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(Scrophulariaceae)**



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EVIDENCE FOR NATURAL SELECTION ON MATING SYSTEM IN MIMULUS (SCROPHULARIACEAE)

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We used two approaches to examine the role of natural selection in the evolution of selfing in *Mimulus*. First, using cpDNA and isozyme variation we investigated the phylogenetic relationships among a number of populations of two selfing (*M. micranthus* and *M. laciniatus*) and two outcrossing (*M. guttatus* and *M. nasutus*) species to determine the frequency of evolution of selfing within the genus. We hypothesized that the independent evolution of selfing taxa from outcrossing taxa would be strong evidence that the mating system is under the influence of natural selection. Second, we used phenotypic selection analysis to measure the presence and intensity of selection on floral characters associated with the mating system in extant populations of the outcrossing species *M. guttatus*. An unrooted Wagner tree constructed from cpDNA variation and a dendrogram constructed from Nei's genetic distances at isozyme loci both indicated the independent origin of the two selfing taxa, *M. micranthus* and *M. laciniatus*, from different ancestral *M. guttatus* populations. Phenotypic selection analysis demonstrated that both direct and indirect directional selection intensities were variable between populations but could be high. Decreased stigma-anther separation was favored in the one population that was likely pollinator-limited. Our results indicated that natural selection was strong enough to cause the repeated evolution of selfing in *Mimulus* and that the selective agent may be inadequate pollinator service.

Introduction

The evolution of selfing from primarily outcrossing ancestors represents one of the most frequent evolutionary transitions in the plant kingdom (Stebbins 1950; Grant 1981). Plants with selfing mating systems often differ dramatically in floral morphology compared to their outcrossing congeners, with selfers having smaller flowers and reduced allocation to male function (Jain 1976; Ritland and Ritland 1989). Selfing often evolves repeatedly in genera containing both selfing and outcrossing species, e.g., *Arenaria uniflora* (Wyatt 1988), *Leavenworthia* (Rollins 1963; Lloyd 1965; Solbrig and Rollins 1977), *Turnera* (Barrett and Shore 1987), *Scutellaria* (Olmstead 1989), and *Eichhornia paniculata* (Husband and Barrett 1993; Fenster and Barrett 1994). These phylogenetically oriented studies quantify the frequency and polarity of mating system evolution and thus provide evidence for an important role of natural selection in mating system evolution (Endler 1986). However, they are unable to reveal the selective agents or type or intensity of selection responsible for the major changes in morphology associated with mating system evolution (Lauder et al. 1993).

Reproductive assurance is one of the most commonly invoked selective agents for the evolution of selfing. Selfers may enjoy greater colonizing ability since they do not have to rely on conspecifics for pollination (Baker's rule [Baker 1955]). Lack of adequate numbers of pollinators may place a premium on selfing (Barrett and Shore

1987; Barrett 1988; Husband and Barrett 1992), and selfing may be a mechanism for escaping intra- or interspecific competition for pollinators (Schemske et al. 1978; Wyatt 1988). Despite the logic behind the pollinator assurance hypothesis, we have little direct evidence of the role pollinators play in mating system evolution (Epperson and Clegg 1987; Abbott and Irwin 1988).

The genus *Mimulus* (Scrophulariaceae) is a particularly appropriate group in which to study mating system evolution. There is a great diversity of mating systems, which range from highly selfing to highly outcrossing taxa (Ritland and Ritland 1989). Here we assess the role of natural selection in the evolution of traits associated with selfing in *Mimulus* by addressing the following issues. First, using cpDNA and isozyme variation, we attempt to reconstruct phylogenetic relationships among a group of populations from two outcrossing and two selfing species to determine whether selfing evolved independently within the genus and consequently is under the influence of natural selection. Second, using phenotypic selection analysis (Lande and Arnold 1983), we assess the strength and direction of natural selection on floral characters associated with the mating system. By quantifying selection in extant populations of *Mimulus*, we determined whether natural selection could be sufficiently strong to drive mating system evolution. In particular, we were interested in determining whether traits associated with female reproductive assurance, such as less stigma-anther separation, are favored because of low pollinator availability.

Material and methods

STUDY SPECIES

We chose to study *Mimulus guttatus* DC, *M. nasutus* Greene, *M. micranthus* Heller, and *M.*

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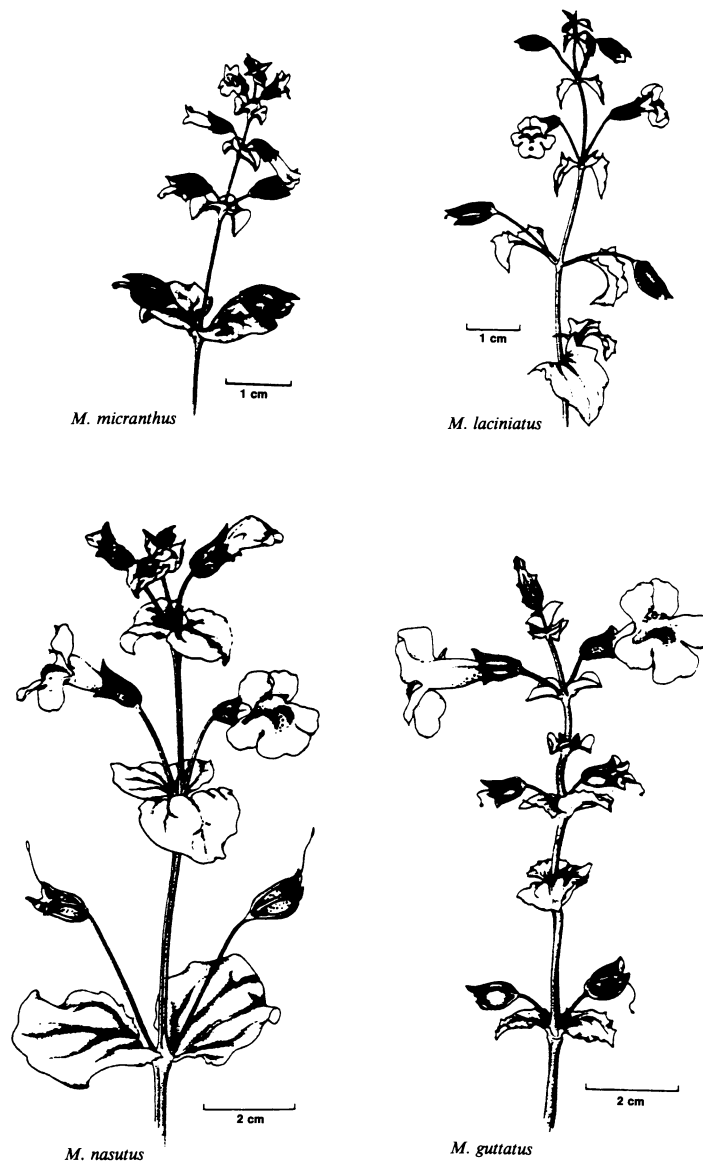


Fig. 1 The four taxa used in the study. The top two are the predominant selfers, *Mimulus micranthus* and *M. laciniatus*, and the bottom two are the higher outcrossing *M. nasutus* and *M. guttatus*. Note change in scale.

laciniatus Gray because their mating systems contrast. The floral architecture differs dramatically among these species (fig. 1). Although all four species are self-compatible and mixed-mating, the large-flowered *M. guttatus* and slightly smaller-flowered *M. nasutus* are herkogamous and have a much higher outcrossing rate ($t = .6-.9$) compared with the much smaller-flowered and autogamous *M. micranthus* ($t = .1-.2$) and *M. laciniatus* ($t = .2-.4$) (Ritland and Ritland 1989).

Populations of these *Mimulus* species are found in moist open habitats such as stream edges and ephemeral pools. *Mimulus guttatus* has an extensive distribution in western North America, and *M. nasutus* is less widely distributed. *Mimulus micranthus* is endemic to the coastal mountain

ranges of central California. *Mimulus laciniatus* is found from 1,000 to 3,000 m in the Sierra Nevada from central to southern California. Most populations of *M. guttatus* are annual except where water is available throughout the year. In these populations perennial growth is by stolons or creeping root stalks. The other three species are strictly annual.

Because of the polytypic nature of *M. guttatus*, the taxonomy of section *Simiolus* has undergone several revisions. *Mimulus micranthus* and *M. nasutus* have been classified as subspecies of *M. guttatus* (Campbell 1950) or as a distinct species (Grant 1924). The precise identity of *M. nasutus* is also in dispute (F. Ganders, personal communication). In keeping with previous taxonomic

treatments and with Ritland and Ritland (1989), as well as for notational brevity, we refer to each of these entities as distinct "taxa," which implies no specific status to any.

PHYLOGENETIC ANALYSIS

To determine patterns of cpDNA variation, we sampled one plant from each of five, three, and five populations in *M. guttatus*, *M. nasutus*, and *M. micranthus*, respectively, and one population of *M. laciniatus*. Localities are given in the Appendix. Methods for isolating cpDNA and observing variation in restriction digests are given in Fenster and Ritland (1992). The following 27 enzymes were used in single digests: AvaII, BamHI, BanI, BclI, BglII, ClaI, DraI, EcoRI, EcoRV, HincII, HindIII, HpaI, KpnI, MluI, NheI, NrvI, PstI, PvuI, SacI, SacII, SalI, ScaI, SmaI, SphI, StuI, XbaI, and XhoI. An unrooted Wagner tree of the populations was constructed from the interpopulation pattern of cpDNA variation.

Fourteen isozyme loci known to be polymorphic in *Mimulus* were assayed in the same populations sampled for cpDNA (Ritland and Ganders 1987; Vickery and Wullstein 1987; Ritland 1989; Ritland and Ritland 1989). These enzymes were Dia 1, Dia 2, Est 1, Est 2, Idh 1, Mdh 1, Mdh 2, Pgm 1, Pgm 2, 6Pgd 1, 6Pgd 2, Pgi, Tpi 1, and Tpi 2. Gel buffers and staining conditions were given in Ritland and Ganders (1987). Enzymes were extracted from a minimum of 30 individuals per population (three seedlings per maternal family from a minimum of 10 field-collected plants). Nei's genetic distances (Nei 1978) were computed between populations, and a dendrogram portraying the phylogeny of populations and taxa was constructed using the unweighted pair-group method. Standard errors of branch lengths within the dendrogram were computed using the procedure of Ritland (1989).

PHENOTYPIC SELECTION

We used multivariate regression techniques developed by Lande and Arnold (1983) to determine the strength and form of natural selection acting on floral traits associated with mating system evolution in three populations of *M. guttatus*: Tullock ($n = 178$ individuals), Guenoc ($n = 274$), and Hough Springs ($n = 241$) (specific localities provided in the Appendix). The Tullock population was a hybrid swarm between *M. guttatus* and a selfing form of *M. nasutus*; the other populations were pure *M. guttatus*. Populations were censused for 5–7-d periods during the height of the flowering period in mid-May 1988. Simultaneously, an investigation of pollen limitation at Guenoc and Hough Springs populations and a "pure" population of *M. guttatus* (population 124)

1 mi east of the Tullock hybrid swarm was conducted (Dudash and Ritland 1991).

The evolution of selfing taxa in *Mimulus* is associated with smaller flower size and close proximity of the stigma to the anthers (Ritland and Ritland 1989). In addition, variation of stigma-anther separation is correlated with levels of autogamy rates at the within- (Carr and Fenster 1994) and between- (Dole 1992) population levels. Thus three floral measurements quantifying flower size and potential for autogamous fruit production were made on each of two flowers per plant, using digital calipers to the nearest 0.1 mm: (1) corolla tube length, (2) widest width of corolla, and (3) stigma-anther separation. Measurement of two flowers per plant was adequate to characterize the phenotype of the plant because of the high repeatability for these floral characters on plants grown in both field and greenhouse environments (unpublished data).

All plants were collected from the field after the flowering season but before fruits dehisced in early June. Because seed mass/fruit was correlated with seed number/fruit ($r^2 = .75$, $P < .001$; D. E. Carr and M. R. Dudash, unpublished data), total seed production per plant, an estimate of female reproductive success in these annual populations, was estimated by weighing all seed for a plant. We also measured plant vegetative biomass to control for the effect of plant size on the expression of floral traits.

The strength of directional selection acting directly on a trait was quantified by examining the multivariate relationship between the standardized fitness of an individual (total seed mass of an individual divided by the population mean seed mass) and four characters: the means of each of the three floral characters measured per individual and plant vegetative biomass. This provided the direct relationship between a specific trait and fitness, and removed spurious associations with the other measured characters. The effect of total selection, i.e., selection acting directly on the trait and selection acting indirectly through correlated traits, was determined by univariate regression of the standardized fitness of an individual on each of the single traits. The univariate and multivariate regression coefficients, β , provide the average slope of the relationship between the trait and standardized fitness. The standardized selection differential or intensity of selection, i (Falconer 1981), for both direct and total selection was calculated by multiplying the regression coefficient for the relationship between standardized fitness and the three floral characters by the SD of the trait for each population (Lande and Arnold 1983). The intensity of selection describes the relationship between standardized fitness (here in terms of

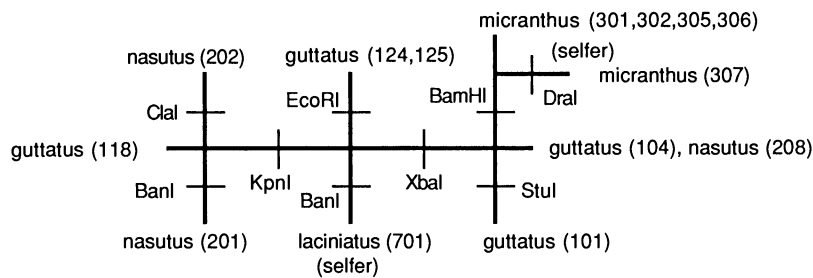


Fig. 2 Phylogenetic relationships among populations of *Mimulus guttatus*, *M. nasutus*, *M. micranthus*, and *M. laciniatus* based on cpDNA variation. Hash marks refer to cpDNA differences uncovered by restriction digests.

seed mass) and variation of the trait in units of SD. To simplify the analysis of stabilizing/disruptive selection (see below), character values were transformed to a mean of zero by subtracting the character mean of a population from each value.

Stabilizing/disruptive selection was measured by regressing standardized fitness on the square of the deviation of the trait from the population mean after the effects of directional selection were taken into account (Lande and Arnold 1983). Because the assumptions of parametric significance testing could not be made for either the analysis of directional or stabilizing/disruptive selection, the relationship between floral characters and standardized fitness was tested using the delete one jackknife procedure of Free-Stat (T. Mitchell-Olds, unpublished data). Selection was measured on untransformed character values.

To help clarify differences between the univariate and multivariate selection analysis, Pearson correlation analysis was conducted among the five traits: (1) corolla tube length, (2) corolla width, (3) stigma-anther separation, (4) plant biomass, and (5) seed biomass in each of the three populations. To meet the assumptions of this parametric analysis, total seed weight, corolla length, and plant biomass were log transformed, and stigma-anther separation distance was square root transformed.

Results

PHYLOGENETIC ANALYSIS

Eight of the 27 tested restriction digests revealed cpDNA polymorphisms either within or among *Mimulus guttatus*, *M. nasutus*, *M. micranthus*, and *M. laciniatus*: BamHI, BanI (2), ClaI, DraI, EcoRI, KpnI, StuI, and XbaI. Two mutation site losses were unique to *M. micranthus*, BamHI (see fig. 1B of Fenster and Ritland [1992]) and DraI, of which the latter was limited to one population. Aside from this one mutation, no other cpDNA polymorphisms were found in *M. micranthus*. By contrast, the *M. guttatus*–*M. nasutus* “complex” had six polymorphisms, three

(BanI, ClaI, and StuI) unique to each of three populations, one (EcoRI; see fig. 1C of Fenster and Ritland [1992]) shared by two populations, and two (KpnI and XbaI; see fig. 1D of Fenster and Ritland [1992]) shared by two different groups of three populations. The BanI digest also revealed that the representative population of *M. laciniatus* is differentiated from the other *Mimulus* populations by a unique mutation. An unrooted Wagner tree of the populations of the four taxa based on the pattern of cpDNA variation is presented in figure 2. The two selfing taxa, *M. micranthus* and *M. laciniatus*, are found on different regions of the tree and appear to be derived from different ancestral populations of *M. guttatus*. In contrast, there is no clear differentiation among the populations of *M. guttatus* and *M. nasutus*.

Thirteen of the fourteen isozyme loci (all but Pgm 1) were polymorphic across the four taxa. A dendrogram portraying the relationships between the populations is presented in figure 3. The two selfing taxa represented by five populations of *M. micranthus* and one population of *M. laciniatus* appeared in different regions of the dendrogram. The dendrogram also supported the lack of distinction between *M. guttatus* and *M. nasutus*. Hence, from here on, *M. guttatus* and

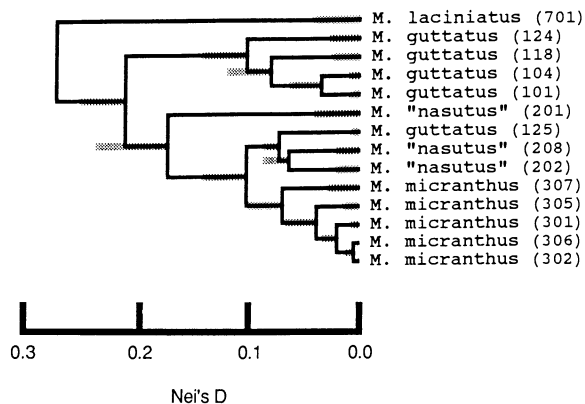


Fig. 3 Phenetic relationships among populations of *Mimulus* constructed from isozyme variation. Shaded areas represent SEs of the branch lengths.

Table 1

TRAIT MEANS AND SDs OF TWO POPULATIONS OF *MIMULUS GUTTATUS* AND THE TULLOCK HYBRID SWARM POPULATION OF *M. GUTTATUS* × *M. NASUTUS*

Character	Guenoc	Hough Spring	Tulloch
Corolla width	15.0 _A (4.6)	15.2 _A (4.3)	15.7 _A (6.3)
Corolla tube length	14.5 _A (4.4)	14.9 _A (3.7)	16.3 _B (4.7)
Stigma-anther separation	1.0 _A (0.8)	0.8 _A (0.7)	0.8 _A (0.9)
Total fruit mass	26.9 _A (62.4)	18.1 _A (54.0)	174.1 _B (239.1)
Plant biomass	56.8 _A (130.9)	61.2 _A (133.7)	411.7 _B (695.3)

Note. SDs are in parentheses. Floral characters are measured in mm and fruit mass and biomass in mg. Means followed by different letters are significantly different (Tukey's test, $P < .05$).

M. nasutus will be referred to as the *M. guttatus* complex.

PHENOTYPIC SELECTION

Considerable phenotypic variation was observed for all traits measured (table 1). Floral traits were similar across populations, but plants were larger and produced more fruit in the Tullock population. Both measurements of flower size—corolla width and tube length—were positively correlated with each other in all three populations (table 2). Plant biomass, a measure of plant size, was significantly associated with flower

size only in the Guenoc and Hough Springs populations. The degree of herkogamy, measured as the distance separating the stigma from the anthers, was positively correlated with the other floral characters in the hybrid swarm population and much less so in the Hough Springs population. In addition, in the Hough Springs and Guenoc populations, flower size but not stigma-anther separation distance was positively correlated with seed production. In contrast, all three floral characters were negatively correlated with seed production in the hybrid swarm population.

Although direct selection favored individuals with smaller flower size in all three populations (table 3), only in the Guenoc population was a significant negative association observed between corolla tube length and seed set. In the two allopatric, or true breeding populations of *M. guttatus*, indirect selection, through the positive correlation of flower size with plant size or biomass (table 2), offset direct selection, resulting in a net strongly significantly positive relationship between flower size and seed set (table 3). Both direct and indirect selection on stigma-anther separation distance was weak in the two non-pollen-limited populations (table 3). However, where pollen limitation is inferred (Tulloch population), both direct and indirect directional selection favored individuals with decreased stigma-anther separation distance. No significant stabilizing or disruptive selection was observed in any of the three populations. In addition, there was no evidence of correlational selection or selection acting on the phenotypic correlations

Table 2

PEARSON CORRELATIONS BETWEEN SEED PRODUCTION, MEASURED AS TOTAL SEED MASS, PLANT BIOMASS, A MEASURE OF PLANT SIZE, AND THREE FLORAL CHARACTERS IN THREE POPULATIONS OF *M. GUTTATUS*

	Corolla width	Corolla tube length	Stigma-anther separation	Plant biomass
Total seed mass:				
Guenoc723***	.617***	.046	.803***
Hough Springs517***	.499***	.133*	.677***
Tulloch hybrid swarm	-.326***	-.233***	-.367***	.701***
Corolla width:				
Guenoc905***	.071	.870***
Hough Springs906***	.259***	.790***
Tulloch hybrid swarm761***	.721***	.091
Corolla tube length:				
Guenoc070	.819***
Hough Springs275***	.778***
Tulloch hybrid swarm594***	.100
Stigma-anther separation:				
Guenoc069
Hough Springs230***
Tulloch hybrid swarm003

* $P < .05$.

*** $P < .001$.

Table 3
DIRECTIONAL SELECTION GRADIENTS (β) AND STANDARDIZED SELECTION INTENSITIES (i)
ON THREE FLORAL TRAITS IN THREE POPULATIONS OF *M. GUTTATUS*

Trait and population	Direct selection			Total selection		
	β	SE	i	β	SE	i
Corolla width:						
Guenoc (NPL)	-.149	.630	-.053	3.137***	.498	1.106
Hough Springs (NPL)	-2.167+	1.576	-.827	1.990***	.317	.629
Tulloch hybrid swarm (PL)	-.542+	.277	-.312	-.814**	.266	-.420
Corolla tube length:						
Guenoc (NPL)	-2.289*	.942	-.718	3.157**	.539	.991
Hough Springs (NPL)	-.835	1.549	-.232	2.300***	.351	.639
Tulloch hybrid swarm (PL)	-.038	.215	-.015	-.684**	.248	-.265
Stigma-anther separation:						
Guenoc (NPL)019	.388	.007	.012	.393	.004
Hough Springs (NPL)076	.046	.029	.758	.549	.278
Tulloch hybrid swarm (PL)	-.574**	.215	-.256	-.896***	.252	-.400

Note. NPL = non-pollen-limited; PL = pollen limitation inferred.

+ .05 < P < .10.

* P < .05.

** P < .01.

*** P < .001.

(Lande and Arnold 1983) among the three floral characters.

Discussion

Self-fertilizing taxa within *Mimulus* have evolved the loss of spatial separation between stigma and anthers and reduced allocation to male function (Ritland and Ritland 1989). The primary objective of our study was to determine the role of natural selection in the evolution of these traits. Analysis of both cpDNA and isozyme variation demonstrates that selfing has evolved independently in *M. laciniatus* and *M. micranthus*, indicative that floral morphology is under the influence of natural selection in *Mimulus*. Phenotypic selection analysis in three populations of *M. guttatus* demonstrates that directional selection intensities are strong enough in extant populations to drive mating system evolution toward a selfing morphology. A possible selective agent for the evolution of selfing taxa may be variation in the availability of pollinators for *Mimulus* populations. Below, we discuss these findings in the context of studies documenting the genetic basis for floral trait differences at both the within-population (Dole 1992; Carr and Fenster 1994) and between-species levels (Macnair and Cumbes 1989; Fenster and Ritland 1994).

PHYLOGENETIC ANALYSES

Although only a limited number of cpDNA variants were detected, several patterns emerge from the data. The mutation at the BamHI site shared by all *M. micranthus* populations indicates a single origin of *M. micranthus*. This single mutation differentiates *M. guttatus*-*M. nasutus* complex populations 104 and 208 from *M. mi-*

cranthus populations 301, 302, 305, and 306 in the cpDNA tree. The placement of *M. micranthus* and *M. laciniatus* in different portions of the cpDNA Wagner tree indicates that the two selfing taxa evolved independently from two different ancestral *M. guttatus* populations.

Placement of *M. micranthus* and *M. laciniatus* in different portions of the isozyme dendrogram is further evidence that the two selfing taxa evolved independently. The genetic distance data are also consistent with the hypothesis that the selfer *M. micranthus* is derived from *M. guttatus*. *Mimulus micranthus* contains only a subset of the allelic variation found within *M. guttatus*, and for all loci the presumed derivative *M. micranthus* is either fixed or polymorphic for the most common alleles of *M. guttatus* (Fenster and Ritland 1992).

In contrast to the cpDNA data, which indicates that *M. laciniatus* is no more different from *M. guttatus* populations than they are to one another, the genetic distance analysis demonstrates *M. laciniatus* to be least related to all other surveyed *Mimulus* populations. Furthermore, the populations of the *M. guttatus*-*M. nasutus* complex are arranged differently in the different trees. These differences between the cpDNA and nuclear isozyme data may reflect differences in their modes of transmission and in their rates of evolution, in addition to different patterns of introgression among the species for the two genomes.

Convergent evolution of character states is stronger evidence for natural selection when the two taxa are unrelated. Although *M. micranthus* and *M. laciniatus* appear in different parts of the cpDNA and isozyme trees, their selfing mating systems may reflect common genetic and devel-

opmental backgrounds. Thus, selfing in the two *Mimulus* species may be associated with nonselection processes such as drift. However, the shift from outcrossing to selfing in *Mimulus* is controlled by many genes (Macnair and Cumbes 1989; Fenster and Ritland 1994). Consequently, drift is an unlikely explanation for the independent evolution of selfing within the genus. Rather, the evolution of selfing through the fixation of alternative alleles at many loci is more likely to result from the action of consistent directional selection. Polygenic inheritance also implies that convergent evolution in mating system may occur by natural selection for reasons unrelated to mating system per se but, rather, may reflect a correlated or indirect response resulting from genetic correlations with other traits under selection. A correlated response to selection will depend on the genetic correlations between mating system and traits that are under selection. Crossing data indicate that stigma-anther separation, a determinant of mating system, is genetically independent of other floral characters (Carr and Fenster 1994; Fenster and Ritland 1994). Thus, the evolution of selfing in *Mimulus* probably reflects selection acting directly on mating system and is discussed in greater detail below.

PHENOTYPIC SELECTION

The direction and strength of natural selection varied among traits and among populations. The three populations also differed in their degree of pollen limitation (Dudash and Ritland 1991). Neither Guenoc or Hough Springs populations exhibited pollen limitation, but an allopatric population 1 mi distant from the Tullock hybrid swarm population was pollen-limited. In the Guenoc and Hough Springs populations, indirect selection, through the positive correlation of flower size with plant biomass, offset direct selection, resulting in a net strong positive relation between flower size and seed set. In contrast, flower size in the hybrid swarm population is positively correlated with stigma-anther separation. Hence, indirect selection favored smaller-flowered individuals through the negative correlation of stigma-anther separation and flower size. Selection for decreased stigma-anther separation in the hybrid swarm may reflect linkage disequilibrium between stigma-anther separation and other unmeasured traits. However, selection favoring decreased stigma-anther separation is not due to a correlation with reproductive effort since the correlation of stigma-anther separation with flower production was small ($r = -.153$, $P = .04$).

It is not clear why direct selection favored smaller flowers in the Guenoc or Hough Springs populations. Cleistogamous flowers, or flowers

that self-fertilize within a closed bud, often represent heterochrony in that they have a shorter period of development compared to outcrossing flowers on the same plant (Lord and Hill 1987). Similarly, the much smaller flower size of *M. micranthus* compared to *M. guttatus* is a product of greatly reduced development time (Fenster et al. 1994). Therefore, selection for reduced flower size in the Guenoc and Hough Springs populations may represent selection for shorter development time.

The observation of significant selection to decrease stigma-anther separation in the population that is likely pollen-limited indicates that reproductive assurance may be a selective agent in the evolution of selfing in *Mimulus*. However, these results must be qualified since the hybrid swarm itself was not directly examined for pollinator limitation. Mating system has also been demonstrated to be affected by differential pollinator visitation on different morphs in artificial arrays of *Ipomoea purpurea* (Clegg and Epperson 1988) and *Senecio vulgaris* (Abbott and Irwin 1988).

Although we did not quantify selection on floral traits acting through male reproductive success, it is unlikely that decreased stigma-anther separation alone would adversely affect male siring ability. We observed low phenotypic correlations between stigma-anther separation and flower size in the Guenoc and Hough Springs populations. The high correlation observed in the Tullock hybrid swarm population is likely an artifact of linkage disequilibrium in the hybrid zone. Low phenotypic correlations combined with the low genetic correlations (citations above) of stigma-anther separation with other floral traits in *Mimulus* indicate that the loss of herkogamy may not result in a proportional reduction in male fertility because of correlated changes in floral features associated with pollinator attraction (Epperson and Clegg 1987). Closer proximity of stigma and anthers actually promoted the export of pollen analogues to females in *Ipomopsis aggregata* (Campbell 1989), presumably because pollen was placed on a location of the pollinator which increased its transfer efficiency. Clearly further work is needed to document the relationship between male siring ability and stigma-anther separation in *Mimulus*. Lack of correlation of stigma-anther separation with other floral characters also indicates that the evolution of small flowers and decreased stigma-anther separation may be independent of each other.

Large positive phenotypic correlations between plant size and floral characters were observed in two of the *Mimulus* populations and account for the large indirect selection pressures on these traits. High correlations between flower number and floral traits also contributed to rel-

atively high total selection intensities on floral characters in populations of *Lobelia cardinalis* (Johnson 1991). These results indicate that positive phenotypic correlations between flower size and vegetative biomass may be a constraint in the evolution of floral morphology. Similarly, large correlations between juvenile and adult body size in Darwin's Medium Ground finches (*Geospiza fortis*) may prevent the evolution of an optimal life history of small juvenile and large adult body size (Price and Grant 1984). Thus, "body size" constraints may be a reoccurring obstacle in the evolution of optimal phenotypes for both plants and animals.

For all three floral characters, large direct selection intensities were observed in one or more populations. Other studies have also documented significant selection intensities on floral characters: corolla tube length, $i = -.256$ (Schemske and Horvitz 1989); corolla width, $i = .14$ (Campbell 1989); pollen export, $i = .42$, visitation rate, $i = .23$ (Campbell et al. 1991); and nectar-stigma distance, $i = .19$ (Johnston 1991). These standardized selection gradients were the highest observed in these studies and were often much lower in other years or populations. However, they do indicate that the intensity of natural selection on floral characters, although temporally and spatially variable, may approach intensities used in animal breeding and artificial selection experiments (geometric mean selection intensity = .71; Endler 1986). Thus natural selection may rapidly drive the evolution of selfing in *Mimulus*, depending on the type and amount of genetic variation present.

EVOLUTIONARY CONSEQUENCES

Mimulus guttatus populations contain substantial amounts of genetic variation for floral characters associated with the mating system (Dole 1992; Carr and Fenster 1994). Therefore, the large selection intensities that we observe for these same characters indicates that *M. guttatus* populations can rapidly respond to selection (Falconer 1981; Lande and Arnold 1983). The independent evolution of selfing documented here is further evidence of a lack of genetic constraints to mating system evolution in *Mimulus*.

Theoretical models of mating system evolution demonstrate that an important selective agent in the evolution of selfing is the immediate 50% fertility advantage enjoyed by selfers (Fisher 1941; Nagylaki 1976; Lloyd 1979; Wells 1979; Lande and Schemske 1985). These studies also demonstrated that the lower progeny fitness of selfed offspring and the reciprocal loss of male siring ability can balance the transmission bias of selfers. Recent models of mating system evolution indicate that the evolution of selfing is more likely

to occur under all circumstances if the switch from outcrossing to selfing is controlled by one or a few loci of major effect. Major gene control increases the likelihood of chance associations developing between loci that modify the mating system and genotypes with lower genetic load, thus nullifying inbreeding depression as an obstacle to the evolution of selfing (Holsinger 1988; Charlesworth et al. 1990). Consequently, the observation that large phenotypic shifts in floral traits associated with the evolution of selfing in *Mimulus* are under polygenic control (Macnair and Cumbes 1989; Fenster and Ritland 1994) indicates that, in addition to the intrinsic advantage of selfing, there must be other selective agents, such as inadequate pollinator availability documented here, responsible for the evolution of selfing in *Mimulus*. The independent evolution of selfing in *M. micranthus* and *M. laciniatus* also allows for the possibility of multiple selective agents.

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Appendix

Mimulus populations surveyed for cpDNA and isozyme variation.

Mimulus guttatus: **101**: 1 mi S of Pine Flat Rd., off Empire Grade, Santa Cruz Co.; **104**: 20202 Skyline Blvd., San Mateo Co.; **118**: near Point Reyes lighthouse at crossroad leading to Chimney Rock, Marin Co.; **124**: Tullock Reservoir Rd., Tuolumne Co.; **125**: 10 mi S of Copperopolis, Tuolumne Co. *Mimulus nasutus*: **201**: 5 mi SE of Middletown, Napa Co.; **202**: along Hwy. 20 at Lake Co./Calousa Co. line; **208**: Hough Springs, Lake Co. *Mimulus micranthus*: **301**: 7 mi W of Bartlett Springs, Lake Co.; **302**: 0.7 mi S of N entrance to Stevens Creek Park, Santa Cruz Co.; **305**: 14 mi W of Willets on road to Fort Bragg, Mendocino Co.; **306**: 1.7 mi W on Saratoga Rd. from intersection with State Rds. 9 and 85, Santa Cruz Co.; **307**: 2 mi N of Laytonville on old Hwy. 101, Mendocino Co. *Mimulus laciniatus*: **701**: Lake Eleanor Rd., 6 mi from Hetch Hetchy Dam, Tuolumne Co.

Phenotypic selection measured in the following *M. guttatus* populations: **Tullock**: 1 mi W of *M. guttatus* **124**; **Guenoc**: 5 mi SE of Middletown in Napa Co.; **Hough Springs**: near Hough Springs, Lake Co.

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