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ECOLOGICAL CONTEXT OF THE EVOLUTION OF SELF-POLLINATION IN *CLARKIA XANTIANA*: POPULATION SIZE, PLANT COMMUNITIES, AND REPRODUCTIVE ASSURANCE

DAVID A. MOELLER[1] AND MONICA A. GEBER
Department of Ecology and Evolutionary Biology, Cornell University, Ithaca, New York 14853

Abstract.—The repeated evolutionary transition from outcrossing to self-pollination in flowering plants has been suggested to occur because selfing provides reproductive assurance. Reports from biogeographical and ecological surveys indicate that selfing taxa are often associated with stressful and ephemeral environments, situations in which plant abundance is low (e.g., Baker's law) and with novel plant communities, however experimental tests of ecological hypotheses are few. In this study, we examined the ecological context of selection on mating system traits (herkogamy and protandry) in a California annual, *Clarkia xantiana*, where natural selfing populations differ from outcrossing populations in that they are often of small size or low density and occur mainly outside the range of pollinator-sharing congeners. We constructed artificial populations of plants with broad genetic variation in floral traits and manipulated two ecological factors, plant population size, and the presence versus absence of pollinator-sharing congeners, in the center of the geographic range of outcrossing populations. We found evidence for context-dependent selection on herkogamy and protandry via female fitness in which reduced traits, which promote autonomous selfing, were favored in small populations isolated from congeners whereas selection was comparatively weak in large populations or when congeners were present. In small, isolated populations, the fertility of plants with low herkogamy or protandry was elevated by 66% and 58%, respectively, compared to those with high herkogamy or protandry. The presence of pollinator-sharing congeners augmented bee visitation rates to *C. xantiana* flowers by 47% for all bees and by 93% for pollen specialists. By facilitating pollinator visitation, congeners mitigated selection on mating system traits in small populations, where outcross mating success is often low (the Allee effect). We also found support for the hypothesis that pollinator availability directly influenced variation in the strength of selection on herkogamy among populations. The striking parallels between our experimental results and patterns of variation in ecological factors across the geographic range of outcrossing and selfing populations suggest that reproductive assurance may play a central role in directing mating system evolution in *C. xantiana*.

Key words.—Allee effect, competition, density dependence, facilitation, mating system, natural selection, pollen limitation, self-fertilization.

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A dominant pattern in the diversification of angiosperms is the repeated transition from outcrossing to self-pollination (Stebbins 1974). From the earliest papers on plant breeding system evolution, biologists have recognized that the relative advantage of outcrossing versus selfing depends on genetic factors that affect the transmission of gametes across generations (Knight 1799; Darwin 1876), as well as ecological factors that influence the likelihood of mating (Darwin 1859; Henslow 1879; Müller 1883; Stebbins 1970).

The framework in which breeding system evolution is currently examined has been shaped by Fisher's (1941) suggestion that selfing genotypes have an inherent advantage over outcrossing genotypes because they can transmit alleles across generations in three ways, through ovules and pollen in selfed matings and pollen in outcrossed matings, whereas outcrossers can only transmit alleles in two ways (automatic selection hypothesis, reviewed in Jain 1976; Uyenoyama et al. 1993). To explain the apparent contradiction between the inherent advantage of selfers and the prevalence of mechanisms that promote outcrossing in angiosperms, an extensive body of research has developed on potential factors that oppose the 3:2 advantage of selfing variants. For example, inbreeding depression that reduces progeny survivorship and performance is widespread (Charlesworth and Charlesworth 1987; Byers and Waller 1999). In addition, selfing can sometimes preclude pollen dispersal for outcrossing (pollen discounting, Holsinger et al. 1984), and therefore offsets the transmission bias of selfing alleles (Holsinger and Thomson 1994; Kohn and Barrett 1994; Chang and Rausher 1998; Fishman 2000). Taken together, the genetic advantage of selfing may not always be a powerful force favoring the evolutionary transition to selfing because assumptions of Fisher's model are often violated in nature.

A second advantage of selfing arises when outcross success is limited due to consistently low or unpredictable pollinator or mate availability (reproductive assurance hypothesis). When opportunities for outcrossing decline, theoretical models predict that reproductive assurance can outweigh the disadvantage of inbreeding depression (Lloyd 1992; Schoen et al. 1996). Field studies have begun to reveal that selfing mechanisms commonly elevate seed production in natural populations (e.g., Herlihy and Eckert 2002; Elle and Carney 2003; Kalisz and Vogler 2003). However, we know much less about the ecological context in which plants suffer from pollen limitation and mating system traits are under strong selection, in part because putative selective agents are challenging to manipulate in experiments.

It has been suggested that selfing is advantageous in long-distance dispersal (Baker 1955), when plant populations fluctuate dramatically in size such as in marginal environments (Lewis 1953, 1962), or are sparsely distributed across a landscape (Pannell and Barrett 1998). Empirical support for this

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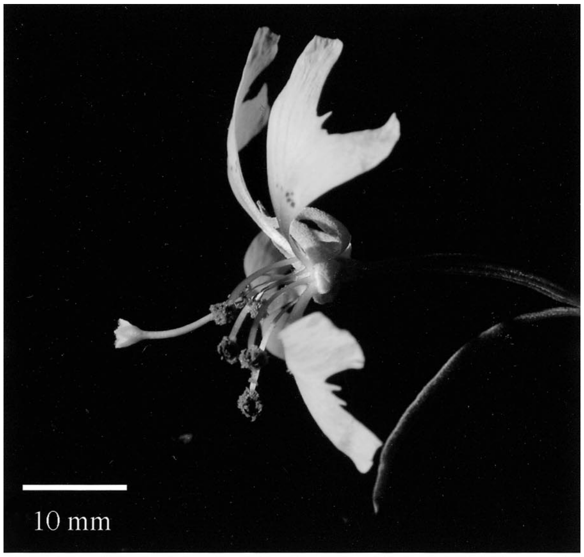
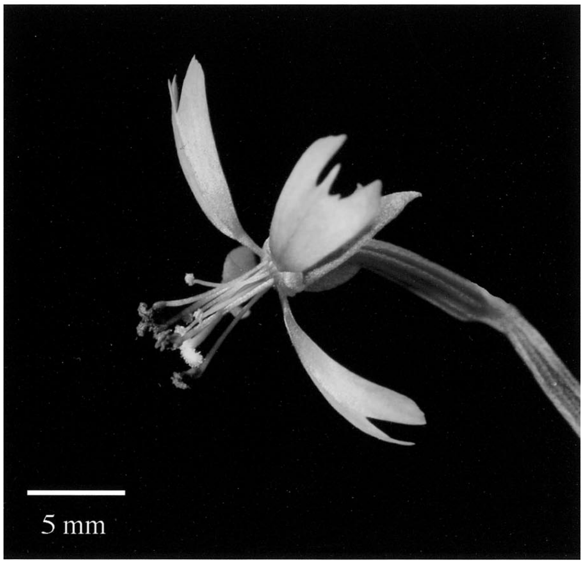
[1] Present address: Department of Plant Biology, University of Minnesota, 1445 Gortner Avenue, Saint Paul, Minnesota 55108; E-mail: moell021@umn.edu.



Clarkia xantiana ssp. *xantiana*



Clarkia xantiana ssp. *parviflora*

FIG. 1. Flowers of the two subspecies of *Clarkia xantiana*. The outcrossing subspecies, *xantiana* (top), has larger petals and pronounced herkogamy and protandry. The selfing subspecies, *parviflora* (bottom), has smaller petals and reduced herkogamy and protandry. Note that photographs appear at different magnifications.

set of ideas draws largely from biogeographic surveys showing that selfing taxa often occur at the periphery of the geographic ranges of sister outcrossing taxa where conditions for plant growth and pollination are often poor (Vasek 1964; Solbrig and Rollins 1977; Schoen 1982; Wyatt 1988; Barrett et al. 1989; Guerrant 1989) and from floristic studies documenting a high proportion of self-compatible taxa on islands (Rick 1966; Carlquist 1974; McMullen 1987; Webb and Kelly 1993).

One emerging theme is that attributes of plant populations, especially plant density and population size, may affect both pollinator attraction and the availability of compatible mates, and therefore the circumstances in which selfing is advantageous. There is growing evidence for Allee effects in plant populations where plant reproductive success increases with population size (Sih and Baltus 1987; Groom 1998; Hendrix and Kyhl 2000; Hackney and McGraw 2001; Moeller 2004) and the density of neighboring plants (e.g., Kunin 1993; Fausto et al. 2001; Knight 2003; Moeller 2004). In addition, outcrossing rates measured using molecular markers are sometimes correlated with population attributes (Farris and Mitton 1984; Motten and Antonovics 1992; Van Treuren et al. 1993; Karron et al. 1995; Routley et al. 1999) but the processes generating these patterns require further study.

Because plant populations are often sympatric with pollinator-sharing plant species, interspecific interactions may have important modifying influences on pollinator attraction, pollen movement between conspecifics, and therefore the likelihood of outcrossing. Indirect interactions via shared pollinators can be competitive when pollinator visitation to one species reduces pollinator availability to another or when pollinator movement between species results in interspecific pollen transfer (Campbell 1985; Caruso 2000). However, interactions can be facilitative when coexisting plant species jointly attract or maintain pollinator populations (Johnson et al. 2003; Moeller 2004). Competitive and facilitative influences on outcross success lead to contrasting predictions about the community context in which selfing is favored. When interactions are competitive, selfing may evolve in sympatry as a form of reproductive assurance (exploitation competition, Levin 1972) or hybridization avoidance (interference competition, Antonovics 1968; Fishman and Wyatt 1999). Alternatively, when pollinator-sharing enhances outcross success, selfing is more likely to be advantageous in isolated populations (allopatry) provided that pollinator availability is concomitantly low.

In this paper, we report on a field experiment that examined the effect of plant population size and the presence of pollinator-sharing congeners on the reproductive assurance value of selfing and patterns of selection on mating system traits in an annual plant, *Clarkia xantiana* (Onagraceae). In the genus *Clarkia*, selfing has evolved independently from outcrossing more than 10 times (Lewis and Lewis 1955; Sytsma et al. 1990; Gottlieb and Ford 1996). Selfing taxa are most commonly found at the geographic margins of the distribution of sister outcrossing taxa and in areas where, or at times when, few other *Clarkia* species flower (Lewis and Lewis 1955; Vasek 1964). In this study, we use *Clarkia xantiana* as an experimental system because its distribution and biology have been characterized in detail and its breeding system varies widely over a small geographic area (Moore and Lewis 1965; Eckhart and Geber 1999; Runions and Geber 2000). The species consists of an outcrossing subspecies, *C. xantiana* ssp. *xantiana*, and a selfing subspecies, *C. xantiana* ssp. *parviflora* (Fig. 1; hereafter ''outcrosser'' and ''selfer''). The subspecies have parapatric distributions that overlap in a narrow zone along their range margins (Fig. 2A). Populations range widely in size and community associations with pollinator-sharing congeners. Previous studies suggest that seed set in natural populations of the outcrosser is higher in large populations, because of increased mate availability, and in populations sympatric with congeners, because of enhanced pollinator availability (Moeller 2004).

We manipulated population size and plant community context (presence vs. absence of congeners) in experimental populations in the center of the outcrosser's geographic range (Fig. 2A). Experimental populations were composed of plants generated from crosses within and between five populations; the populations were chosen to span the species' natural range of floral trait variation. We focused our studies on two floral characters that are functionally important to self-pollination, herkogamy, and protandry and a secondary sexual character involved in pollinator attraction, petal size. Herkogamy is the spatial separation between dehiscing anthers and the receptive stigma, whereas protandry is the temporal precedence of pollen maturation over stigma maturation. Reduced herkogamy and protandry promote autonomous self-pollination. Our objectives were to examine the influence of population size and pollinator-sharing congeners on (1) visitation by specialist and generalist bee pollinators, (2) the reproductive assurance value of reduced herkogamy and protandry, and (3) selection on mating system and secondary sexual traits. To investigate the causal relationship between pollinator limitation and the pattern of selection via female fitness on herkogamy and protandry, we made direct comparisons between the magnitude of selection on mating system traits and pollinator availability across populations.

MATERIALS AND METHODS

Natural History of Clarkia xantiana

Clarkia xantiana A. Gray (Onagraceae) is a winter annual endemic to southern California. The outcrossing and selfing subspecies are distinguished primarily by floral characters and flowering time (Fig. 1; Eckhart and Geber 1999; Runions and Geber 2000). Both subspecies are self-compatible and cross-compatible (M. A. Geber and A. Senft, unpubl. data). In the selfer, there is little or no protandry and anthers make contact with the stigma as the flower opens or shortly thereafter to effect pollination (i.e., prior or competing selfing). In the outcrosser, gender expression is protandrous, in that anthers dehisce prior to stigma receptivity. In addition, the stigma is exserted beyond the anthers (herkogamy) before becoming receptive (Fig. 1; see also Runions and Geber 2000). Both protandry and herkogamy are highly variable among populations of *C. xantiana* (Eckhart and Geber 1999). Self-pollination is derived from outcrossing in *C. xantiana* (Gottlieb 1984) and in other *Clarkia* taxa (Lewis and Lewis 1955; Vasek 1964; Allen et al. 1991). In the absence of pollinators, rates of autonomous selfing (% fruit set) decline with increasing herkogamy and protandry for individuals sampled across 30 populations of *C. xantiana* (herkogamy: $R^2 = 0.62$, $P < 0.0001$; protandry: $R^2 = 0.84$, $P < 0.0001$; D.A. Moeller, unpubl. data).

Clarkia xantiana populations may coexist and overlap in flowering time with as many as three other outcrossing *Clarkia* species (*Clarkia cylindrica* ssp. *clavicarpa* W. Davis, *Clarkia unguiculata* Lindley, and *Clarkia speciosa* ssp. *polyantha* Harlan Lewis and M. Lewis). We characterized geographic patterns in community associations between *C. xantiana* and pollinator-sharing congeners across the range of both subspecies using a survey of 130 populations. Overall, outcrossing populations of *C. xantiana* coexist with congeners more often than selfing populations (Fig. 2B; outcrosser vs. selfer: 47.6%, $n = 84$ vs. 6.5% of populations, $n = 46$, Fisher's exact test, $P < 0.0001$). In the outcrossing subspecies, the frequency of associations with congeners is higher in its exclusive range (western) than where subspecies overlap (72.5%, $n = 40$ vs. 25.0%, $n = 44$; Fisher's exact test, $P < 0.0001$). Selfing populations are only infrequently associated with congeners in the zone of subspecies overlap and populations in the selfer's exclusive range (eastern) occur outside the geographic distribution of congeners (Fig. 2B; 16.7%, $n = 18$ vs. 0.0%, $n = 28$; Fisher's exact test, $P = 0.05$). In this study, we focus on interactions between *C. xantiana* and two congeners, *C. cylindrica* and *C. unguiculata*, because previous work has shown that these species are the most common and abundant associates of *C. xantiana* (Moeller 2004). The three species share a phylogenetically diverse group of bee pollinators including both specialist and generalist foragers (MacSwain et al. 1973; Fausto et al. 2001; Moeller 2005) but do not form hybrids under natural conditions or with controlled crosses (Lewis and Lewis 1955).

Experimental Design

The effects of population size and the presence of congeners on pollinator availability and selection on floral traits were examined using experimental populations of greenhouse-grown plants. First, we describe the crosses that we performed to generate progeny with a broad phenotypic range of floral trait variation. Second, we describe the experimental treatments, data collection, and statistical analyses.

Manipulation of phenotypic variation.—Populations were composed of plants derived from crosses among five natural populations that were chosen to span a large portion of floral trait variation in the species (see Fig. 2 for the location of populations). In a common environment, populations exhibit genetic differences in herkogamy, protandry, and petal size; variation in these traits across subspecies is bimodal (Fig. 3A–C; D. A. Moeller, unpubl. data; see also Eckhart and Geber 1999). The five populations differed in trait means and patterns of trait covariation. We selected four populations from ssp. *xantiana* and one population from ssp. *parviflora* because mating system traits are considerably more variable in the former compared to the latter. Reciprocal crosses were conducted within and between all five populations by randomly pairing plants from each of five maternal families per population. When plants from highly selfing populations acted as dams, bud emasculations were performed to prevent self-pollination. The resulting F_1 progeny exhibited high phenotypic variance and novel intermediate phenotypes between subspecies and populations (Fig. 3D–F). The use of experimental populations with the full range of floral variation should improve the statistical power to detect selection and identify the targets of selection (Mitchell-Olds and Shaw 1987; Wade and Kalisz 1990).

In March 2000, 120 individuals from each of the 15 crosstypes were grown in pots (164 mL Cone-tainers, Stuewe and Sons, Corvallis, OR) in a greenhouse at the University of

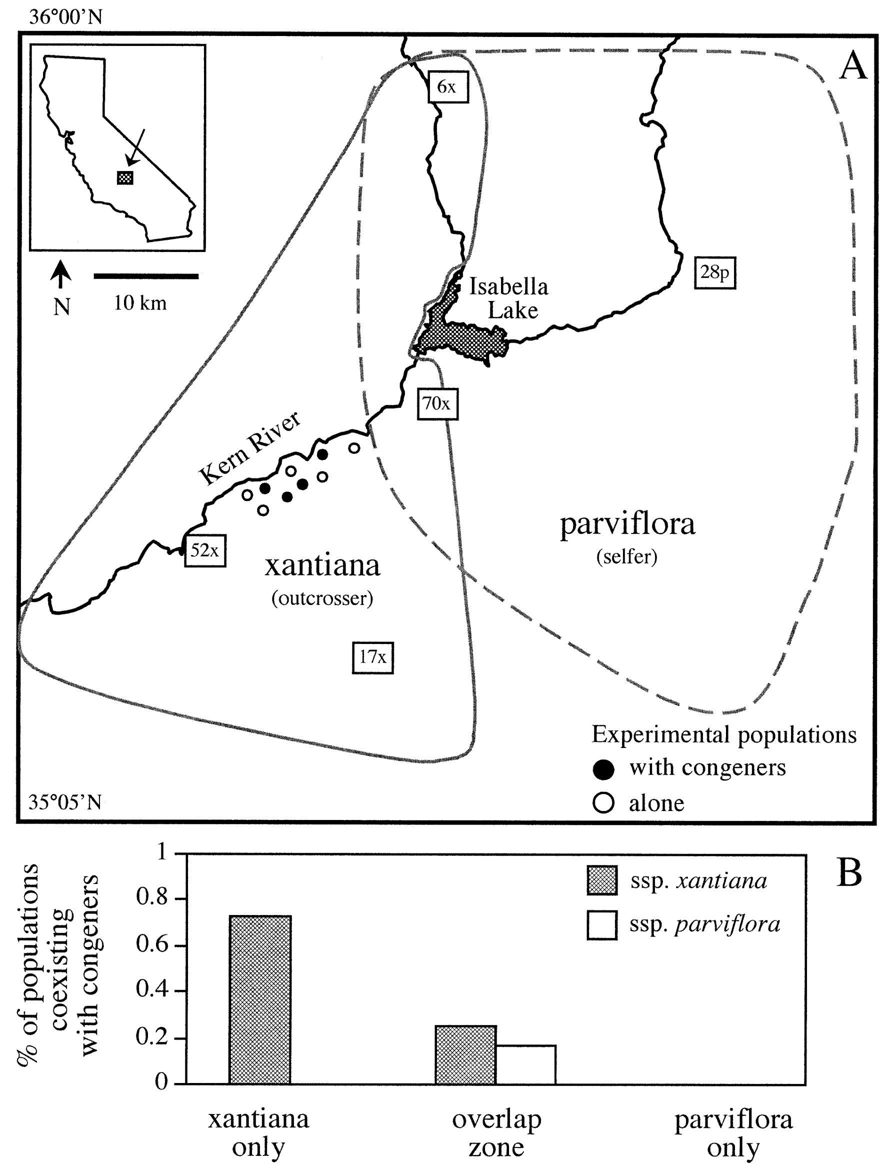


FIG. 2. (A) Geographic distribution of *Clarkia xantiana* ssp. *xantiana* (outlined by solid line) and ssp. *parviflora* (dashed line) in the southern Sierra Nevada of California. Five populations used in crosses to generate plants for the experiment are identified by population number in boxes (x: ssp. *xantiana*, p: ssp. *parvifora*). Experimental sites are labeled with circles and coded according to plant community type. (B) The frequency of associations between natural *C. xantiana* populations and pollinator-sharing congeners in the exclusive range of *C. x. xantiana*, the zone of overlap between subspecies, and in the exclusive range of *C. x. parviflora*.

California, Davis. After three weeks, plants were transported to a shade house near field sites in the southern Sierra Nevada, California, fertilized once per week using a 10–60–10 (N-P-K) fertilizer, and maintained until flowering. Because vegetative and early flower development occurred in a common environment prior to exposure to different biotic environments, phenotypic differences among plants were expected to be largely genetic.

Experimental treatments.—Experimental populations were either small (50 individuals) or large (150 individuals). Small populations were comparable in size to small natural populations; large population size was maximized based on feasibility. Populations were either placed into natural populations of *C. cylindrica* and *C. unguiculata* with >500 plants (''with congeners'') or into unoccupied, but otherwise suitable sites, where no *Clarkia* individuals were present (''alone''). Unoccupied sites were carefully chosen to match the slope, aspect, and dominant vegetation of sites occupied by natural populations and were all located in the vicinity of natural *Clarkia* communities. Experimental populations flowered when *C. cylindrica* and *C. unguiculata* populations had just passed their peak in flowering, as occurs in natural communities (Moeller 2004). The experiment was conservative in that all populations were placed into the portion of the species' range with the greatest abundance of *Clarkia* species and bee pollinators (Fig. 1; Moeller 2004, 2005) and where natural populations have pronounced herkogamy and protandry (Eckhart and Geber 1999).

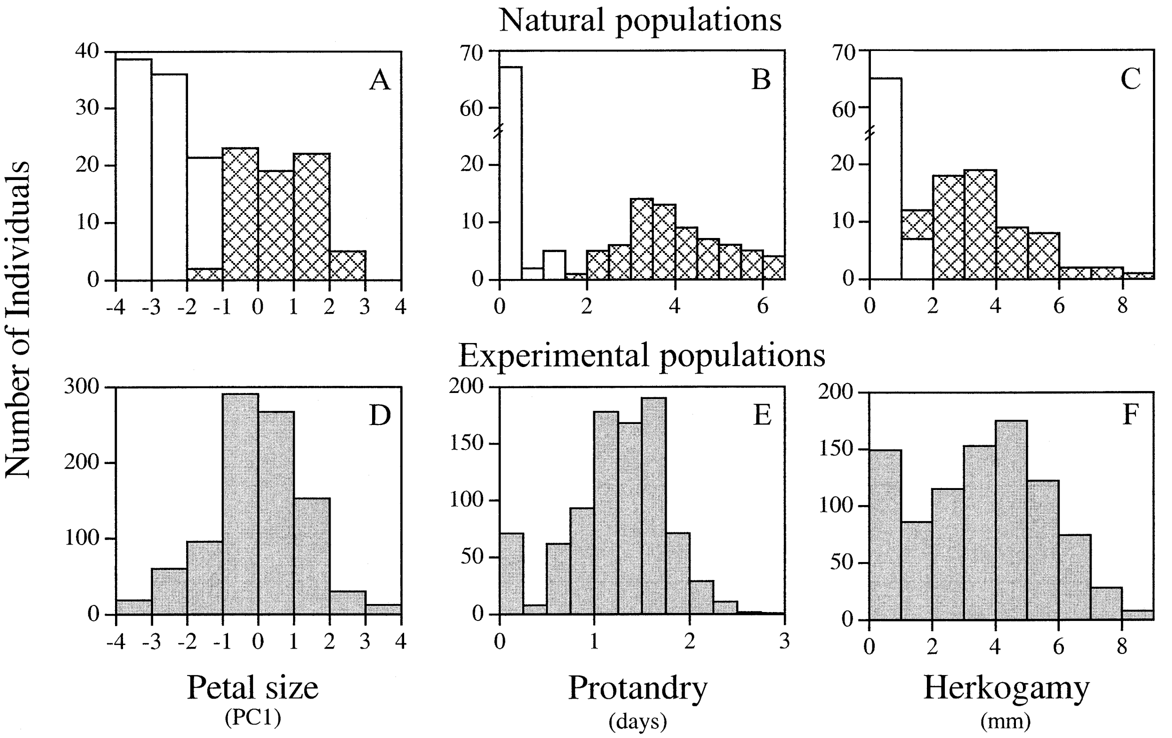


FIG. 3. Frequency distribution of floral traits (petal size, protandry, and herkogamy) from natural populations and experimental populations. The top three panels (A–C) show plants from 30 natural populations sampled from across the species' geographic range (D. A. Moeller, unpubl. data). Trait means differ between subspecies for all three traits: petal size (*t*-test, $t = 22.3$, $P < 0.0001$), protandry (Welch's *t*-test, $t = 27.0$, $P < 0.0001$), and herkogamy (Welch's *t*-test, $t = 16.6$, $P < 0.0001$). The bottom three panels (D–F) show plants from the nine experimental populations in this study. For both datasets, plants were grown in a common environment. Petal size is the first principal component of petal length and width.

Ten experimental populations were placed in the field in June 2000; one population was destroyed by small mammals during the experiment. We took advantage of variation in flowering time among natural populations of *C. xantiana* in different sections of the Kern Canyon (Sierra Nevada mountains) to perform the experiment in three temporal blocks. We synchronized each block of the experiment with the peak flowering time of nearby natural populations. Individual populations remained in the field for one week. The first two temporal blocks contained one replicate of each of the four treatments (small and large populations occurring either with congeners or alone). The third temporal block contained only small populations (in each community type) because there were insufficient numbers of additional experimental plants to construct large populations.

Each population in a given block was identical in composition. Plants from each of the 15 cross-types were randomly assigned to experimental populations such that each population in a given block was composed of the same proportion of each cross-type. Several cross-types were underrepresented in the third block of the experiment but the range and variance in traits were similar across blocks. In each population, plants were placed in multiple 0.6 m × 0.3 m plastic racks (Stuewe and Sons, Corvallis, OR) and racks were spaced 0.6 m apart such that plant density was constant across populations (3.6 plants/m^2); this density falls into the upper end of the range observed in natural populations (Fausto et al. 2001; Moeller 2004).

Floral Measurements, Pollinator Observations, and Female Fitness

We measured four floral traits on two flowers per plant during field exposure. Petal length and petal width were measured for the uppermost petal on each flower. Herkogamy and protandry were measured using daily records of individual flower phenology. Herkogamy was measured as the distance between the surface of a receptive stigma and the nearest anther. Protandry was measured as the difference in time between the onset of long-anther dehiscence and stigma receptivity.

Pollinator visitation was observed on six days in each population from the second day of each experimental block through the seventh day. The order in which we observed populations on any given day was systematically rotated to avoid confounding effects of observation time with experimental treatments. Pollinator visitation was observed for one hour per population per day during the peak visitation period (0900–1400 hrs); observation time was divided among racks of plants so that all plants were observed each day. We recorded the number of visits to each rack of plants, the number of consecutive visits to flowers on individual plants, and identified visitors by sight based on previous experience with the bee pollinators of *Clarkia* (Moeller 2005). To obtain visitation rates, the number of visits during each 10-min observation period was divided by the number of flowers observed.

Female fitness was estimated from the seed set of flowers that opened and senesced during the period of the experiment. Following field exposure, we returned plants to a shade house where they were maintained until fruits were harvested. We counted the number of fully developed seeds and the remaining undeveloped ovules in each fruit. Female fitness was measured as the ratio of fully developed seeds to the overall number of ovules (seeds plus remaining ovules) because populations vary in the number of ovules per flower.

Statistical Analyses

Treatment effects on pollinator visitation and female fitness.—The effect of the plant community and population size on pollinator visitation rates (visits/flower/10 min.) was analyzed by repeated-measures mixed-model analysis of variance (The MIXED procedure, SAS 1997). Plant community, population size, and day of observation were considered as fixed effects whereas block and population, the subject upon which repeated measures were taken, were considered random effects. The covariance structure of the data was taken to be compound symmetric after comparing the fit of alternative models (Littell et al. 1996). The significance of random effects was tested using likelihood ratio tests. The block effect was nonsignificant for all analyses of pollinator visitation and all other analyses from the experiment ($P \geq 0.2$), thus it was dropped from all models. In addition to an overall analysis of visitation rates by all bee visitors, we conducted separate analyses for specialist and generalist bees. All visitation rates were square-root transformed to improve the homoscedasticity of the residuals. We used a two-way ANOVA to ask whether treatments (pop. size and plant community) influenced the number of consecutive visits by bees to flowers on individual plants and a one-way ANOVA to test for differences among bee species.

We tested for treatment effects on mean female fitness using a two-way ANOVA including population size and plant community. To compare the potential strength (upper bound) of selection acting in individual populations, we calculated the opportunity for selection (I), that is, the variance in relative fitness, for each experimental population and examined treatment effects on the variance in fitness using a two-way ANOVA.

Selection on floral traits.—The direction and magnitude of selection on floral traits was estimated from simple and multiple regressions of relative fitness on standardized trait values (Lande and Arnold 1983). Selection analyses included three floral traits: herkogamy, protandry, and petal size (first principal component of petal length and petal width). Correlations among floral traits were moderate and therefore each was retained in the analyses (petal size × protandry, $r = 0.53$; petal size × herkogamy, $r = 0.57$; protandry × herkogamy, $r = 0.56$; $P < 0.05$ for all). Selection differentials and gradients were first estimated for each population separately, with traits standardized within populations and relative fitness calculated as individual fitness divided by mean fitness in each population. Linear selection differentials provide a measure of the net directional selection on traits whereas linear selection gradients measure direct selection on each trait and better identify the targets of selection among a suite of traits. Linear selection differentials (S) were estimated from simple regression coefficients of relative fitness against standardized trait values ($\mu = 0$, $\sigma = 1$). Linear selection gradients (β) were estimated from partial regression coefficients obtained from multiple regressions of relative fitness against all three floral traits.

Nonlinear selection coefficients describe selection affecting trait variances and covariances. Nonlinear differentials (C_{ii}) were estimated from separate multiple regression models including linear and quadratic terms for individual traits. Nonlinear gradients were estimated from models including linear, quadratic (γ_{ii}), and bivariate (γ_{ij}) terms. The residuals from analyses were normally and independently distributed. Multicollinearity was low as indicated by variance inflation factors (all VIFs ≤ 3), which measure the degree to which variance in parameter estimates is elevated by interrelationships among predictor variables (Neter et al. 1996). For significant quadratic gradients, we used nonparametric cubic splines to determine whether there was a fitness maximum or minimum within the range of observed phenotypes signifying stabilizing or disruptive selection (Schluter and Nychka 1994). For significant bivariate gradients, we visualized fitness surfaces with mesh plots of predicted fitness using SigmaPlot (2000).

Tests for context-dependent selection.—We examined the effects of experimental treatments on selection on floral traits using two approaches. In the first analysis, individuals from each treatment were combined across blocks in an ANCOVA including treatments, traits, and interaction terms (an individual-level analysis). A significant treatment by trait interaction indicates that the covariance between relative fitness and trait values differs between experimental treatments. We standardized trait values and calculated relative fitness within populations (as in the analyses above). This approach removes the block and treatment effects on mean fitness (mean fitness = 1 for all populations), but allows for a test of treatment effects on selection gradients. Because no interactions between treatments and quadratic or bivariate terms were significant, they were dropped from the model as they did not affect the significance of other effects.

In the second analysis, we submitted selection gradients, calculated separately for each population (see Table 3), to a two-way ANOVA including population size and plant community (population-level analysis). This approach provides a more conservative test of context-dependent selection compared to the individual-level analysis because the experimental unit is the whole plant population.

Treatment effects on the magnitude of selection on mating system traits were predicted to be mediated by variation in pollinator or mate availability among populations. Slopes of the relationship between selection gradients for floral traits and mean pollinator visitation rates were estimated using reduced major axis (RMA) regressions with 95% confidence intervals obtained by bootstrapping. RMA was used because of considerable measurement error in the independent variable (Sokal and Rohlf 1995). If *pollinator* limitation of reproduction has an important influence on the strength of selection, we expect selection gradients and pollinator visitation rates to covary; whereas, covariation is not necessarily

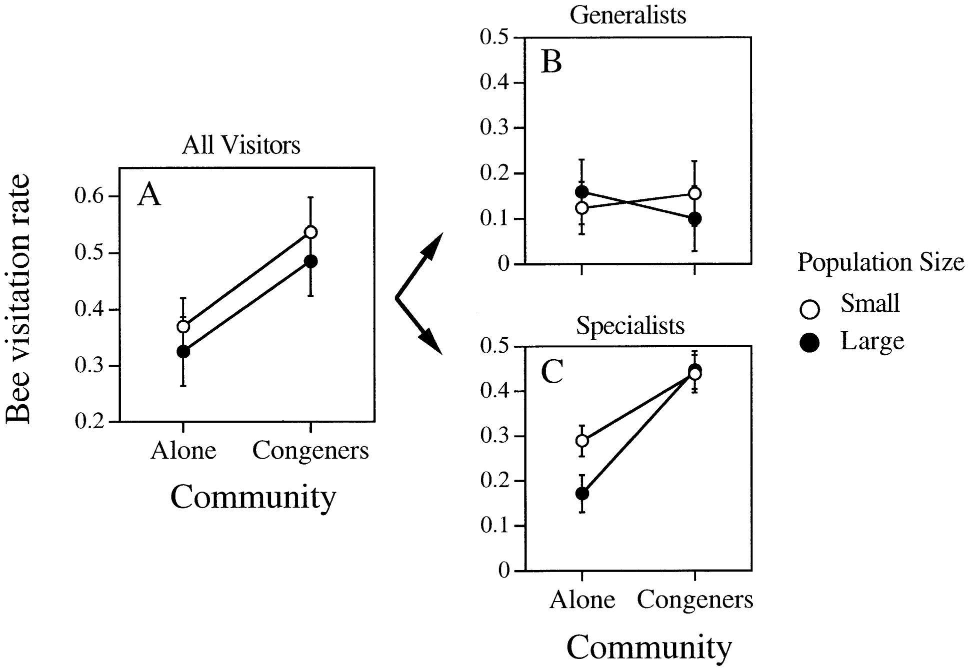


FIG. 4. The effect of the plant community and population size on visitation rates by bees (least-square means ± SE). Results are shown for (A) all visitors, (B) generalist foragers, and (C) specialist foragers. Visitation rates were calculated as the number of bee visits per flower per 10-min observation period (square-root transformed).

expected when *mate* limitation (or some other phenomenon) is the principal cause of selection on a floral trait.

RESULTS

Pollinator Visitation and Female Fitness

We observed a total of 2755 bee visits to experimental populations; bees were the only visitors of flowers. Pollen specialists accounted for 78.2% ($n = 1990$) of the bees identified ($n = 2543$). Populations co-occurring with congeners had significantly greater pollinator visitation rates than those occurring alone (Fig. 4A; Table 1). Visitation by generalist bees did not differ significantly among experimental treatments, although visitation by specialists was significantly greater, by a factor of 1.9, for populations with congeners compared to those isolated from congeners (Fig. 4B, C; Table 1). There was some variation in the response of individual specialist pollinator taxa to community treatments: *Hesperapis regularis*, *Megachile gravita*, *Megachile pascoensis*, and *Ceratina sequoiae* were between 3.3 and 8.5 times more common in populations co-occurring with congeners (Table 1). There was no significant effect of population size and its interaction with community on pollinator visitation rates. However, the composition of pollinator assemblages did differ between small, isolated populations and all other treatments. *Lasioglossum pullilabre* accounted for 70% of bee visitors in small, isolated populations and less than 23% of visitors in all other treatments ($\chi^2 = 501.8$, df = 3, $P < 0.0001$).

Bees visited a single flower per plant on 1779 of 2288 foraging bouts (77.7%; mean ± SE, 1.28 ± 0.013 flowers/plant). The number of consecutive visits made to flowers within an inflorescence was significantly greater in populations with congeners compared to those isolated from con-

TABLE 1. F-values for fixed effects and χ^2-values for random effects from repeated-measures ANOVAs of visitation rates for all visitors, specialists, generalists, and for the three principal groups of specialists: *Hesperapis* and *Megachile* species (*H. regularis*, *M. gravita*, and *M. pascoensis*, $n = 787$ visits), *Ceratina sequoiae* ($n = 333$), and *Lasioglossum pullilabre* ($n = 870$).

					Principal specialist taxa		
Source	df	All visitors	Specialists	Generalists	*H. regularis*, *Megachile* spp.	*C. sequoiae*	*L. pullilabre*
Fixed effects							
Community	1, 5	7.72*	27.74**	0.04	22.04**	8.54*	0.31
Population size	1, 5	0.66	1.87	0.02	5.25	0.07	2.61
Community × pop. size	1, 5	0.00	2.42	0.44	0.97	0.05	1.23
Day	5, 50	1.28	1.69	0.38	0.28	0.80	1.73
Random effect							
Population	1	0.04	0.40	4.98*	0.13	1.52	5.52*

* $P < 0.05$, ** $P < 0.01$.

TABLE 2. Linear (S) and quadratic (C) selection differentials, opportunity for selection (I), and mean female fitness (±SE), for the nine experimental populations. Levels of significance are indicated by asterisks.

Pop. size, community population	Selection differentials						Opportunity for selection	Mean female fitness
	Petal size		Protandry		Herkogamy			
	S	C_{ii}	S	C_{ii}	S	C_{ii}	I	
Small, alone								
A	−0.09*	0.08*	−0.17***	0.02	−0.17***	0.00	0.10	0.52 (0.02)
B	−0.08	0.03	−0.32***	0.04	−0.29***	0.02	0.30	0.32 (0.02)
C	−0.08	−0.08	−0.20***	0.00	−0.20***	−0.06*	0.17	0.36 (0.02)
Small, congener								
D	−0.29***	0.10	−0.24**	0.19**	−0.23**	0.05	0.37	0.38 (0.03)
E	−0.03	0.00	0.07	−0.07	0.05	−0.12	0.25	0.38 (0.03)
Large, alone								
F	−0.04	0.02	−0.06*	0.03	−0.11***	0.02	0.10	0.59 (0.02)
G	−0.02	−0.01	−0.02	−0.02	−0.03	0.00	0.06	0.56 (0.01)
Large, congener								
H	−0.06***	0.00	−0.05**	0.03*	−0.08***	0.02	0.06	0.62 (0.01)
I	0.00	0.02	−0.08**	0.05*	−0.10**	0.06*	0.12	0.48 (0.01)

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

geners, but only by a factor of 1.1 (pop. size, $F_{1,5} = 0.44$, $P = 0.53$; community, $F_{1,5} = 17.58$, $P = 0.006$; interaction, $F_{1,5} = 0.158$, $P = 0.71$). Bee species differed in the number of consecutive visits ($F_{6,1922} = 3.45$, $P = 0.002$) chiefly because bumble bees, the most common generalist pollinators of *C. xantiana*, more often visited multiple flowers on a plant compared to specialists (*Bombus* spp. vs. specialists; 1.45 vs. 1.29 flowers/plant; $F_{1,1672} = 7.58$, $P = 0.006$).

Plants in experimental populations ($n = 818$ plants, 3526 flowers) produced 103,405 seeds from a total of 192,428 ovules (53.7% seed set). Mean female fitness for populations was influenced significantly by population size but not the plant community or the interaction between these factors (pop. size: $F_{1,5} = 10.27$, $P = 0.02$; community: $F_{1,5} = 0.13$, $P = 0.74$; interaction: $F_{1,5} = 0.002$, $P = 0.97$). Individuals in small populations suffered lower reproductive success (large vs. small pops. 0.56 vs. 0.39 seeds/ovule). Variance in relative fitness was also higher in small populations compared to large populations indicating a greater opportunity for selection (pop. size: $F_{1,5} = 9.8$, $P = 0.03$; community: $F_{1,5} = 1.5$, $P = 0.27$; interaction: $F_{1,5} = 1.1$, $P = 0.34$; Table 2).

Selection on Floral Traits

Linear selection differentials for mating system traits, herkogamy and protandry, were highly variable among populations (range: −0.39–0.05 and −0.42–0.07, respectively) and significantly less than zero in seven of nine populations for both traits (Table 2). Small populations occurring alone had uniformly and strongly negative linear differentials for herkogamy and protandry. Net selection on these traits in other treatments was weakly negative or variable among replicate populations. Selection differentials for petal size were significantly different from zero in three small populations; as with mating system traits, we detected little selection acting on petal size in large populations in either community context (Table 2). Several quadratic differentials were significant; however, visualization of fitness surfaces revealed that relative fitness was an accelerating or decelerating function of floral trait variation, rather than stabilizing or disruptive selection, or that quadratic differentials were heavily influenced by high leverage points in parametric regressions.

Multiple regressions revealed strong direct selection on herkogamy and protandry in all small populations occurring alone; all linear selection gradients were significantly less than zero indicating that high relative fitness was associated with reduced herkogamy and protandry (Table 3). For the remaining six populations in other treatments, estimates of direct linear selection for herkogamy and protandry were comparatively weak and significantly less than zero in only two cases for herkogamy. We detected significant direct selection on petal size in only one population (pop. A). Quadratic selection gradients were weak across all populations (Table 3). As with nonlinear selection differentials, the one significant quadratic gradient (protandry in pop. G) was heavily influenced by high leverage points and therefore provided little evidence of stabilizing selection. We also did not detect significant bivariate (correlational) selection for any individual population.

Because statistical power was low for estimates of selection on trait variances and covariances in individual populations, we also conducted analyses by combining data across replicate populations within treatments. There were significant quadratic gradients for petal size in large populations with congeners ($\gamma_{ii} = -0.045$, $F_{1,282} = 4.0$, $P = 0.045$) and small populations isolated from congeners ($\gamma_{ii} = -0.052$, $F_{1,138} = 4.16$, $P = 0.043$) and a significant bivariate selection gradient for petal size and protandry in small populations isolated from congeners ($\gamma_{ij} = 0.078$, $F_{1,138} = 4.0$, $P = 0.049$). However, visualization of fitness surfaces failed to provide supporting evidence of stabilizing, disruptive, or correlational selection.

Causes of Selection

Both analytical approaches testing for treatment effects on patterns of selection revealed strong context-dependent se-

TABLE 3. Linear (β), quadratic (γ_{ii}), and bivariate (γ_{ij}) selection gradients for the nine experimental populations.

	Selection gradients								
Pop. size, community population	Petal size		Protandry		Herkogamy		Petal size × protandry	Petal size × herkogamy	Protandry × herkogamy
	β	γ_{ii}	β	γ_{ii}	β	γ_{ii}	γ_{ij}	γ_{ij}	γ_{ij}
Small, alone									
A	0.14*	0.02	−0.16**	−0.08	−0.17***	−0.04	0.11	0.00	0.03
B	0.12	−0.03	−0.24**	0.03	−0.20*	−0.13	0.05	−0.14	0.26
C	−0.04	−0.06	−0.14**	0.04	−0.13*	−0.07	−0.11	0.11	−0.09
Small, congener									
D	−0.23	0.13	−0.07	0.16	−0.01	−0.14	−0.17	−0.08	0.21
E	−0.05	0.03	0.06	−0.13	0.04	−0.17	−0.24	0.08	0.16
Large, alone									
F	−0.05	−0.03	−0.02	0.03	−0.13***	0.00	0.03	0.01	0.02
G	−0.01	−0.01	−0.01	−0.04*	−0.02	−0.02	−0.01	0.00	0.08
Large, congener									
H	−0.02	−0.08	−0.01	0.01	−0.06*	0.01	0.10	0.03	−0.04
I	0.05	−0.01	−0.06	0.05	−0.07	0.04	0.03	−0.04	0.02

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

lection on mating system traits. In the population-level analysis, where selection gradients were submitted to ANOVAs, there was a statistically significant interaction between the effect of population size and the plant community on selection on herkogamy and protandry (Table 4). Selection for reduced herkogamy and protandry was strongest in small populations isolated from congeners. Selection gradients for petal size did not differ significantly among experimental treatments (Table 4).

The individual-level analysis yielded very similar results overall, but provided several additional insights. Context-dependent selection on herkogamy and protandry was detected from significant two- and three-way interactions between experimental treatments and mating system traits, consistent with the results described above (Fig. 5A–D, Table 4). The fitness advantage conferred by reduced herkogamy and protandry was pronounced in small populations isolated from congeners. In this environment, the relative fitness of plants with low herkogamy and protandry (lower 10th percentile) was elevated by 66.1% and 58.0%, respectively, compared to those with high herkogamy and protandry (upper 10th percentile).

In contrast to the population-level analysis, selection on petal size did differ among treatments (Table 4). There was little evidence of selection on petal size in large populations in either community context (Fig. 5E). In small populations, however, selection favored larger petals when populations occurred alone but smaller petals when congeners were present (Fig. 5F).

The slope of the relationship between selection gradients for herkogamy and mean pollinator visitation rates was significantly greater than zero (RMA slope = 0.84, 95% confidence interval: 0.50–1.27, $R^2 = 0.49$) suggesting that *pollinator* limitation of reproduction influenced the strength of selection on herkogamy (Fig. 6A). We did not detect a significant relationship between protandry or petal size selection gradients and mean pollinator visitation rates (Fig. 6B; protandry: 95% CI of RMA slope = −0.96–1.55, $R^2 = 0.14$; petal size: 95% CI of RMA slope = −2.1–0.44, $R^2 = 0.13$).

DISCUSSION

There is growing evidence that selfing mechanisms provide reproductive assurance in natural populations (e.g., Herlihy

TABLE 4. Tests for context-dependent selection. In the population-level analyses, selection gradients for each trait were submitted to two-way ANOVAs (df = 1, 5). In the individual-level analysis, individuals were combined in an ANCOVA including treatments, traits, and interactions between treatments and traits (df = 1, 794); only tests of context-dependent selection are shown. Significant effects appear in bold.

	Petal size		Protandry		Herkogamy	
	F	*P*	*F*	*P*	*F*	*P*
Population-level analysis (response: selection gradients)						
Population size	0.7	0.439	3.2	0.13	0.0	0.85
Community	3.2	0.131	4.3	0.09	10.2	**0.02**
Pop. size × community	3.0	0.146	6.8	**0.05**	8.2	**0.04**
Individual-level analysis (response: relative fitness)						
Population size × trait	5.2	**0.023**	8.5	**0.004**	0.0	0.961
Community × trait	11.1	**<0.001**	1.6	0.199	6.5	**0.011**
Pop. size × community × trait	15.9	**<0.001**	4.2	**0.042**	7.6	**0.006**

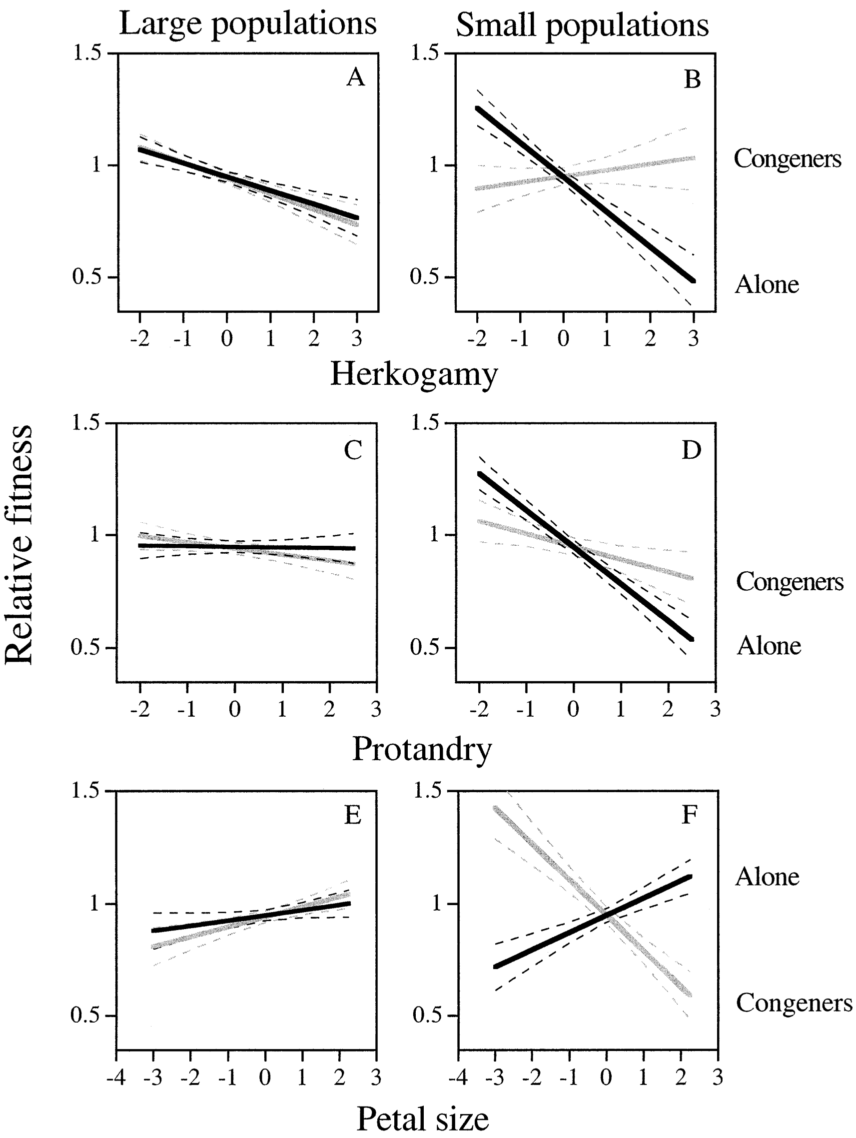


FIG. 5. Estimated directional selection gradients for herkogamy, protandry, and petal size from the ANCOVA testing for context-dependent selection (Table 4). Selection gradients for populations with congeners are indicated by gray lines and those isolated from congeners by black lines. Standard errors are indicated by dashed lines. Selection gradients in each panel span only the range of trait values observed in each population.

and Eckert 2002; Elle and Carney 2003; Kalisz and Vogler 2003), consistent with long-standing expectations, and recent evidence that population variation in selfing rates is related to the probability of reproductive failure (Kalisz et al. 2004). We know less about the ecological context in which selfing provides reproductive assurance and has a relative advantage over outcrossing. Our results show that plant population size and positive interactions with pollinator-sharing congeners have strong effects on selection on mating system traits. We also found direct support for the hypothesis that variable selection on mating system traits was mediated, in part, by the availability of pollinators. In combination with studies of pollen limitation and pollinator communities in natural populations of *C. xantiana* (Fausto et al. 2001; Moeller 2004, 2005), these results suggest that reproductive assurance may play a central role in mating system evolution in *C. xantiana*.

Mating System Evolution in the Context of Communities and Populations

Our finding that pollinator-sharing plant species enhance pollinator visitation and relax selection on mating system traits runs counter to previous reports where competitive interactions resulted in selection favoring self-pollination. In theory, pollinator sharing among plant species can have contrasting influences on mating system evolution depending upon the nature of plant species interactions. Competitive interactions are likely to select for self-pollination either when pollinators recurrently avoid one plant species in favor of another co-occurring species (selfing confers reproductive assurance; Levin 1972) or when pollinator movement between species leads to interspecific pollen transfer and low female fertility (selfing prevents hybridization, Antonovics

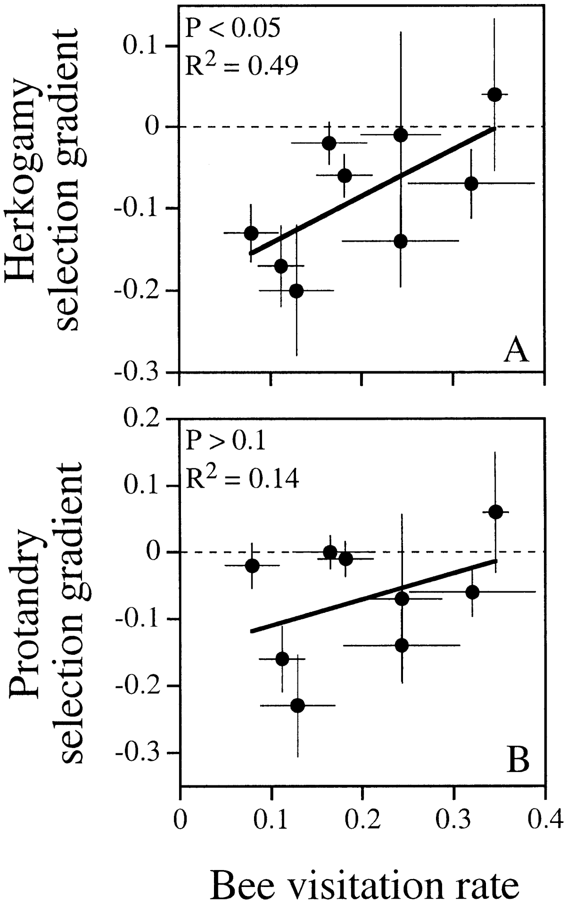


FIG. 6. The relationship between selection gradients for (A) herkogamy and (B) protandry and mean pollinator visitation rates for the nine experimental populations. Standard errors are shown for both variables.

1968). Although we are not aware of support for the first mechanism, there is evidence in *Arenaria uniflora* that outcross reproductive success is depressed due to interspecific pollen transfer where its geographic range overlaps with a pollinator-sharing congener (Fishman and Wyatt 1999). By contrast, when co-occurring species interact in a facilitative manner by enhancing the supply of pollinators (Moeller 2004), as in this study, mating system traits that promote selfing are not under selection. Consistent with experimental results, geographic surveys indicate that the selfing subspecies of *C. xantiana* occurs almost exclusively outside the range of congeners or flowers prior to congeners where they are sympatric (D. A. Moeller, pers. obs.). The association between selfing and environments with low *Clarkia* species diversity appears to be widespread across the genus *Clarkia* (M. A. Geber and D. A. Moeller, unpubl. data). Although competition among pollinator-sharing species may favor selfing as a form of reproductive character displacement, positive interactions may promote the mutual maintenance of outcrossing (reproductive character convergence).

Positive interactions in plant communities are likely to be important to the maintenance of outcrossing under a restricted set of conditions. The strength of facilitative effects on patterns of selection in this study was clearly dependent upon population size and previous work in natural populations has also shown that pollen limitation of reproduction decreases as population size increases (Moeller 2004). In small populations, there was considerable variance in fitness, and selection tended to favor reduced herkogamy and protandry whereas in large populations, we detected little variance in fitness and comparatively weaker selection. Therefore, facilitative interactions may be most important in the presence of the Allee effect. The importance of population size in determining selection on mating system traits is consistent with Baker's law, the suggestion that selfing is advantageous in the colonization of new sites (Baker 1955; 1967), and Lewis' hypothesis that selfing evolves during periods of catastrophic selection in marginal environments, where plant populations experience frequent reductions in size and density (Lewis 1962). The strong effects of community context and population size on patterns of selection in our study are especially striking given that the experiment was conducted in the center of the outcrosser's range where specialist pollinators of *Clarkia* are most abundant and *Clarkia* populations are at a high density across the landscape (all experimental populations were < 0.75 km from neighboring populations of congeners).

Although variation in female fitness is closely associated with mating system traits in small populations isolated from congeners, our study does not establish that higher seed production in individuals with low herkogamy and protandry is due to autonomous selfing as opposed to other forms of pollen transfer: outcrossing, facilitated selfing (pollen transfer within-flowers by pollinators), or geitonogamous selfing (pollen transfer between flowers within an inflorescence) (Lloyd 1992). However, observations of pollinator visitation suggest that autonomous selfing is the most likely explanation for our results. In small populations isolated from congeners, we found no evidence that pollinator visitation rates were related to variation in mating system traits (herkogamy: $F_{1,137} = 0.69$, $P = 0.406$; protandry: $F_{1,137} = 1.48$, $P = 0.226$) suggesting that higher outcross success or facilitated selfing cannot explain the fertility advantage of plants with reduced herkogamy or protandry. Pollinator movement between flowers within inflorescences was uncommon and did not covary with mating system traits (herkogamy: $F_{1,125} = 1.14$, $P = 0.287$; protandry: $F_{1,125} = 0.01$, $P = 0.925$) providing little evidence for geitonogamous selfing. The parallel between selection gradients for herkogamy and pollinator visitation rates indicates that the advantage conferred by reduced herkogamy was greatest when pollinator visitation was low. Last, seed set of unmanipulated flowers in natural populations of the selfing subspecies is 32–43% higher than emasculated flowers (D.A. Moeller, unpubl. data). Collectively, these results are more consistent with the conclusion that autonomous selfing provided reproductive assurance. Establishing the paternity of the progeny of experimental plants using molecular markers would provide a direct method for confirming that plants with reduced mating system traits had elevated fertility due to selfing rather than outcrossing.

Our results suggest that both population size and pollinator-sharing congeners enhance outcross success; however, different underlying mechanisms may account for these results. Consistent with previous work in natural populations

(Moeller 2004), we found a significant effect of population size on mean seed set but not pollinator visitation rates. However, bee visitors to small, isolated populations were dominated by a single bee (*L. pullilabre*) that collects pollen from *C. xantiana* (Moeller 2005) but often fails to transfer pollen to receptive stigmas (G.M. Hart and V. M. Eckhart, unpubl. data). Therefore, small population size may limit outcross success through both low mate availability and a paucity of highly effective pollinators. Conversely, pollinator-sharing congeners strongly elevated pollinator visitation rates but we did not find a significant effect on mean seed set; whereas, previous work in natural populations did reveal a positive effect of pollinator-sharing congeners on seed set (Moeller 2004). One explanation is that when congeners overlap in flowering time, interspecific pollen transfer may reduce seed production particularly for the minority plant species in a community. Because of our experimental setup, it is possible that the positive effects of congeners on pollinator visitation were counteracted by pollen movement from highly abundant natural populations of *Clarkia* congeners to our smaller experimental populations of *C. xantiana* (D.A. Moeller, pers. obs.). However, competitive effects on mean seed set need not generate selection favoring selfing phenotypes if interspecific pollen transfer occurs at random with respect to individual variation in mating system traits. In sum, these results suggest that interactions with pollinator-sharing plant species can be both facilitative (through increased pollinator availability) and competitive (through interspecific pollen transfer) under certain circumstances.

Patterns of Selection on Secondary Sexual Traits

Relative to outcrossing taxa, sister selfing taxa typically have highly reduced secondary sexual characters (e.g., petals) involved in pollinator attraction (Ornduff 1969; Jain 1976). Reductions in secondary sexual characters may evolve because of (1) direct selection for reduced traits during the evolutionary transition to self-pollination (e.g., inconspicuous flowers may reduce interspecific pollen transfer); (2) indirect selection arising from genetic or developmental correlations between attractive traits and mating system traits; or (3) direct selection subsequent to the evolution of selfing when attractive traits are no longer important to mating success and are costly to produce. In this study, when ecological conditions favored mating system traits that promote self-pollination (in small, isolated populations), direct selection via female fertility tended to favor large petals, a pattern that is inconsistent with the first hypothesis. However, net selection differentials for petal size were weakly negative in small, isolated populations. The difference between direct and net selection suggests that indirect selection from developmental or genetic correlations with mating system traits may constrain the evolution of attractive features, in support of the second hypothesis. There is also some evidence that artificial selection for small petals leads to reduced anther size as a correlated response (M. A. Geber and C. M. McGuire, unpubl. data). Our results do not address the third hypothesis but it is plausible given that patterns of sex allocation differ between outcrossers and selfers (Runions and Geber 2000).

Patterns of selection on petal size, when considered independent of mating system traits, provide some insight into the potential role of plant communities in shaping floral form. We found that when small populations were isolated from congeners, selection favored individuals with large corollas; whereas, when sympatric with congeners, we found either that selection favored a reduction in mean petal size or no evidence of selection. In small, isolated populations, large corollas may be favored because pollinators are more discriminating when competition among pollinators for floral resources is low. By contrast, in populations with congeners, small corollas may be favored when rates of interspecific pollen transfer are high and pollinators prefer large corollas; that is, the most attractive flowers have reduced seed set because they disproportionately receive heterospecific pollen. Consistent with this argument, in the one population where we observed strong selection for small petals (popn. D), *Clarkia* congeners were highly abundant, pollinator availability was high, and pollinators strongly preferred large flowers of *C. xantiana* ($F_{1,47} = 63.14$, $R^2 = 0.57$, $P < 0.001$), suggesting that interspecific pollen transfer, not competition for pollinator service, suppressed the seed production of large-petaled plants.

Although further study is required to verify the mechanism underlying our results, it is notable that positive interactions among plants for pollinator service appear to differ in their effects on patterns of selection compared to situations where plants compete for pollinators. For example, in the presence of a competitor, *Ipomopsis aggregata* received less conspecific pollen and experienced selection for longer corollas compared to populations isolated from competitors (Caruso 2000). Whether interactions are competitive or facilitative, these studies suggest that variation in female fertility influences the evolution of secondary sexual traits just as male function does (reviewed in Ashman and Morgan 2004). Information on patterns of male fitness in *C. xantiana* is needed to assess whether the direction of selection on attractive traits is concordant or discordant via male versus female function, and whether the answer depends on the ecological context.

Spatial Population Dynamics and Geographic Patterns of Mating System Variation

Our demonstration that selfing provides reproductive assurance and that selection on mating system traits is context dependent is an important first step in elucidating the drivers of mating system evolution. However, ecological processes affecting pollinator abundance and plant reproduction are also likely to operate at much larger spatial scales than can be manipulated in experiments. In *C. xantiana*, as in many other examples of the evolution of selfing (e.g., *Lycopersicon*, Rick et al. 1977; *Leavenworthia*, Solbrig and Rollins 1977; *Gilia*, Schoen 1982), mating system varies over an environmental gradient with selfers occupying more stressful, marginal habitats. In parallel with this gradient, populations also exhibit adaptive differentiation in developmental, life history, and morphological traits (Eckhart et al. 2004; Geber and Eckhart 2005) and plants and plant populations become increasingly sparse in arid regions (Fausto et al. 2001; D.A. Moeller, unpubl. data). In view of the fact that outcross success is often positively density-dependent, we propose that the oft-cited association between selfing and marginal environments results from the synergistic effects of (1) declines in population carrying capacity and increased patchiness across environmental gradients, and (2) the presence of Allee effects limiting outcross success and population persistence.

For outcrossers, small population size and population isolation can amplify mate limitation of reproduction, as suggested by this study, and may have important consequences for the population dynamics of pollinators (e.g., Steffan-Dewenter et al. 2002). For selfers, populations may persist in environments where conditions for growth are unpredictable and populations are small because mating success is not influenced by density-dependent or stochastic demographic processes. Therefore, selfers may replace outcrossers at the stressful ends of environmental gradients due to the cascading effects of the abiotic environment on plant distribution and abundance, and in turn, on the availability of pollinators and mates for outcrossing. Consistent with this logic, theoretical work by Keitt et al. (2001) suggests that the presence of Allee effects can produce abrupt species' range limits when populations are patchily distributed, even without a broad-scale environmental gradient; whereas in the absence of Allee effects, range expansion can occur. In addition, metapopulation dynamics (frequent colonization and extinction of populations) are predicted to favor selfing over obligate outcrossing (Pannell and Barrett 1998). Although empirical tests of these hypotheses will be challenging, there is a need for greater focus on the role of demographic processes in mating system evolution and on the links between ecological and evolutionary processes affecting species interactions in the context of landscapes.

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LITERATURE CITED

Allen, G. A., L. D. Gottlieb, and V. S. Ford. 1991. Electrophoretic evidence for the independent origins of two self-pollinating subspecies of *Clarkia concinna* (Onagraceae). Can. J. Bot. 69: 2299–2301.

Antonovics, J. 1968. Evolution in closely adjacent plant populations. V. Evolution of self-fertility. Heredity 23:219–238.

Ashman, T.-L., and M. T. Morgan. 2004. Explaining phenotypic selection on plant attractive characters: male function, gender balance, or ecological context? Proc. R. Soc. Lond. B Biol. Sci. 271:553–559.

Baker, H. G. 1955. Self-compatibility and establishment after ''long-distance'' dispersal. Evolution 9:347–349.

———. 1967. Support for Baker's law-as a rule. Evolution 21: 853–856.

Barrett, S. C., M. T. Morgan, and B. C. Husband. 1989. The dissolution of a complex genetic polymorphism: the evolution of self-fertilization in tristylous *Eichhornia paniculata* (Pontederiaceae). Evolution 43:1398–1416.

Byers, D. L., and D. M. Waller. 1999. Do plant populations purge their genetic load? Effects of population size and mating history on inbreeding depression. Annu. Rev. Ecol. Syst. 30:479–513.

Campbell, D. R. 1985. Pollinator sharing and seed set of *Stellaria pubera*: competition for pollination. Ecology 66:544–553.

Carlquist, S. 1974. Island biology. Columbia Univ. Press, New York.

Caruso, C. M. 2000. Competition for pollination influences selection on floral traits of *Ipomopsis aggregata*. Evolution 54: 1546–1557.

Chang, S.-M., and M. D. Rausher. 1998. Frequency-dependent pollen discounting contributes to maintenance of a mixed mating system in the common morning glory *Ipomea purpurea*. Am. Nat. 152:671–683.

Charlesworth, D., and B. Charlesworth. 1987. Inbreeding depression and its evolutionary consequences. Annu. Rev.Ecol. Syst. 18:237–268.

Darwin, C. D. 1859. On the origin of species by means of natural selection, or preservation of favoured races in the struggle for life. John Murray, London.

———. 1876. The effects of cross- and self-fertilization in the vegetable kingdom. John Murray, London.

Eckhart, V. M., and M. A. Geber. 1999. Character variation and geographic distribution of *Clarkia xantiana* A. Gray (Onagraceae): flowers and phenology distinguish two subspecies. Madroño 46:117–125.

Eckhart, V. M., M. A. Geber, and C. M. McGuire. 2004. Experimental studies of adaptation in *Clarkia xantiana*. I. Sources of trait variation across a subspecies border. Evolution 58:59–70.

Elle, E., and R. Carney. 2003. Reproductive assurance varies with flower size in *Collinsia parviflora* (Scrophulariaceae). Am. J. Bot. 90:888–896.

Farris, M. A., and J. W. Mitton. 1984. Population density, outcrossing rate, and heterozygote superiority in Ponderosa pine. Evolution 38:1151–1154.

Fausto, J. A., V. M. Eckhart, and M. A. Geber. 2001. Reproductive assurance and the evolutionary ecology of self-pollination in *Clarkia xantiana* (Onagraceae). Am. J. Bot. 88:1794–1800.

Fisher, R. A. 1941. Average excess and average effect of a gene substitution. Ann. Eugen 11:53–63.

Fishman, L. 2000. Pollen discounting and the evolution of selfing in *Arenaria uniflora* (Caryophyllaceae). Evolution 54: 1558–1565.

Fishman, L., and R. Wyatt. 1999. Pollinator-mediated competition, reproductive character displacement, and the evolution of selfing in *Arenaria uniflora* (Caryophyllaceae). Evolution 53: 1723–1733.

Geber, M. A., and V. M. Eckhart. 2005. Experimental studies of adaptation in *Clarkia xantiana*. II. Fitness variation across a subspecies border. Evolution 59:521–531.

Gottlieb, L. D. 1984. Electrophoretic analysis of the phylogeny of the self-pollinating populations of *Clarkia xantiana*. Plant Syst. Evol 147:91–102.

Gottlieb, L. D., and V. S. Ford. 1996. Phylogenetic relationships among the sections of *Clarkia* (Onagraceae) inferred from the nucleotide sequences of PgiC. Syst. Bot. 21:45–62.

Groom, M. J. 1998. Allee effects limit population viability of an annual plant. Am. Nat. 151:487–496.

Guerrant, E. O. 1989. Early maturity, small flowers, and autogamy: a developmental connection? Pp. 61–84 *in* J. H. Bock and Y. B. Linhart, eds. The evolutionary biology of plants. Westview Press, Boulder, CO.

Hackney, E. E., and J. B. McGraw. 2001. Experimental demonstration of an Allee effect in American ginseng. Conserv. Biol. 15:129–136.

Hendrix, S. D., and J. F. Kyhl. 2000. Population size and reproduction in *Phlox pilosa*. Conserv. Biol. 14:304–313.

Henslow, G. 1879. On the self-fertilization of plants. Trans. Linn. Soc. Lond. Ser. 2, Bot. 1:317–339.

Herlihy, C. R., and C. G. Eckert. 2002. Genetic cost of reproductive assurance in a self-fertilizing plant. Nature 416:320–323.

Holsinger, K. E., and J. D. Thomson. 1994. Pollen discounting in *Erythronium grandiflorum*: mass-action estimates from pollen transfer dynamics. Am. Nat. 144:799–812.

Holsinger, K. E., W. M. Feldman, and F. B. Christiansen. 1984. The evolution of self-fertilization in plants: a population genetics model. Am. Nat. 124:446–453.

Jain, S. K. 1976. The evolution of inbreeding in plants. Annu. Rev. Ecol. Syst. 7:469–495.

Johnson, S. D., C. I. Peter, L. A. Nilsson, and J. Agren. 2003. Pollination success in a deceptive orchid is enhanced by co-occurring rewarding magnet plants. Ecology 84:2919–2927.

Kalisz, S., and D. W. Vogler. 2003. Benefits of autonomous selfing under unpredictable pollinator environments. Ecology 84: 2928–2942.

Kalisz, S., D. W. Vogler, and K. M. Hanley. 2004. Context-dependent autonomous self-fertilization yields reproductive assurance and mixed mating. Nature 430:884–887.

Karron, J. D., N. N. Thumser, R. Tucker, and A. J. Hessenauer. 1995. The influence of population density on outcrossing rates in *Mimulus ringens*. Heredity 75:175–180.

Keitt, T. H., M. A. Lewis, and R. D. Holt. 2001. Allee effects, invasion pinning, and species' borders. Am. Nat. 157:203–216.

Knight, T. 1799. Experiments on the fecundation of vegetables. Philos. Trans. R. Soc. Lond. B Biol. Sci. 89:195–204.

Knight, T. M. 2003. Floral density, pollen limitation, and reproductive success in *Trillium grandiflorum*. Oecologia 137: 557–563.

Kohn, J. R., and S. C. H. Barrett. 1994. Pollen discounting and the spread of a selfing variant in tristylous *Eichornia paniculata*: evidence from experimental populations. Evolution 48: 1576–1594.

Kunin, W. E. 1993. Sex and the single mustard: population density and pollinator behavior effects on seed set. Ecology 74: 2145–2160.

Lande, R., and S. J. Arnold. 1983. The measurement of selection on correlated characters. Evolution 37:1210–1226.

Levin, D. 1972. Competition for pollinator service: a stimulus for the evolution of autogamy. Evolution 26:668–674.

Lewis, H. 1953. The mechanism of evolution in *Clarkia*. Evolution 7:1–20.

———. 1962. Catastrophic selection as a factor in speciation. Evolution 16:257–271.

Lewis, H., and M. E. Lewis. 1955. The Genus Clarkia. Univ. of California Press, Berkeley, CA.

Littell, R. C., G. A. Milliken, W. W. Stroup, and R. D. Wolfinger. 1996. SAS system for mixed models. SAS Institute, Cary, NC.

Lloyd, D. G. 1992. Self- and cross-fertilization in plants. II. The selection of self-fertilization. Int. J. Plant Sci. 153:370–380.

MacSwain, J. W., P. H. Raven, and R. W. Thorp. 1973. Comparative behavior of bees and Onagraceae. IV. *Clarkia* bees of the western United States. Univ. Calif. Publ. Entomol. 70:1–80.

McMullen, C. K. 1987. Breeding systems of selected Galapagos Islands angiosperms. Am. J. Bot. 74:1694–1705.

Mitchell-Olds, T., and R. G. Shaw. 1987. Regression analysis of natural selection: statistical inferences and biological interpretation. Evolution 41:1149–1161.

Moeller, D. A. 2004. Facilitative interactions among plants via shared pollinators. Ecology 85:3289–3301.

———. 2005. Pollinator community structure and sources of spatial variation in plant-pollinator interactions in *Clarkia xantiana* ssp. *xantiana*. Oecologia 142:28–37.

Moore, D. M., and H. Lewis. 1965. The evolution of self-pollination in *Clarkia xantiana*. Evolution 19:104–114.

Motten, A. F., and J. Antonovics. 1992. Determinants of outcrossing rate in a predominantly self-fertilizing weed, *Datura stramonium* (Solanaceae). Am. J. Bot. 79:419–427.

Müller, H. 1883. The fertilisation of flowers. Macmillan, London, U.K.

Neter, J., M. H. Kutner, C. J. Nachtsheim, and W. Wasserman. 1996. Applied linear statistical models. McGraw-Hill, Burr Ridge, IL.

Ornduff, R. 1969. Reproductive biology in relation to systematics. Taxon 18:121–133.

Pannell, J. R., and S. C. H. Barrett. 1998. Baker's law revisited: reproductive assurance in a metapopulation. Evolution 52: 657–668.

Rick, C. M. 1966. Some plant-animal relations in the Galapagos. Pp. 215–224 *in* R. Bowman, ed. The Galapagos. The Univ. of California Press, Berkeley, CA.

Rick, C. M., J. F. Fobes, and M. Hollé. 1977. Genetic variation in *Lycopersicon pimpinellifolium*: evidence for evolutionary change in mating systems. Plant Syst. Evol. 127:139–170.

Routley, M. B., K. Mavraganis, and C. G. Eckert. 1999. Effect of population size on the mating system in a self-compatible, autogamous plant, *Aquilegia canadensis* (Ranunculaceae). Heredity 82:518–528.

Runions, C. J., and M. A. Geber. 2000. Evolution of the self-pollinating flower in *Clarkia xantiana* (Onagraceae). I. size and development of floral organs. Am. J. Bot. 87:1439–1451.

Schluter, D., and D. Nychka. 1994. Exploring fitness surfaces. Am. Nat. 143:597–616.

Schoen, D. J. 1982. The breeding system of *Gilia achilleifolia*: variation in floral characteristics and outcrossing rate. Evolution 36:352–360.

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.

Sih, A., and M. Baltus. 1987. Patch size, pollinator behavior, and pollinator limitation in catnip. Ecology 68:1679–1690.

Sokal, R. R., and F. J. Rohlf. 1995. Biometry. W. H. Freeman, San Francisco, CA.

Solbrig, O. T., and R. C. Rollins. 1977. The evolution of autogamy in species of the mustard genus *Leavenworthia*. Evolution 31: 265–281.

Stebbins, G. L. 1970. Adaptive radiation of reproductive characteristics in angiosperms. 1. Pollination mechanisms. Annu. Rev. Ecol. Syst. 1:307–326.

———. 1974. Flowering plants: evolution above the species level. Harvard Univ. Press, Cambridge, MA.

Steffan-Dewenter, I., U. Munzenberg, C. Burger, C. Thies, and T. Tscharntke. 2002. Scale-dependent effects of landscape context on three pollinator guilds. Ecology 83:1421–1432.

Sytsma, K. J., J. F. Smith, and L. D. Gottlieb. 1990. Phylogenetics in *Clarkia* (Onagraceae): restriction site mapping of chloroplast DNA. Syst. Bot. 15:280–295.

Uyenoyama, M. K., K. E. Holsinger, and D. M. Waller. 1993. Ecological and genetic factors directing the evolution of self-fertilization. Pp. 327–381 *in* D. Futuyma and J. Antonovics, eds. Oxford surveys in evolutionary biology. Oxford Univ. Press, Oxford, U.K.

Van Treuren, R., R. Bijlsma, N. J. Ouborg, and W. Van Delden. 1993. The effects of population size and plant density on outcrossing rates in locally endangered *Salvia pratensis*. Evolution 47:1094–1104.

Vasek, F. C. 1964. The evolution of *Clarkia unguiculata* derivatives adapted to relatively xeric environments. Evolution 18:26–42.

Wade, M. J., and S. Kalisz. 1990. The causes of natural selection. Evolution 44:1947–1955.

Webb, C. J., and D. Kelly. 1993. The reproductive biology of the New Zealand flora. Trends Ecol. Evol. 8:442–447.

Wyatt, R. 1988. Phylogenetic aspects of the evolution of self-pollination. Pp. 109–131 *in* L. D. Gottlieb and S. K. Jain, eds. Plant evolutionary biology. Chapman and Hall, London.

Corresponding Editor: S. Kalisz