosser *A. uniflora* ovules. Patterns of reproduction in the field experiments were also consistent with receipt of heterospecific pollen, rather than simple loss of pollinator service.

For heterospecific pollen transfer to generate selection for preemptive self-fertilization, interspecific pollen-ovule interactions must be both costly and common. Heterospecific pollen is often at a competitive disadvantage, either in pollen tube growth rate (Carney et al. 1996) or fertilization ability (Williams and Rouse 1988; Rieseberg et al. 1995; Emms et al. 1996). By assessing paternity after handicapped pollen tube races, several studies have shown that disproportionately few hybrid seeds result from mixed pollinations (Rieseberg et al. 1995; Emms et al. 1996). In such systems, the transfer of heterospecific pollen in natural mixed loads would have little effect on recipient fitness. Hybrid inviability precludes paternity analysis of the relative competitive ability of conspecific and heterospecific pollen in *Arenaria*, but it is clear that *A. glabra* pollen has profound effects on *A. uniflora* reproduction even when delivered in mixtures. Pollination with

mixed heterospecific and conspecific loads decreased seed set by 38% relative to outcross pollination. Because the pure heterospecific crosses (and observations of pollen tube growth) indicate that *A. glabra* can reach and fertilize *A. uniflora* ovules, direct usurpation of ovules is the most likely source of the seed set reduction in the field. Clogging of the stigmatic surface or style and/or other prefertilization interactions can also contribute to lower seed set following mixed pollinations (Stucky 1985; Randall and Hilu 1990). In *Arenaria*, conspecific pollen may have a slight competitive advantage because fewer than 50% of ovules were lost on average after mixed pollination. However, titration of pollen mixtures would be necessary to determine this precisely.

The competitive effects observed in the greenhouse crosses translate into substantial losses in female fitness during natural pollination. In the field, any insect visitor carrying *A. glabra* pollen is a potential threat to outcrosser seed production. Because pollinators were observed to move freely between outcrosser *A. uniflora* and *A. glabra* in the field experiments, heterospecific pollen transfer certainly contributes to the lower female fitness in the +*glabra* competition treatments. Competition for pollinator service may also account for some of the decrease in reproductive success, particularly the fruit set reductions. However, no significant pollinator preferences were detected in 30 person-hours of observation, suggesting that insects do not visit *A. glabra* to the exclusion of *A. uniflora* (L. Fishman and R. Wyatt, unpubl. data). Indeed, *A. glabra* plants may actually facilitate visitation to outcrossers in the competition arrays, because fruit set and seed set increased with total display size within the two controls in the 1996 experiment. Coflowering may result in facilitation almost as often as it generates competition (Poole and Rathcke 1979; Schemske 1981; Thomson 1982).

Further costs of the interaction cannot be ruled out without explicit measures of the male fitness consequences of heterospecific pollen transfer. Campbell (1985b) found that *Stellaria pubera* plants in mixed arrays with *Claytonia virginica* exported pollen-mimicking dye to conspecifics at lower rates and over shorter distances than plants grown in single-species arrays. This suggests that pollinator-mediated competition could have large effects on pollen success as well as female fitness. However, movement of pollen between incompletely compatible congeners should affect male fitness no more than movement between unrelated species.

It is difficult to untangle the effects of competition for pollinator service and competition through pollen transfer in this system because autonomous self-pollination in outcrossers can produce patterns of seed set similar to heterospecific pollination (Fig. 3). However, in the 1996 competition experiment, the processes affecting female fitness in the +*glabra* treatment appear to be fundamentally different from those that lowered fruit set in the low-frequency control (Fig. 5). The low-frequency control showed little reduction in seed set despite a large reduction in fruit set, whereas the competition treatment experienced substantial decreases in both components of female fitness relative to the high-frequency control. Plants in the competition treatment also had more variable seed set relative to both controls, thus further suggesting that pollen load composition (rather than autonomous selfing) mediates the effects of competition on seed set. Het-

erospecific pollen transfer may also contribute to the fruit set reductions, because the empty or few-seeded hybrid fruits that formed in the greenhouse crosses may abort more often in the field.

The Evolution of Autogamy as Reproductive Character Displacement

The evolutionary interaction of pre- and postzygotic isolating mechanisms is a fundamental issue in the study of speciation. Dobzhansky (1937) first articulated the idea that premating barriers evolve to reinforce incomplete postzygotic barriers when incipient species meet in secondary sympatry and produce less fit hybrids. Whether such reinforcement can occur in the face of ongoing gene flow and whether the conditions for reinforcement do occur regularly remains controversial (Butlin 1987; Howard 1993; Liou and Price 1994). Evidence for the action of reinforcement has primarily come from increased mate discrimination and reproductive character displacement in animal populations in sympatry with congeners (Coyne and Orr 1989; Noor 1995; Rundle and Schluter 1998). The evolution of mating avoidance through autonomous self-pollination in marginal populations of *A. uniflora* appears to be a similar case of reproductive character displacement. The evolution of such an isolating mechanism in the face of inbreeding depression suggests that pollinator-mediated interactions between coflowering plants can be an important factor in both mating system evolution and the development of reproductive isolation.

Floral divergence frequently precedes the development of recognition systems and/or hybrid inviability (Bradshaw et al. 1995; Hodges and Arnold 1995). In taxa with more generalized floral morphologies, however, floral differentiation may not develop even in widely allopatric populations. If the accumulation of genetic incompatibility outpaces floral divergence, the conditions for reinforcement in secondary sympatry are met. If postzygotic reproductive isolation is absolute prior to secondary contact, the speciation process is complete and reinforcement, in a strict sense, cannot be said to operate (Butlin 1987). However, this semantic distinction should not obscure the potential for profound effects on the ecology and evolution of the intermating species.

In a review of the evidence for pollinator-mediated competition, Waser (1983) cited numerous examples of floral divergence in sympatric populations that are consistent with reproductive character displacement, but that lack experimental evidence of selection for premating isolation. Since then, only a few studies (e.g., Levin 1985) have experimentally examined such patterns. Our confirmation of predictions based on the geographical distribution of floral variation in *Arenaria* (Wyatt 1984, 1986) suggests that pollinator-mediated processes may have sparked divergence within these other taxa.

This study focused on the negative impact of interactions with *A. glabra* on outcrosser *A. uniflora* female fitness. It did not address the fitness consequences of pollen transfer from *A. uniflora* to *A. glabra* or the potential male fitness costs to *A. glabra* of wasting pollen on *A. uniflora* ovules. The observed pattern of reproductive character displacement is unilateral, which suggests that *A. uniflora* was either more af-

ected by ancestral interactions or quicker to respond to selection against intermating. Asymmetry of both effect and response have been documented in other studies of competition through interspecific pollen transfer. In *Impatiens*, Randall and Hilu (1990) found that *I. pallida* pollen tubes grow through *I. capensis* styles and reduce seed set in mixed pollinations, but that *I. pallida* was unaffected by pollen transfer from *I. capensis*. Pollinator-mediated competition between *A. uniflora* and *A. glabra* may be similarly asymmetric, particularly because *A. glabra* has longer styles. With the exception of the Louisiana iris hybrid complex (Emms et al. 1996), the taxon with the longer styles is generally the more successful pollen parent in hybrid fertilizations (Kiang and Hamrick 1978; Levin 1978; Gore et al. 1990). Alternatively, the asymmetry of reproductive character displacement in *Arenaria* may simply depend on which species responded to selection first.

Given selection for the avoidance of heterospecific mating, why evolve autonomous self-pollination? A number of non-exclusive explanations present themselves. Genetic or developmental constraints may limit floral variation along certain axes. Most *Arenaria* species have pale, bowl-shaped, radially symmetric flowers, and variation in flower color or shape may be less available than variation in flower size or protandry. Ecological conditions also constrain certain evolutionary trajectories. The granite outcrops on which *A. uniflora* and *A. glabra* occur have extremely short spring growing seasons, limiting opportunities for flowering time displacement. The pollinator community may be limiting as well, because the small bees and flies that visit *Arenaria* are generalists and may not discriminate between color variants (Campbell 1985a). Furthermore, autonomous selfing provides the additional ecological benefits of complete reproductive assurance, rather than simply decreasing the probability of heterospecific pollen transfer.

Although pollinator-mediated competition alone appears to generate a selfer advantage sufficient to overcome the costs of inbreeding in *A. uniflora*, other selective processes may have also favored the evolution and maintenance of selfing. Selfers with extremely reduced floral morphology may be able to allocate limited resources to other functions, thus increasing fitness relative to outcrossers (Lloyd 1988). The cleistogamy of current *A. uniflora* selfers also produces a complete trade-off between self and outcross pollen donation (Fishman 1998), eliminating the 50% male transmission advantage accorded selfing genotypes in many models of mating system evolution (Fisher 1941; Lande and Schemske 1985; Charlesworth et al. 1990). If ancestral variants selfed prior to anthesis, but had showy long-lived flowers, some male transmission advantage may have contributed to the initial spread of selfers. Because the floral morphology of ancestral selfer variants is unknown, it is difficult to assess the timing and contribution of each mode of selection to the establishment of nearly cleistogamous selfers. However, quantitative genetic analyses of floral morphology and mating behavior in these populations suggest that trade-offs between self-pollination and pollinator attraction are an integral part of the genetic architecture of *A. uniflora* (Fishman 1998). This supports the argument that localized selection for preemptive self-pollination, rather than an constant transmission advan-

tage, favored the evolution of selfing in *A. uniflora* populations sympatric with *A. glabra*. The joint evolution of outcrossing rate and inbreeding depression below a threshold set by pollinator-mediated selection for self-fertilization (Lande and Schemske 1985) could have further accelerated the establishment of completely selfing populations.

One important outcome of the evolution of autogamy is the development of reproductive isolation between selfer and outcrosser *A. uniflora* populations. Although *A. glabra* and *A. uniflora* are distinct species themselves, reproductive character displacement in areas of sympatry has led to morphological and genetic divergence within *A. uniflora* (Fishman 1998). Such localized ecological selection for selfing may play a major role in the frequent generation of selfing species and subspecies within outcrossing lineages (Barrett et al. 1996; Schoen et al. 1997). This suggests that models that incorporate ecological factors (Lloyd 1988, 1992; Holsinger 1992; Schoen et al. 1996) may be the most appropriate framework for the study of mating system evolution.

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LITERATURE CITED

- Aide, T. M. 1986. The influence of wind and animal pollination on variation in outcrossing rates. *Evolution* 40:434–435.
- Antonovics, J. 1967. Evolution in closely adjacent plant populations. V. Evolution of self-fertility. *Heredity* 23:219–238.
- Armbruster, W. S., M. E. Edwards, and E. M. Debevec. 1994. Floral character displacement generates assemblage structure of Western Australian triggerplants (*Stylidium*). *Ecology* 75:315–329.
- Arnold, M. L. 1997. Natural hybridization and evolution. Oxford Univ. Press, Oxford.
- Baker, H. G. 1955. Self-compatibility and establishment after "long-distance" dispersal. *Evolution* 9:347–348.
- Barrett, S. C. H., and C. G. Eckert. 1990. Variation and evolution of mating systems in seed plants. Pp. 229–254 in S. Kawano, ed. *Biological approaches and evolutionary trends in plants*. Academic Press, London.
- Barrett, S. C. H., M. T. Morgan, and B. C. Husband. 1989. The dissolution of complex genetic polymorphism: the evolution of self-fertilization in tristylous *Eichhornia paniculata* (Pontederiaceae). *Evolution* 43:1398–1416.
- Barrett, S. C. H., L. D. Harder, and A. C. Worley. 1996. The comparative biology of pollination and mating in flowering plants. *Phil. Trans. R. Soc. Lond. B Biol. Sci.* 351:1271–1280.
- Baskin, J. M., and C. C. Baskin. 1987. Seed germination and flowering requirements of the rare plant *Arenaria fontinalis* (Caryophyllaceae). *Castanea* 52:291–299.
- Bradshaw, H. D., Jr., S. M. Wilbert, K. G. Otto, and D. W. Schemske. 1995. Genetic mapping of floral traits associated with reproductive isolation in monkeyflowers (*Mimulus*). *Nature* 376:762–765.
- Butlin, R. K. 1987. Speciation by reinforcement. *Trends Ecol. Evol.* 2:8–13.
- Campbell, D. R. 1985a. Pollen and gene dispersal: the influences of competition for pollination. *Evolution* 39:418–431.
- . 1985b. Pollinator sharing and seed set of *Stellaria pubera*: competition for pollination. *Ecology* 66:544–553.
- Carney, S. E., S. A. Hodges, and M. L. Arnold. 1996. Effects of differential pollen tube growth on hybridization in the Louisiana irises. *Evolution* 50:1871–1878.
- Charlesworth, D. 1985. Distribution of dioecy and self-incompatibility in angiosperms. Pp. 237–268 in J. J. Greenwood, P. Harvey, and M. Slatkin, eds. *Evolution—essays in honor of John Maynard Smith*. Cambridge Univ. Press, Cambridge, U.K.
- Charlesworth, D., and B. Charlesworth. 1987. Inbreeding depression and its evolutionary consequences. *Annu. Rev. Ecol. Syst.* 18:237–268.
- Charlesworth, D., M. T. Morgan, and B. Charlesworth. 1990. Inbreeding depression, genetic load, and the evolution of outcrossing rates in a multilocus system with no linkage. *Evolution* 44:1469–1489.
- Coyne, J. A., and H. A. Orr. 1989. Patterns of speciation in *Drosophila*. *Evolution* 43:362–381.
- Darwin, C. R. 1876. The effects of cross and self-fertilization in the vegetable kingdom. Murray, London.
- Dobzhansky, T. 1937. *Genetics and the origin of species*. Columbia Univ. Press, New York.
- Dole, J. A. 1992. Reproductive assurance in the three taxa of the *Mimulus guttatus* complex (Scrophulariaceae). *Am. J. Bot.* 79:650–659.
- Emms, S. K., S. A. Hodges, and M. L. Arnold. 1996. Pollen-tube competition, siring success, and consistent asymmetric hybridization in Louisiana irises. *Evolution* 50:2201–2206.
- Feinsinger, P., and W. H. Busby. 1987. Pollen carryover: experimental comparisons between morphs of *Palicourea lasiorrachis* (Rubiaceae), a distylous, bird-pollinated treelet. *Oecologia* 73:231–235.
- Feinsinger, P., and H. M. Tiebout III. 1991. Competition among plants sharing hummingbird pollinators: laboratory experiments on a mechanism. *Ecology* 72:1946–1952.
- Fisher, R. A. 1941. Average excess and average effect of a gene substitution. *Ann. Eugen.* 11:53–63.
- Fishman, L. 1998. Ecological and genetic factors in the evolution of autogamy in *Arenaria uniflora* (Caryophyllaceae). Ph.D. diss., Princeton Univ., Princeton, NJ.
- Galen, C., and T. Gregory. 1989. Interspecific pollen transfer as a mechanism of competition: consequences of foreign pollen contamination for seed set in the alpine wildflower, *Polemonium viscosum*. *Oecologia* 81:120–123.
- Gore, P. L., B. M. Potts, P. W. Volker, and J. Megalos. 1990. Unilateral cross-compatibility in *Eucalyptus*: the case of hybridization between *E. globulus* and *E. nitens*. *Aust. J. Bot.* 38:383–394.
- Hodges, S. A., and M. L. Arnold. 1995. Spurring plant diversification: are floral nectar spurs a key innovation? *Proc. R. Soc. Lond. B Biol. Sci.* 262:343–348.
- Holsinger, K. E. 1992. Ecological models of plant mating systems: the evolutionary stability of mixed mating systems. Pp. 169–191 in R. Wyatt, ed. *Ecology and evolution of plant reproduction: new approaches*. Chapman and Hall, New York.
- Holsinger, K. E., and J. D. Thomson. 1995. Pollen discounting in *Erythronium grandiflorum*: mass-action estimates from pollen transfer dynamics. *Am. Nat.* 144:799–812.
- Howard, D. J. 1993. Reinforcement: the origin, dynamics and fate of an evolutionary hypothesis. Pp. 46–69 in R. G. Harrison, ed. *Hybrid zones and the evolutionary process*. Oxford Univ. Press, Oxford.
- Husband, B. C., and D. W. Schemske. 1996. Evolution of the magnitude and timing of inbreeding depression in plants. *Evolution* 50:54–70.
- Jain, S. K. 1976. The evolution of inbreeding in plants. *Ann. Rev. Ecol. Syst.* 7:469–95.
- Kiang, Y. T., and J. L. Hamrick. 1978. Reproductive isolation in the *Mimulus guttatus*-*M. nasutus* complex. *Am. Midl. Nat.* 100:269–276.
- Kohn, J. R., and S. C. H. Barrett. 1994. Pollen discounting and

- the spread of a selfing variant in tristylous *Eichhornia paniculata*: evidence from experimental populations. *Evolution* 48:1576–1594.
- Lande, R., and D. W. Schemske. 1985. The evolution of self-fertilization and inbreeding depression in plants. I. Genetic models. *Evolution* 39:24–40.
- Lefebvre, C. 1970. Self-fertility in marine and zinc mine populations of *Armeria maritima* (Mill.) Willd. *Evolution* 24:571–577.
- Levin, D. A. 1978. The origins of isolating mechanisms in flowering plants. *Evol. Biol.* 11:185–317.
- . 1985. Reproductive character displacement in *Phlox*. *Evolution* 39:1275–1281.
- Levin, D. A., and W. W. Anderson. 1970. Competition for pollinators between simultaneously flowering species. *Am. Nat.* 104:455–467.
- Levin, D. A., and B. A. Schaal. 1970. Corolla color as an inhibitor of interspecific hybridization in *Phlox*. *Am. Nat.* 104:273–283.
- Liou, L. W., and T. D. Price. 1994. Speciation by reinforcement of premating isolation. *Evolution* 48:1451–1459.
- Lloyd, D. G. 1979. Some reproductive factors affecting the selection of self-fertilization in plants. *Am. Nat.* 113:67–79.
- . 1980. Demographic factors and mating patterns in angiosperms. Pp. 67–88 in O. Solbrig, ed. *Demography and evolution of plant populations*. Univ. California Press, Berkeley.
- . 1988. Benefits and costs of biparental and uniparental reproduction in plants. Pp. 233–252 in R. E. Michod and B. R. Levin, ed. *The evolution of sex*. Sinauer, Sunderland, MA.
- . 1992. Self- versus cross-fertilization in plants. II. The selection for self-fertilization. *Int. J. Plant Sci.* 153:370–380.
- McCormick, J. F., J. R. Bozeman, and S. Spongberg. 1971. A taxonomic revision of the granite outcrop species of *Minuartia* (*Arenaria*). *Brittonia* 23:149–160.
- McNeill, J. 1962. Taxonomic studies in the Alsinoideae. I. Generic and infrageneric groups. *Notes R. Bot. Gard. Edinburgh* 24:79–155.
- Noor, M. A. 1995. Speciation driven by natural selection in *Drosophila*. *Nature* 375:674–675.
- Ornduff, R. 1980. Heterostyly, population composition, and pollen flow in *Hedyotis caerulea*. *Am. J. Bot.* 67:95–103.
- Poole, R. W., and B. J. Rathcke. 1979. Regularity, randomness and aggregation in flowering phenologies. *Science* 203:470–471.
- Randall, J. L., and K. W. Hilu. 1990. Interference through improper pollen transfer in mixed stands of *Impatiens capensis* and *I. palida* (Balsaminaceae). *Am. J. Bot.* 77:939–944.
- Rathcke, B. 1983. Competition and facilitation among plants for pollination. Pp. 305–329 in C. E. Jones and R. J. Little, eds. *Handbook of experimental pollination biology*. Van Nostrand Reinhold, New York.
- . 1988. Interactions for pollinators among coflowering shrubs. *Ecology*. 69:446–457.
- Rathcke, B., and L. Real. 1993. Autogamy and inbreeding depression in mountain laurel, *Kalmia latifolia* (Ericaceae). *Am. J. Bot.* 80:143–146.
- Rice, W. R. 1989. Analyzing tables of statistical tests. *Evolution* 43:223–225.
- Rieseberg, L. H., A. M. Desrochers, and S. J. Youn. 1995. Interspecific pollen tube competition as a reproductive barrier between sympatric species of *Helianthus* (Asteraceae). *Am. J. Bot.* 82:515–519.
- Ritland, K. 1991. A genetic approach to measuring pollen discounting in natural plant populations. *Am. Nat.* 138:1049–1057.
- Rundle, H. D., and D. Schluter. 1998. Reinforcement of stickleback mate preferences: sympatry breeds contempt. *Evolution* 52:200–208.
- SAS Institute. 1994. JMP 3.0.2 user's guide. SAS Institute, Inc., Cary, NC.
- Schemske, D. W. 1981. Floral convergence and pollinator sharing in two bee-pollinated tropical herbs. *Ecology* 62:946–954.
- Schemske, D. W., and R. Lande. 1985. The evolution of self-fertilization and inbreeding depression in plants. II. Empirical observations. *Evolution* 39:41–52.
- Schoen, D. J., M. T. Morgan, and T. Bataillon. 1996. How does self-pollination evolve? Inferences from floral ecology and molecular genetic variation. *Phil. Trans. R. Soc. Lond. B Biol. Sci.* 351:1281–1290.
- Schoen, D. J., M. O. Johnston, A.-M. L'Heureux, and J. V. Marsolais. 1997. Evolutionary history of the mating system in *Amsinckia* (Boraginaceae). *Evolution* 51:1090–1099.
- Stebbins, G. L. 1957. Self-fertilization and population variability in higher plants. *Am. Nat.* 91:337–354.
- Stucky, J. M. 1985. Pollination systems of sympatric *Ipomoea hederacea* and *I. purpurea* and the significance of interspecific pollen flow. *Am. J. Bot.* 72:32–43.
- Thomson, J. D. 1982. Patterns of visitation by animal pollinators. *Oikos* 39:241–250.
- Thomson, J. D., and B. Thomson. 1989. Dispersal of *Erythronium grandiflorum* pollen by bumblebees: implications for gene flow and reproductive success. *Evolution* 43:657–661.
- Waser, N. M. 1983. Competition for pollination and floral character differences among sympatric plant species: a review of evidence. Pp. 277–293 in C. E. Jones and R. J. Little, eds. *Handbook of experimental pollination biology*. Van Nostrand Reinhold, New York.
- Waser, N. M., and M. L. Fugate. 1986. Pollen precedence and stigma closure: a mechanism of competition for pollination between *Delphinium nelsonii* and *Ipomopsis aggregata*. *Oecologia* 70:573–577.
- Weaver, R. E. 1970. The *Arenarias* of the southeastern granitic flatrocks. *Bull. Torrey Bot. Club* 97:40–52.
- Williams, E. G., and J. L. RoUse. 1988. Disparate style lengths contribute to isolation of species in *Rhododendron*. *Aust. J. Bot.* 36:183–191.
- Wyatt, R. 1977. *Arenaria alabamensis*: a new combination for a granite outcrop endemic from North Carolina and Alabama. *Bull. Torrey Bot. Club* 104:243–244.
- . 1984. The evolution of self-pollination in granite outcrop species of *Arenaria* (Caryophyllaceae). I. Morphological correlates. *Evolution* 38:804–816.
- . 1986. Ecology and evolution of self-pollination in *Arenaria uniflora* (Caryophyllaceae). *J. Ecol.* 74:403–418.
- . 1990. The evolution of self-pollination in granite outcrop species of *Arenaria* (Caryophyllaceae). V. Artificial crosses within and between populations. *Syst. Bot.* 15:363–369.
- Wyatt, R., E. A. Evans, and J. C. Sorenson. 1992. The evolution of self-pollination in granite outcrop species of *Arenaria* (Caryophyllaceae). VI. Electrophoretically detectable genetic variation. *Syst. Bot.* 17:201–209.