

MATING SYSTEM AND THE EVOLUTION OF QUANTITATIVE TRAITS: AN EXPERIMENTAL STUDY OF *MIMULUS GUTTATUS*

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Abstract.—The mating system of a population profoundly influences its evolution. Inbreeding alters the balance of evolutionary forces that determine the amount of genetic variation within a population. It redistributes that variation among individuals, altering heritabilities and genetic correlations. Inbreeding even changes the basic relationships between these genetic statistics and response to selection. If populations differing only in mating system are exposed to the same selection pressures, will they respond in qualitatively different ways? Here, we address this question by imposing selection on an index of two negatively correlated traits (flower size and development rate) within experimental populations that reproduce entirely by outcrossing, entirely by self-fertilizing, or by a mixture of outcrossing and selfing. Entirely selfing populations responded mainly by evolving larger flowers whereas outcrossing populations also evolved more rapid development. Divergence occurred despite an equivalent selection regime and no direct effect of mating system on fitness. The study provides an experimental demonstration of how the interaction of selection, genetic drift, and mating system can produce dramatic short-term changes in trait means, variances, and covariances.

Key words.—Epistasis, *Mimulus guttatus*, mixed mating, selection, self-fertilization.

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For centuries, biologists have been captivated by the diversity of plant mating systems (Sprengel 1793; Darwin 1876; Barrett 2003). Most studies have focused on the evolution of mating systems and on how the balance of evolutionary advantages and costs determines the tendency for plants to self-fertilize versus outcross (Uyenoyama et al. 1993). An important question that has received less empirical study is how differences in mating system impact the evolution of quantitative traits. In fact, the characters that determine mating system (e.g., floral morphology and developmental timing) are usually quantitative. Through their effects on mating system, these traits subsequently influence inbreeding depression and the distribution of genetic diversity within and between populations (Charlesworth and Charlesworth 1987, 1995; Hamrick and Godt 1996; Charlesworth and Wright 2001).

Inbreeding reduces the frequency of heterozygotes relative to homozygotes at polymorphic loci within a population. It thus alters the “presentation” of genetic variation at the phenotypic level and hence the balance between natural selection and other evolutionary forces (Robertson 1952; Mitchell-Olds and Rutledge 1986; Kelly 1999a). This is most obvious for loci exhibiting genetic dominance, where inbreeding exposes recessive alleles more directly to selection. Deleterious alleles may be more rapidly “purged” and advantageous alleles more rapidly fixed with partial or complete self-fertilization (Caballero and Hill 1992a; Charlesworth 1992; Byers and Waller 1999).

The experiment described here evaluates the most immediate effects of mating system on quantitative trait evolution, that is, short-term response to selection. The simplest genetic model, a single locus with alleles that act additively, predicts that inbreeding should accelerate response to directional selection. The genetic variance contributed by an additive locus is directly proportional to the inbreeding coefficient of the population (Wright 1951; Falconer and Mackay 1996). If this locus is entirely responsible for trait variation, inbreeding

should accelerate response because the segregation of alternative alleles into homozygotes increases the efficiency of selection in changing allele frequencies. With dominance, the genetic variance contributed by a locus will usually increase with inbreeding, but there are cases in which it can decline (Robertson 1952). Also, only a fraction of the genetic variance is available to selection with dominance. This fraction is itself dependent on the mating system, with dominance components becoming increasingly important as the level of inbreeding increases (Harris 1964; Pederson 1969; Jacquard 1974; Cockerham and Matzinger 1985).

When genetic variation is caused by multiple loci, two different kinds of interlocus associations influence response to selection: linkage disequilibrium and identity disequilibrium. Most forms of selection will generate linkage disequilibria among quantitative trait loci (QTL). Recombination effectively diminishes linkage disequilibrium in randomly mating populations (Bulmer 1980; Turelli and Barton 1994). However, with inbreeding, the frequency of doubly heterozygous genotypes is reduced and thus so is the rate that crossing-over generates recombinant gametes. This allows disequilibria to persist and selection can substantially alter the genetic variance, V_G , in the short term even when allele frequency changes are insubstantial (Hayashi and Ukai 1994; Kelly 1999a). The magnitude and direction of the changes in V_G depend on how selection affects the phenotypic variance and how different QTL combine to determine the phenotype, that is, the presence and nature of epistasis. Stabilizing selection, as well as most forms of directional selection, will tend to reduce the phenotypic variance. If QTL combine additively (there is no epistasis), associations among loci will tend to be negative and V_G will be reduced. The magnitude of the reduction depends on the trait heritability, the strength of selection, and the mating system (for estimates under random mating see Bulmer 1980, pp. 155–160; for estimates with different levels of selfing see Kelly 1999a; Kelly and Williamson 2000; Nomura 2005). It is more dif-

ficult to predict changes in V_G with epistatic interactions among QTL. However, a number of authors have argued that inbreeding allows selection to act more directly on gene combinations, facilitating the evolution of co-adapted gene complexes (Clegg et al. 1972; Allard 1975).

A second kind of interlocus association, identity disequilibrium, is likely to be important when individuals vary in the extent to which they are inbred (Haldane 1949; Bennett and Binet 1956). Variation among individuals in their respective inbreeding coefficients is inevitable in a mixed mating population (Wright and Cockerham 1985; Kelly 1999a). For traits that exhibit directional dominance (e.g., inbreeding depression) much of the phenotypic variation present in such a population may be due to differences in inbreeding coefficients rather than in the allelic composition of genotypes, as it would be in a randomly mating population (Willis 1996). Under directional selection, allele frequency evolution is determined primarily by the relationship between phenotype and fitness among individuals with the same inbreeding coefficient (Kelly 1999a). Thus, genetic variation due to identity disequilibrium in a mixed mating population is largely unavailable to selection, analogous to how nonadditive variation is inaccessible in a randomly mating population.

The preceding discussion focuses on the presentation of genetic variation to selection, but mating system also affects genetic drift and the interaction between selection and drift. In the absence of selection, inbreeding will tend to reduce the effective population size (N_e) and thus increase the impact of genetic drift. The magnitude of this effect depends on the amount and kind of inbreeding, as well as the variance in family sizes (Caballero and Hill 1992b and references therein). Selection can further magnify the effect of inbreeding on genetic drift. Caballero and Santiago (1995) show that high selfing rates combined with intense truncation selection can reduce N_e to a tenth of the actual population size. Much of this theory concerns the effect of mating system on the fixation probability of new mutations (e.g., Caballero et al. 1991; Charlesworth 1992; Pollak and Sabran 1992; Caballero and Santiago 1995) and is thus not directly applicable to the results of this study. Here, the immediate response to selection (four generations in duration) is due to the recruitment of standing variation. However, it is reasonable to hypothesize that the same basic processes will affect selection on standing variation and that inbreeding might accelerate the stochastic divergence of replicate populations. We directly evaluate this hypothesis by comparing the variation in response to selection among populations within mating system categories: outcrossing, mixed-mating, and selfing.

Evolution of the Multivariate Phenotype

In this experiment, we impose selection on an index of two genetically correlated traits, flower size and development rate. Each is a quantitative character, and we expect the various factors discussed above to influence the genetic variance in each. However, selection on multiple traits introduces the additional complexity of correlated responses to selection (Lande and Arnold 1983; Kelly 1999b). Like variances, genetic covariances among traits are affected by associations among QTL (linkage and/or identity disequilibria). With mul-

iple traits, inbreeding can alter not only the rate of evolution (whether trait means change more or less rapidly than a comparable randomly mating population), but also the nature of response (which characters evolve and in what direction).

Consider the standard model of multi-trait selection in a randomly mating population (Lande and Arnold 1983): $\Delta\bar{z} = \mathbf{G}\beta$, where $\Delta\bar{z}$ is the vector of predicted changes in the mean of each trait, \mathbf{G} is the additive genetic variance-covariance matrix, and β is the vector of selection gradients on each trait. Inbreeding clearly alters the elements of \mathbf{G} and even the appropriate definition of these elements (Kelly 1999b; see below). However, it can also affect selection parameters in several distinct ways. For example, the combination of mixed mating and inbreeding depression in fitness can generate false selection gradients on traits with no effect on fitness (Willis 1996). Inbred individuals may have lower fitness due to the exposure of deleterious alleles in homozygous form, but also consistently different trait values (for any character that exhibits directional dominance) than outbred individuals. This will yield an apparent association between trait values and fitness, independent of any effect the trait has on fitness. The same basic mechanism can produce genetic correlations between traits even when there is no pleiotropy or linkage disequilibrium (Kelly 1999b).

More generally, the vector of selection gradients is contingent on both the selection regime and the distribution of phenotypic variation: $\beta = \mathbf{P}^{-1}\mathbf{S}$, where \mathbf{P}^{-1} is the inverse of the phenotypic variance/covariance matrix and \mathbf{S} is the vector of selection differentials on each trait (Lande and Arnold 1983). If inbreeding alters the means, variances, and covariances of traits, it will shift the location of the phenotypic distribution relative to fitness surface. Gradients reflect the (linear) relationship between trait values and relative fitness in the vicinity of the current multivariate mean (Phillips and Arnold 1989). Gradients will thus change as means change if the fitness function is nonlinear, as must usually be the case in nature.

We imposed selection on an index combining flower size and rate of development for several reasons. The first is simply that natural selection typically acts on suites of traits rather than individual characters in isolation. Second, the index allows us to address the question of how mating system might alter response to an evolutionary constraint. The trade-offs that organisms confront (e.g., investment in growth vs. reproduction) manifest themselves at the population level as negative genetic correlations among characters (Arnold 1992). Corolla width and rate of development exhibit a negative genetic correlation, at least under the growth conditions used in this experiment (J. K. Kelly, unpubl. data; Appendix). Rapidly developing plants tend to flower when small. Delayed development allows plants to flower after they have accumulated substantially greater above-ground biomass. Larger plants produce larger flowers. It is noteworthy that flower size and development rate also exhibit a negative relationship among populations and species within the *Mimulus guttatus* species complex. Annual populations of *M. guttatus* tend to have more rapidly developing plants with smaller flowers than perennial populations (L. M. Holeski, unpubl. data). Primarily self-fertilizing species within the complex, such as *M. nasutus* and *M. micranthus*, have greatly reduced

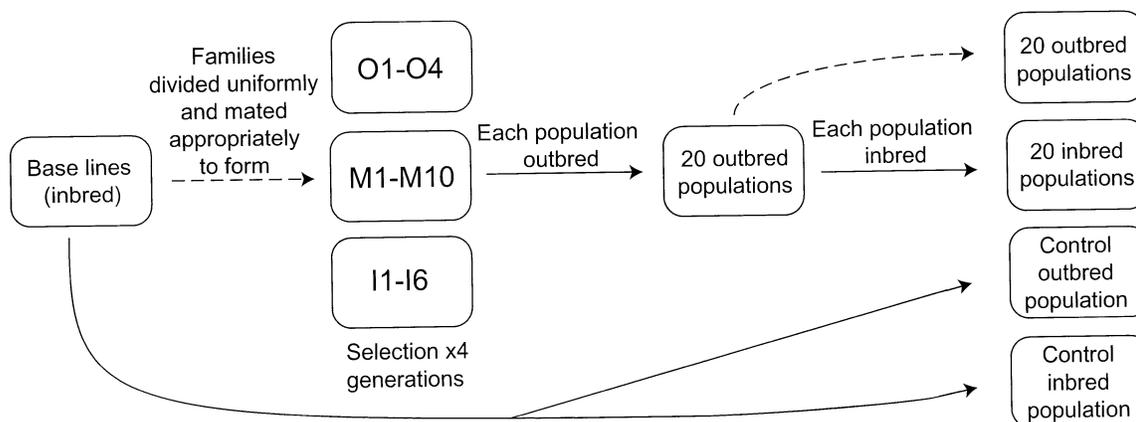


FIG. 1. A schematic of the experimental design: evolutionary change was measured within four fully outcrossing populations (O1–O4), 10 mixed-mating populations (M1–M10), and six fully selfing populations (I1–I6).

flowers and tend to develop more rapidly than *M. guttatus* (Fenster et al. 1995).

Predicting Changes in Mean Phenotypes

Inbreeding can directly change trait means (without selection) by increasing homozygosity within the population. Consider a quantitative character, z , determined by the summed contributions of an arbitrary number of QTL. The mean of z in the population, \bar{z} , has two components:

$$\bar{z} = \mu_O + f(\mu_I - \mu_O), \quad (1)$$

where μ_O is the outbred mean and μ_I is the inbred mean (Wright 1951; Kempthorne 1957; Kelly and Williamson 2000). The statistics μ_O and μ_I are functions of genotypic effects and allele frequencies at QTL, and each will evolve under selection. The relative contribution of each component is determined by f , the average inbreeding coefficient within the population. The value of f can change both in the short term, due to the varying reproductive success of individuals that are inbred to different levels (Wright and Cockerham 1985), and also in the long term, with the evolution of traits that affect the selfing rate. As a consequence, $\Delta\bar{z}$ is a complex mixture of changes in different quantities unless $\mu_O = \mu_I$, that is, the trait exhibits no directional dominance. Changes in μ_O and μ_I cannot be distinguished without controlled matings/self-fertilizations, generating collections of individuals with different but known values for f . We employ this approach here to monitor the evolution of both μ_O and μ_I under different mating systems.

The partitioning of the mean phenotype (eq. 1) can also be used to predict response to multivariate selection with inbreeding. Each trait is associated with a specific value for μ_O and μ_I and the population is described by the two vectors of means (Kelly and Williamson 2000). A distinct matrix of genetic quantities is used to predict changes in the values of μ_O and μ_I for each trait (Kelly 1999b). Like \mathbf{G} of the random mating model, the elements of these matrices can be estimated from comparisons among relatives.

The diversity of potential evolutionary consequences associated with inbreeding is impressive. However, the empirical importance of these myriad factors has yet to be deter-

mined. To evaluate the most immediate effects of mating system, experimental populations of *Mimulus guttatus* were established from a common source and subjected to the same phenotypic selection regime. We imposed artificial selection on replicate populations of three different mating systems: fully outcrossing, mixed mating, and fully selfing (Fig. 1). We documented evolution of six quantities within each experimental population: μ_O and μ_I for each of three traits. These data address two basic questions: Does mating system affect the rate and pattern of quantitative evolution? Does mating system affect the rate that replicate populations diverge?

METHODS

Study Species and Source Population

Mimulus guttatus ($2n = 28$; Phrymaceae) is a self-compatible wildflower that occupies a variety of moist, open habitats throughout western North America. The species is mixed mating, the estimated selfing rate varies from 0 to 0.75 among populations, and it may be annual, winter annual, or perennial (Ritland and Ganders 1987; Willis 1993; Awadalla and Ritland 1997; Sweigart et al. 1999). Previous genetic studies have demonstrated substantial genetic variation in floral traits (Carr and Fenster 1994; Robertson et al. 1994) and inbreeding depression in fitness components (Dole and Ritland 1993; Willis 1993; Latta and Ritland 1994; Willis 1999a,b; Carr and Dudash 1996; Dudash and Carr 1998). There have also been a number of studies examining the genetic basis of differences in floral morphology and mating system between *M. guttatus* and closely related species (Vickery 1978; Fenster and Ritland 1994; Fishman et al. 2002).

Experimental populations were initiated from the same source population, a large collection of randomly extracted, highly inbred lines. J. H. Willis initiated approximately 1200 independent lines from Iron Mountain in August 1995. The Iron Mountain population is located in Oregon's western Cascades and contains several hundred thousand individuals continuously distributed over an area of approximately 400 m² (Willis 1996, 1999a,b). Each line was founded from the seed set of a separate field-collected plant and subsequently main-

tained by single-seed descent (self-fertilization) for seven to nine generations. The lines have an inbreeding coefficient of greater than 0.99 ($f \cong 1$) and, as expected, are almost completely homozygous at highly polymorphic microsatellite loci with different lines fixed for different alleles (Willis 1999a; L. M. Holeski, unpubl. data).

We established a total of 20 experimental populations, six within the fully selfing category (I1–I6), 10 within the mixed-mating category (M1–M10), and four within the fully outcrossing category (O1–O4). The 20 populations were established by sampling progeny from a breeding design (based on the inbred lines) in a way that maximized the genetic variance within populations and minimized allele frequency differences among populations. From our collection, 194 inbred lines were randomly selected, self-fertilized, and then randomly paired and crossed to produce F_1 individuals. The F_1 plants were then selfed to produce F_2 families and backcrossed to each parental line (for a detailed description of the breeding design, see Kelly 2005). Selfed progeny of each line were sampled to initiate populations I1–I6 with each line contributing at most one plant to each population. The fully outcrossing populations (O1–O4) were founded by F_1 plants with each line pair contributing at most two plants to each population. The mixed-mating populations were founded by F_2 and backcross individuals (both of which have $f = 0.5$ relative to the ancestral population), again maximally dispersing lines across populations.

Populations were founded by different line-cross types (e.g., F_1 , F_2 , backcross) to replicate the distribution of genetic variation generated by their respective mating systems. In a randomly mating population, we expect alleles to be distributed into genotypes according to Hardy-Weinberg proportions. These proportions obtain within the population of F_1 families of the breeding design. With complete selfing, variation should be segregated into fully homozygous inbred lines, and our fully selfing populations were founded from such lines. In a mixed-mating species with a constant selfing rate S , the mean inbreeding coefficient (f) is $S/(2 - S)$ (Hartl and Clark 1989, p. 262). In contrast to the other categories, plants within the mixed-mating populations will vary in the extent to which they are inbred. The initial mean inbreeding coefficient of plants initiating our mixed mating populations is slightly greater than the predicted equilibrium ($f = 0.5$ instead of 0.33), but variation in individual inbreeding coefficients is established after the first generation of selection and reproduction. Although the differing initial f -values of populations within different mating system types imply differences in genotype frequencies, the expected allele frequencies do not differ among populations. Of course, there was stochastic variation due to sampling, but such differences would not be associated with mating system category. The implication is that the expected values for μ_O and μ_I (which are functions of allele frequencies and genotypic effects) of each trait are initially the same in each population.

Each population consisted of approximately 120 individuals prior to selection, all of which were measured for number of days to first flower (from day of seeding) and corolla width. These measurements were then used to calculate I , the trait index:

$$I = (\text{corolla width}/\sigma_{\text{corolla width}}) - (\text{number of days to first flower}/\sigma_{\text{day}}), \quad (2)$$

where the σ terms are the estimated trait standard deviations within the original base population: $\sigma_{\text{corolla width}} = 2.34$ mm and $\sigma_{\text{day}} = 3.05$ days. We imposed truncation selection on I values (the top 25% selected to reproduce) within each population subsequent to the completion of flowering. Several alternates, plants just below the cut-line, were also preserved from each population and included only if plants in the top 30 failed to produce seed.

Within the outcrossing and mixed-mating populations, selected plants were randomly paired and crossed via hand pollination. The cross was unidirectional, with pollen transferred from the randomly assigned sire to dam. In the mixed-mating populations, each plant (both sires and dams) was also self-fertilized by hand, as were all selected plants in the fully selfing populations. In the mixed-mating populations, the progeny generation was founded by an equal number of outcrossed and selfed offspring, thus yielding a realized selfing rate of 0.5. Each generation, seed from the selected parents of the previous generation were seeded into two-inch pots in the University of Kansas (Lawrence, KS) greenhouse (18-h day lengths). Eleven days after seeding, the appropriate numbers of individuals from each family were randomly selected from each two-inch pot, transferred to 98-well trays, and placed in a growth room (18-h day length). Seedling mortality was generally low.

Selection was imposed on four successive generations, indexed as generations 0, 1, 2, and 3. The selected plants of generation 3 were randomly paired (within all 20 populations) and crossed. The resulting progeny, 20 outbred populations, were grown to maturity without selection in generation 4. Within each population, plants were randomly paired and crossed, and each was also self-fertilized. This produced a collection of outbred and inbred progeny for each experimental population. The latter were all inbred to the same extent ($f = 0.5$ for selfed progeny of outbred plants) and are thus comparable across populations. Selfed and outbred progeny were grown to maturity and measured in generation 5 and these measurements were used to estimate μ_O and μ_I of each experimental population.

To produce sufficient sample sizes for accurate estimates, generation 5 consisted of two successive grow-ups from the same seed sets, each involving about 2150 individuals. The first grow-up (G-1) consisted of 617 individuals from the inbreeding populations, 1100 individuals from the mixed-mating populations, and 463 individuals from the outcrossing populations. The second grow-up (G-2) consisted of 628 individuals from the inbreeding populations, and 1043 and 446 individuals from the mixed-mating and outcrossing populations, respectively. A total of 941 plants from the base population, 436 F_1 individuals and 505 F_2 individuals, were grown simultaneously with plants from the experimental populations in G-1 and G-2 of generation 5. The mean of F_1 plants estimates the original value for μ_O , and the mean of F_2 plants estimates μ_I .

Statistical Analyses

We separately analyzed data from outbred and inbred plants of each population because they estimate responses of distinct genetic quantities (μ_O and μ_I ; eq. 1). The general linear model (GLM) was fit to each response variable (index, corolla width, and days to flower) using maximum likelihood. Let Y_{ijkm} denote the m th measurement within population k of mating system j in grow-up i :

$$Y_{ijkl} = \mu + \delta_i + \alpha_j + P_{jk} + \varepsilon_{ijkm}, \quad (3)$$

where μ is the grand mean, δ_i is the (fixed) effect of grow-up ($i = 1, 2$), α_j is the (fixed) effect of mating system ($j = S, M, O$), P_{jk} is the (random) effect of population (nested within mating system), and ε_{ijkm} is a normally distributed error. This is a mixed model, and the log-likelihood, l , is

$$l = C - \frac{1}{2} \ln |\mathbf{V}| - \frac{1}{2} (\mathbf{y} - \mathbf{X}\boldsymbol{\eta})^T \mathbf{V}^{-1} (\mathbf{y} - \mathbf{X}\boldsymbol{\eta}), \quad (4)$$

where \mathbf{V} is the variance-covariance matrix of individual measurements, $|\mathbf{V}|$ is the determinant of \mathbf{V} , \mathbf{y} is the vector of values for the response variable, \mathbf{X} is an incidence matrix for fixed effects, $\boldsymbol{\eta}$ is the vector of fixed effects, and C is a constant determined by the total sample size (Searle et al. 1992, p. 234). We ignore C in subsequent calculations given that it has no effect on the difference between log-likelihoods of comparable models.

Our primary aims are to determine whether the average responses differ among mating systems (can we reject $\alpha_j = 0$ for all j ?) and whether the divergence among populations differs among mating systems (is the variance of P_{jk} heterogeneous across mating systems?). We address these hypotheses by comparing the full model to a set of restricted models. The full model has eight estimated parameters, four fixed effects (the means associated with each mating system plus the effect of grow-up), and four variance parameters for random effects (the error variance, σ^2 , and interpopulation variances $\sigma_{P,S}^2$, $\sigma_{P,M}^2$, and $\sigma_{P,O}^2$). Reduced model 1 stipulates that mating system has no effect on the mean phenotypes of populations ($\alpha_j = 0$ for all j) and thus has six parameters: two fixed effects (the grand mean and the effect of grow-up) and four variance components. Reduced model 2 stipulates that mating system has no effect on the stochastic divergence of populations. It also has six parameters: the four fixed effects and two variance components (σ^2 and σ_P^2). Reduced model 3 stipulates that mating system has no effect on either means or interpopulation variances, and thus has only four parameters (two fixed effects and two variance components).

We compare models using two standard approaches, the Akaike information criterion (AIC) and likelihood ratio tests. The AIC value for a model is

$$\text{AIC} = -2l + 2K, \quad (5)$$

where K is the number of parameters (Burnham and Anderson 2002, p. 61). The selected model has the lowest AIC. The likelihood-ratio statistic is equal to twice the difference between the log-likelihood of the alternative model and the log-likelihood of the null model. Here, the null model is derived from the alternative model by eliminating a particular effect. The effect of mating system on the means of populations can

be evaluated with two different model comparisons: the full model versus reduced model 1, or reduced model 2 versus reduced model 3. Likewise, the other null hypothesis, that mating system has no effect on the interpopulation variance, can be evaluated with either of two different contrasts: the full model versus reduced model 2, or reduced model 1 versus reduced model 3. For each trait and type of plant (outbred and inbred), we calculate both likelihood-ratio test statistics for each null hypothesis. We reject the null if the statistic is greater than 5.99 (the critical value from the chi-squared distribution with two df) because the alternative and null models differ by two parameters in each case.

We analyzed the measurements from generation 5 using maximum likelihood instead of analysis of variance (ANOVA) for two reasons. First, the standard nested ANOVA assumes that the variance among subgroups (populations) within groups (mating systems) is homogeneous. It thus does not allow us to address one of our primary questions. The ANOVA test for the effect of group (mating system) is essentially a contrast between reduced models 2 and 3 described above. Second, even with homogeneous interpopulation variance, maximum likelihood is a preferable approach for the analysis of mixed models when the design is unbalanced (Searle et al. 1992, p. 254). The present design is unbalanced at both levels: the number of populations varies among mating system categories, as does the number of measurements per population.

We used ANOVA to test for differences in selection parameters and in the realized heritability of the index among mating systems. For each of the 20 experimental populations in each of the four generations of selection, we calculated the selection differential from the difference in mean phenotype between selected individuals and the entire population. Using estimates for the phenotypic variances and covariances within each population, we subsequently calculated the selection gradients on corolla width and days to flower according to the formula $\beta = \mathbf{P}^{-1}\mathbf{S}$. (The selection gradient for I is \mathbf{S} divided by the phenotypic variance in I .) Cumulative selection differentials and gradients for each trait were then obtained by summing across generations within each population. Two realized heritability estimates were obtained from each population by dividing the cumulative responses, $\Delta\mu_O$ and $\Delta\mu_I$, for the trait index by the cumulative selection differential. In each of these analyses, all measurements from a population distill into a single value per analysis, for example, the cumulative selection gradient on corolla width.

RESULTS

Figure 2 illustrates the change in the mean index value of populations in each mating system category over the first three generations of selection. The differences in generation 0 (prior to any selection) reflect the direct effect of inbreeding on trait mean values. Although there is clearly a response to selection, this response involves a mixture of changes in μ_O , μ_I , and f (although f is only changing in the mixed-mating populations). The controlled crosses and self-fertilizations of adult plants in generations 3 and 4 serve to disentangle these changes. The direct evaluation of mating system effects on

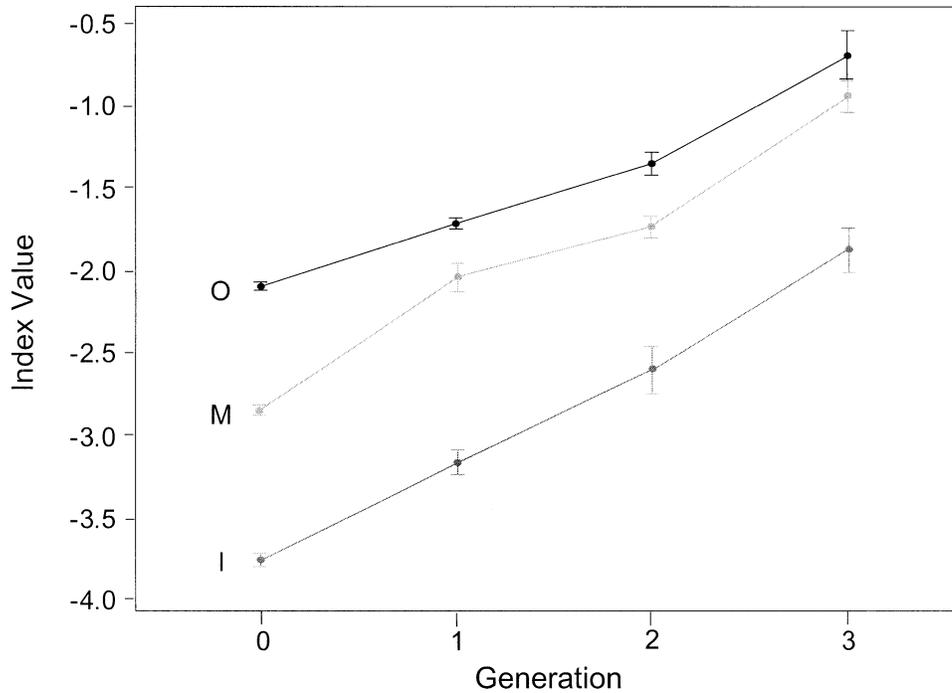


FIG. 2. The mean index values (I) of populations within each mating system category are given for generations 0, 1, 2, and 3 (error bars denote ± 1 SE).

the evolution of μ_O and μ_I is given in Table 1, the parameter estimates from the full model in Table 2.

The full model produces the lowest AIC value in five of six cases, the exception being μ_O for the trait index where reduced model 1 is selected. In all cases, the selected model allows the variance among replicate populations to differ between mating system categories. Inbreeding increases variability, $\sigma_{P,S}^2 > \sigma_{P,M}^2 > \sigma_{P,O}^2$ (Table 2). Mating system affected the mean values of μ_O for corolla width and days to flower, but not for the trait index. Mating system means differed for all three response variables of μ_I (Table 1, lower panel). Likelihood-ratio tests are consistent with AIC selections. Fo-

cusings first on the effect of mating system on interpopulation variation in μ_O , the likelihood-ratio statistic for all six of the possible comparisons is significant (full model vs. reduced model 2, and reduced model 1 vs. reduced model 3 for each trait). The corresponding tests for inbred plants are significant for days and the index, but not for corolla width. Likelihood-ratio tests of mating system effects on the means are more variable in outcome. For outbred plants, three of six comparisons are significant: reduced model 2 versus reduced model 3 for both corolla width and days, and full model versus reduced model 1 for days. For inbred plants, three of six are also significant: reduced model 2 versus reduced mod-

TABLE 1. The log-likelihood and Akaike information criterion (AIC) values for each model, predicting variation in corolla width, days to flower and the trait index. The upper panel gives model fits for outbred plants grown in generation 5 ($\Delta\mu_O$); the lower panel, for inbred plants ($\Delta\mu_I$). Bold type indicates the lowest AIC value, that is, the selected model.

	Corolla width		Days to flower		Index	
	Log-likelihood	AIC	Log-likelihood	AIC	Log-likelihood	AIC
Outbred plants: $\Delta\mu_O$						
Full model	-1003.56	2015.12	-1004.79	2017.58	-1038.00	2084.00
Reduced 1: MS has no effect on mean divergence	-1006.14	2018.28	-1011.61	2029.22	-1038.88	2083.76
Reduced 2: MS has no effect on variance in divergence	-1007.60	2021.20	-1010.78	2027.56	-1042.51	2091.02
Reduced 3: MS has no effect on mean or variance in divergence	-1010.68	2025.36	-1016.12	2036.24	-1042.79	2089.58
Inbred plants: $\Delta\mu_I$						
Full model	-951.93	1911.86	-969.43	1946.86	-1002.20	2012.40
Reduced 1: MS has no effect on mean divergence	-954.17	1914.34	-975.96	1957.92	-1004.14	2014.28
Reduced 2: MS has no effect on variance in divergence	-954.15	1914.30	-970.60	1947.20	-1005.31	2016.62
Reduced 3: MS has no effect on mean or variance in divergence	-956.51	1917.02	-983.36	1970.72	-1008.46	2020.92

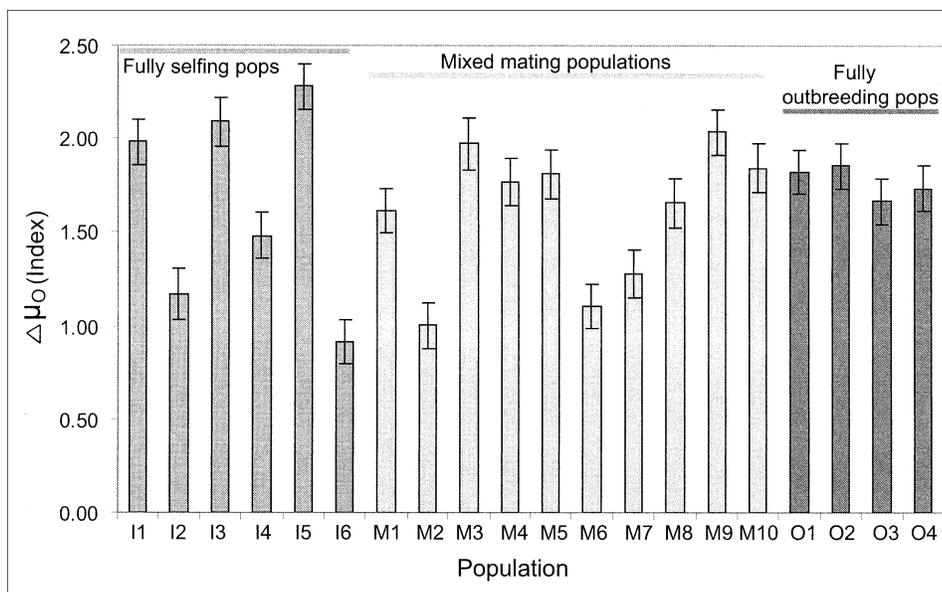


FIG. 3. The estimated $\Delta\mu_O$ for the index value (I) of each population (pops; error bars denote ± 1 SE using the pooled within population variance).

el 3 for both the trait index and days, and full model versus reduced model 1 for days.

Variation among populations in $\Delta\mu_O$ (evolution of the outbred mean) for the trait index is illustrated in Figure 3. The estimated $\Delta\mu_O$ within each experimental population was obtained by subtracting the mean of F_1 (control) plants from the mean of outbred plants (for each response variable). Estimates for $\Delta\mu_I$ (evolution of the inbred mean) were obtained

by calculating the mean phenotype of the inbred progeny within each experimental population and then subtracting the mean of F_2 control plants. The F_2 plants of the base population, with $f = 0.5$, are inbred to the same extent as the selfed progeny of each experimental population. One technical note: the μ_I estimates from generation 5 of our experiment are not equivalent to the variable introduced in equation (1), because $f = 0.5$ for generation 5 plants. However, this

TABLE 2. Parameter estimates from the full model with standard errors extracted from the asymptotic dispersion matrix (Searle et al. 1992). Estimates for fixed effects are given as the following functions of the terms in equation 3: effect of grow-up = $\delta_2 - \delta_1$, mean of selfing populations = $\mu + \alpha_S$, mean of mixed mating populations = $\mu + \alpha_M$, mean of outcrossing populations = $\mu + \alpha_O$.

Outbred plants						
Fixed effects	Corolla width (mm)		Days to flower		Index	
	Estimate	SE	Estimate	SE	Estimate	SE
Grow-up	0.70	0.11	0.53	0.12	0.12	0.06
Selfing mean	18.64	0.41	25.43	0.48	-0.36	0.21
Mixed mating mean	17.82	0.20	24.49	0.17	-0.40	0.11
Outcrossing mean	17.59	0.13	23.68	0.15	-0.24	0.07
Variance components						
σ^2	6.64	0.14	8.18	0.18	1.74	0.04
$\sigma_{P,S}^2$	0.91	0.40	1.30	0.56	0.24	0.10
$\sigma_{P,M}^2$	0.32	0.12	0.17	0.08	0.10	0.04
$\sigma_{P,O}^2$	0.00	0.03	0.00	0.04	0.00	0.01
Inbred plants						
Fixed effects	Corolla width (mm)		Days to flower		Index	
	Estimate	SE	Estimate	SE	Estimate	SE
Grow-up	1.29	0.13	0.31	0.16	0.46	0.07
Selfing mean	17.26	0.40	27.47	0.33	-1.63	0.16
Mixed mating mean	16.62	0.20	25.44	0.19	-1.24	0.09
Outcrossing mean	16.29	0.19	25.24	0.20	-1.31	0.08
Variance components						
σ^2	8.52	0.19	12.54	0.28	2.35	0.05
$\sigma_{P,S}^2$	0.82	0.37	0.49	0.25	0.12	0.06
$\sigma_{P,M}^2$	0.28	0.12	0.18	0.09	0.05	0.02
$\sigma_{P,O}^2$	0.05	0.06	0.03	0.07	0.00	0.01

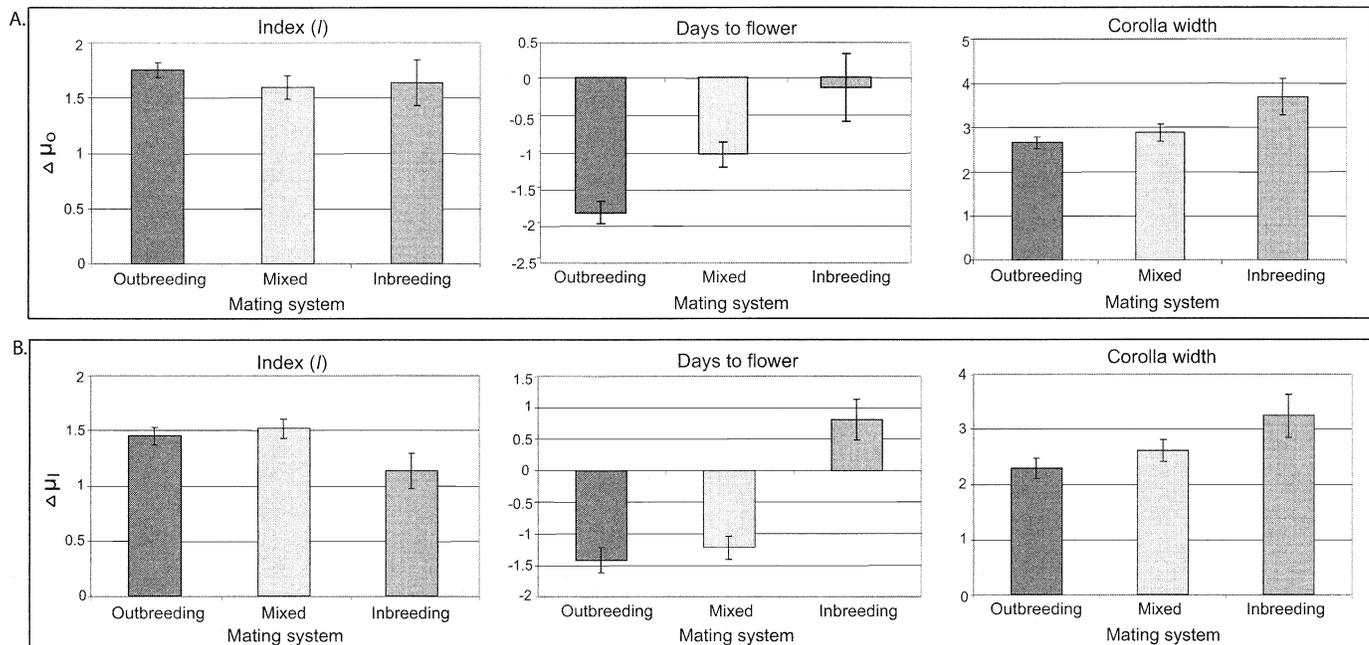


FIG. 4. Mean responses to selection within each mating system category (error bars denote ± 1 SE) for the trait index, days to first flower, and corolla width (in mm). (A) Changes in outbred mean values ($\Delta\mu_O$). (B) Changes in inbred mean values ($\Delta\mu_I$).

is sufficient to predict the mean of fully inbred plants ($f = 1$) when the relationship between trait means and f is linear (which is assumed in the derivation of eq. 1). Given linearity, one can predict the mean of fully inbred plants (μ_I of eq. 1) simply by doubling the difference between our reported estimates of μ_I and μ_O . A previous experiment demonstrated that both flower size and days to flower change in an approximately linear way with f (Kelly 2005).

The nature of mating system effects on rates of evolution, $\Delta\mu_O$ and $\Delta\mu_I$ (averaging across populations within each mating system category), is demonstrated in Figure 4. For the trait index, $\Delta\mu_O$ did not differ significantly among mating systems, but inbreeding and outcrossing populations achieved high index values in different ways. The fully inbreeding populations responded mainly by evolving larger flowers, whereas outcrossing populations evolved both traits. Development time was substantially reduced in outcrossing populations. Mixed-mating populations were intermediate.

The response of the inbred mean, $\Delta\mu_I$, for the trait index was greatest for mixed-mating populations and least for fully selfing populations (Fig. 4B). As with the outbred means, the fully selfing populations yielded the greatest response in $\Delta\mu_I$ for corolla width. Fully outcrossing populations evolved the least for corolla width and mixed mating populations were intermediate. The opposite trend obtained for $\Delta\mu_I$ of days to flower. In fact, the inbreeding populations evolved opposite the direction of selection for this character; that is, populations I1–I6 exhibit significantly delayed flowering (on average) relative to the controls.

The pattern of selection, as measured by cumulative selection gradients and differentials, differed significantly among mating system categories (Table 3, Fig. 5). The cumulative selection differential on I was significantly lower in the fully outcrossing populations than in the mixed-mating

or fully selfing categories. The opposite trend was obtained for the cumulative selection gradient. This reversal reflects the fact that the variance in I within populations was substantially elevated by inbreeding. For days to flower, cumulative differentials did not differ among categories, but the cumulative gradients for fully outcrossing populations were significantly greater than for mixed-mating or fully selfing populations. For corolla width, the magnitude of differentials increases with inbreeding, whereas gradients decline. The realized heritability of the trait index differed significantly among mating systems when evaluated using inbred plants but not outbred plants (Table 3). The estimated mean heritabilities derived from $\Delta\mu_O$ are 0.27, 0.26, and 0.33 for fully selfing, mixed-mating, and outcrossing populations, respectively. The comparable values from $\Delta\mu_I$ are 0.18, 0.25, and 0.27, respectively.

DISCUSSION

This experiment demonstrates that populations experiencing the same selection regime may diverge in their responses due only to differences in mating system. Phenotypic divergence is notable given that our experimental design excluded many of the hypothesized influences of mating system. Our populations were founded from the same source and each had the same initial allele frequencies (approximately). The important effect of mating system on the amount of genetic variation was thus excluded (Stebbins 1957; Lande and Schemske 1985; Charlesworth 2003). The experiment was short in duration, and as a consequence, standing variation determined response. Mating system would likely impact the dynamics of new mutations contributing to long-term response (Caballero and Hill 1992a; Charlesworth 1992; Caballero and Santiago 1995). Finally, we experimentally con-

TABLE 3. Analysis of variance results with selection parameters (cumulative gradients, β , or differentials, S) or realized heritabilities as the response variables and mating system as the factor.

		Source	df	SS	MS	<i>F</i>	<i>P</i>
Section parameters:							
β	corolla width	mating system	2	2.180	1.090	30.53	0.000
		error	17	0.607	0.036		
	day	mating system	2	3.695	1.847	13.37	0.000
		error	17	2.349	0.138		
	index	mating system	2	6.694	3.347	15.12	0.000
		error	17	3.763	0.221		
S	corolla width	mating system	2	0.698	0.349	2.14	0.15
		error	17	2.777	0.163		
	day	mating system	2	0.210	0.105	3.57	0.05
		error	17	0.501	0.029		
	index	mating system	2	0.616	0.308	10.89	0.001
		error	17	0.481	0.028		
Realized heritability for index:							
$\Delta\mu_0$	mating system	2	0.01412	0.00709	1.98	0.17	
	error	17	0.06090	0.00358			
$\Delta\mu_1$	mating system	2	0.02385	0.01193	5.73	0.01	
	error	17	0.03539	0.00208			

trolled the fitness of both inbred and outbred plants based on their phenotypic values. In nature, however, inbreeding often directly affects fitness via inbreeding depression, complicating the relationships between genotype, phenotype, and fitness (Willis 1996).

The experiment identified mating system effects on both the mean and variance of evolutionary responses to selection. Average responses in both outbred and inbred means, that is, the means for $\Delta\mu_0$ and $\Delta\mu_1$ across replicate populations, differed in both magnitude and nature among mating system categories (Fig. 4). In interpreting these differences, it is useful to distinguish the effects of inbreeding on the initial presentation of genetic variation (evident in generation 0)

and the subsequent interaction of selection and inbreeding (accruing over generations 0–4). As described below, two sets of genetic estimates suggest that the immediate effect of inbreeding is to inflate genetic variances. The initial V_G for I and its component traits was thus greater in the fully selfing populations (I1–I6) than in the outcrossing populations (O1–O4). It is therefore noteworthy that inbreeding did not generally accelerate response to selection. For the trait index (I), there was no difference among mating systems in mean $\Delta\mu_0$, whereas $\Delta\mu_1$ was substantially lower in I1–I6 than in O1–O4 (Fig. 3).

The effect of inbreeding on the genetic variance of a quantitative character can be predicted using genetic variance

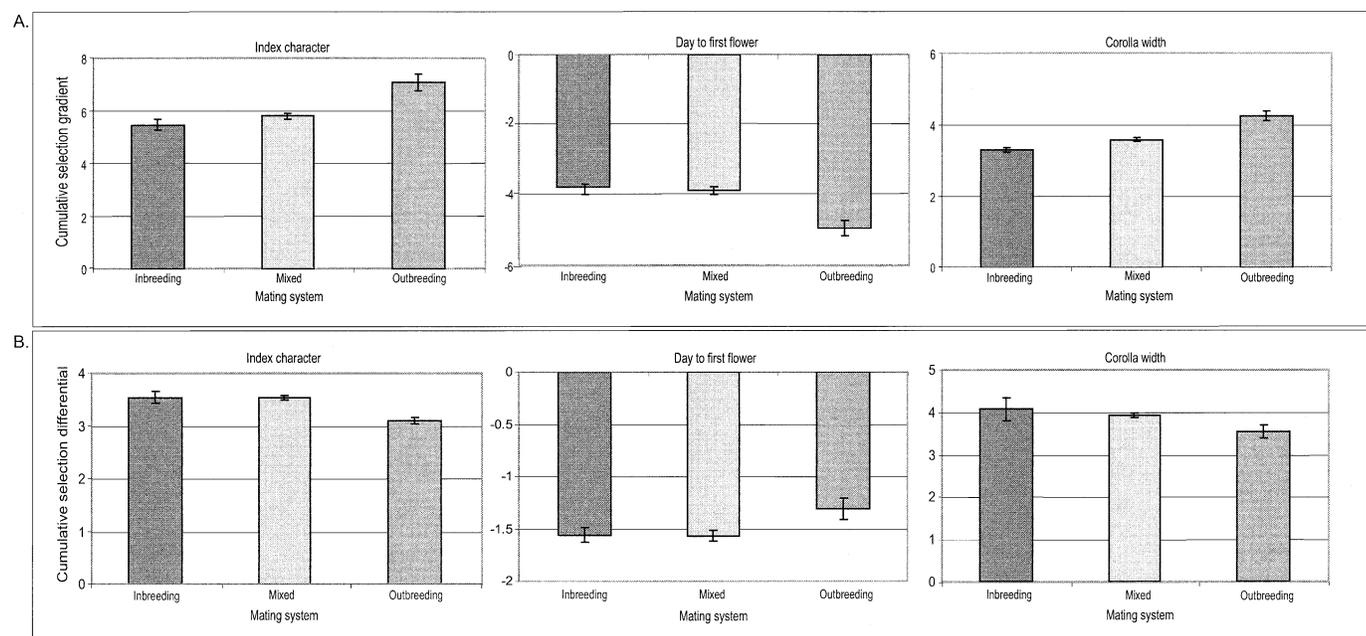


FIG. 5. The average cumulative selection gradients (A) and differentials (B) are given for each trait for each mating system category (error bars denote ± 1 SE).

components (Weir and Cockerham 1977; Cockerham 1983; Cockerham and Weir 1984; Shaw et al. 1998). Previously, Kelly and Arathi (2003) used a breeding design with both inbred and outbred plants from the Iron Mountain population to estimate the relevant components for flower size and the rate of development. These estimates predict that the genetic variance of corolla width should double as f increases from zero to one, whereas the variance in days to flower should change little (see table 2 of Kelly and Arathi 2003). Importantly, values for both traits were transformed in that study (using square root for corolla width and the logarithm for days), whereas selection was imposed on an index of untransformed values in the present experiment. In addition, the plants measured by Kelly and Arathi (2003) were grown in a greenhouse (with a mixture of natural and artificial light), while those of this study matured in a growth chamber. The relevant characters, particularly development rate, are sensitive to growth conditions.

For the growth conditions of this study, we can estimate V_G for both outbred and fully inbred genotypes from measurements of plants in generation 0. This generation consisted of family groups. Each inbred line, as well as the F_1 families derived from crosses between lines, represents a set of genetically identical individuals (or at least nearly identical, see Kelly 2005). We can thus partition variation within and among families to estimate genetic and environmental variances (procedure described in the Appendix). For corolla width, the estimated outbred V_G is 2.54. The variance among fully inbred lines is 3.11, a 22% increase. For days to flower, the estimated V_G is 1.83 among outbred genotypes and 4.44 among fully inbred lines (a 143% increase). For the trait index, the estimated V_G among inbred genotypes is slightly less than twice the outbred V_G (0.95 vs. 0.53). These estimates are associated with sizable standard errors, and, unlike those of Kelly and Arathi (2003), they are potentially contaminated by maternal effects. However, taken in aggregate, these studies suggest that the immediate effect of inbreeding is to inflate the genetic variance in both I and its component traits.

Given the initial effect of inbreeding, the observed $\Delta\mu_O$ and $\Delta\mu_I$ (Fig. 3) suggests that the interaction between selection and inbreeding over the course of the experiment (generations 0–4) must have substantially retarded responses. The realized heritability of I did not differ among mating systems (for $\Delta\mu_O$) or was significantly lower in the fully selfing populations (for $\Delta\mu_I$, Table 3). This result is fully consistent with theoretical predictions (see introduction). Linkage disequilibrium among QTL is one manifestation of the interaction between inbreeding and selection. Truncation selection reduces the phenotypic variance and generates negative associations among alleles (Bulmer 1980). This reduces V_G , an effect that is greatly amplified with inbreeding due to the reduced efficacy of recombination. Reduced V_G limits response to selection.

The most surprising result concerns $\Delta\mu_I$: the inbred mean for I evolved least in the fully selfing populations. Days to flower actually evolved in the opposite direction of selection (Fig. 4B), that is, the selfed progeny from experimental populations I1–I6 developed more slowly (on average) than selfed progeny from the original control population. This result is counterintuitive because μ_I is determined by the

effects of alleles when in homozygous form (Wright 1951). Selection acted most directly on the homozygous effects of alleles in the fully selfing populations and least directly on these effects in the fully outcrossing populations.

This paradoxical result may represent a case of “genetic slippage,” wherein selection gains are reduced ($\Delta\mu_I$ is lowered in this case) because recombination eliminates favorable gene combinations produced by selection (Lynch and Deng 1994; Pfrender and Lynch 2000). Selected adults within populations I1–I6 were randomly paired and mated at the end of generation 3. These outbred progeny (generation 4) were then both outcrossed and selfed to produce the plants used to estimate $\Delta\mu_O$ and $\Delta\mu_I$ (Fig. 1). Because there was no selection, these two meiotic episodes should not have altered allele frequencies in a deterministic way within populations I1–I6. Thus, the same single-locus genotypes present at the end of generation 3 are reproduced in the inbred plants of generation 5. However, the multilocus genotypic combinations are not. Recombination in these two generations might have eliminated, or at least reduced the frequency of, favorable gene combinations established by selection within populations I1–I6. Inbreeding reduces the effect of recombination and facilitates selection on gene combinations if there is epistasis (Allard 1975). Despite the fact that corolla width decreases linearly with f whereas days to flower increases linearly with f (consistent with eq. 1), both traits exhibit considerable epistasis (Kelly 2005). Experiments are currently underway to determine whether this epistasis is of the proper nature to yield genetic slippage.

The second prominent effect of mating system was how selection affected the evolution of the component characters, flower size and rate of development. The regime favored large-flowered plants that develop rapidly. Fully selfing populations responded almost entirely by evolving larger flowers, whereas fully outcrossing populations also evolved more rapid development. The mixed-mating populations evolved intermediate values for both flower size and development rate. We would like to interpret this differential response in terms of mating system effects on genetic parameters, that is, the elements of \mathbf{G} or its generalization (Kelly 1999b), and/or on selection parameters, for example, β . Mating system did alter selection differentials and gradients (Fig. 5), but these effects do not explain the differential responses of μ_O and μ_I . The cumulative selection differentials for corolla width, days to flower, and the character index were smaller in magnitude for the outcrossing populations than in either mixed or fully selfing populations. In contrast, the cumulative selection gradients of outcrossing populations were consistently the largest in magnitude. These differences are due mainly to the inflation of the phenotypic variance (in the index and each component character) caused by inbreeding. Both the genetic and environmental variances of quantitative traits can change with inbreeding (Lerner 1954; Wright 1977; Whitlock and Fowler 1999; Kelly and Arathi 2003).

Can inbreeding-induced changes in the genetic variances of, and covariance between, corolla width and development rate explain their differential responses to selection? As described above, the immediate effects of inbreeding on genetic variances do not obviously explain the result. Inbreeding does not simultaneously inflate the variance in corolla width (ac-

celerating response) and reduce the variance in days to flower (retarding response). Inbreeding could have altered genetic correlations among traits (e.g., Phillips et al. 2001) and the extent to which each component trait determines the index. The immediate effect of inbreeding is to increase the magnitudes of genetic covariances between both component traits and the index (see Appendix). However, just as with variances, the interaction between selection and inbreeding can dramatically alter genetic covariances (Kelly 1999b). This interaction is likely responsible for the differential response.

The third major mating system effect was that inbreeding consistently increased the variance among replicate populations (Fig. 3; Table 2). This observation is also consistent with theoretical predictions (see Introduction). Drift is an important factor in most selection experiments (Robertson 1961) and its effect is magnified by inbreeding of selected adults (Caballero and Santiago 1995). We expect drift to limit long term response, but it is unlikely that the inbreeding effect on drift can explain the differences in mean responses of mating system categories (Fig. 4). First, the experiment was short in duration (four generations) with response based on standing variation. As a consequence, the pronounced effect of drift on new mutations is not relevant (see Caballero and Hill 1992a; Merchante et al. 1995). Second, the responses of inbreeding populations were not uniformly lower than those of fully outcrossing populations. Fully selfing populations produced the most evolutionary change in corolla width (both $\Delta\mu_0$ and $\Delta\mu_1$), whereas mixed-mating populations exhibited the highest average $\Delta\mu_1$ for the trait index (Fig. 4).

Implications

The experiment demonstrates short-term changes in trait means, variances, and covariances that result from the interaction of selection, genetic drift, and mating system. These results bear, at least indirectly, on broader examinations of evolutionary pattern. Genetic variances and covariances provide a critical linkage between microevolutionary processes and macroevolutionary patterns (Arnold 1992). Lande (1979) developed an explicit quantitative method for relating genetic variances and covariances within species to differences in mean trait values among species. This approach assumes constancy of the relevant genetic parameters, an assumption that has received substantial empirical scrutiny (Steppan et al. 2002; Phelan et al. 2003; Begin and Roff 2004; Manuel Cano et al. 2004). Our results demonstrate that genetic variances and covariances are likely to change rather dramatically, over short time spans, if the mating system of a population changes simultaneously with selection.

Consider the evolutionary transition from outcrossing to self-fertilization, one of most common in the history of Angiosperms (Stebbins 1950, 1974; Barrett 2002). Selfing species are typified by numerous physiological and morphological characteristics including accelerated floral development, smaller corollas, decreased stigma and anther separation, and changes in timing of anther dehiscence and stigma receptivity (Wyatt 1983; Karron et al. 1997; van Kleunen and Ritland 2004). These characteristics may evolve prior to, simultaneous with, or following the transition to a self-fertilizing mating system. Given that many of these characters will ex-

hibit genetic correlations, determining the actual targets of selection at different stages in the progression becomes difficult. Disentangling cause and consequence requires a detailed consideration of the interaction between mating system and natural selection in determining phenotypic evolution.

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APPENDIX

The plants grown in generation 0 were part of a larger experiment investigating epistasis in *Mimulus guttatus* (Kelly 2005). Plants from the same families (F_1 and inbred line) were measured in two additional grow-ups, one before and one after generation 0. Here, we combine these data to estimate variances within and among families. Inbred lines and F_1 families are considered in separate analyses to estimate the variance among fully inbred and outbred genotypes, respectively. Across all three grow-ups, there were 1156 plants within F_1 families and 1841 from inbred lines. A mixed model was fit to data from each response variable with grow-up (fixed) and family (random) as the factors. Using the statistical package JMP 5.1 (SAS Institute, Cary, NC), we applied restricted maximum likelihood for estimation of variance components and assignment of standard errors. The estimates for genetic and environmental variances for corolla width (CW), days to flower (days) and I are given for each analysis in Table A1.

We also estimated variance components for sums that combine

TABLE A1. The variance among families estimates V_G , while the intrafamily variance estimates V_E (see text). Separate model fits were conducted for each trait within each dataset (outbred vs. inbred plants).

Trait	V_G (SE)	V_E
Outbred plants		
Corolla width	2.54 (0.51)	3.40
Days to flower	1.83 (0.40)	6.08
I	0.53 (0.12)	1.34
CW + days	5.07 (1.00)	9.09
CW + I	5.02 (1.03)	7.77
Days + I	1.51 (0.30)	3.25
Inbred plants		
Corolla width	3.11 (0.44)	3.48
Days to flower	4.44 (0.69)	8.68
I	0.95 (0.14)	1.59
CW + days	8.31 (1.21)	12.02
CW + I	6.52 (0.94)	8.10
Days + I	2.80 (0.42)	4.52

each pair of characters (Table A1). The variance estimates for sums can be to estimate genetic and environmental covariances by noting that for two traits (z_1 and z_2) the following relationship holds:

$$\text{Var}[z_1 + z_2] = \text{Var}[z_1] + \text{Var}[z_2] + 2 \text{Cov}[z_1, z_2].$$

The genetic covariance can be obtained by substituting V_G estimates from Table A1 for the first three terms and solving for the fourth term. These estimates, in addition to the associated genetic correlation estimates, are given in Table A2.

TABLE A2. The genetic covariance and correlation between each pair of measurements are given for outbred and inbred plants, respectively.

Trait	Genetic covariance	Genetic correlation
Outbred plants		
CW/days	0.35	0.16
CW/ I	0.98	0.84
Days/ I	−0.43	−0.43
Inbred plants		
CW/days	0.38	0.10
CW/ I	1.23	0.72
Days/ I	−1.295	−0.63