Quantitative genetics of mating system divergence in the yellow monkeyflower species complex

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To study the genetic basis of phenotypic changes in floral characters associated with mating system divergence in the yellow monkeyflowers (*Mimulus*, section *Simiolus*), all pairwise crosses were conducted between four taxa, of which two were independently evolved inbreeders. The effective number of genetic factors differentiating taxa for each of six characters was estimated from back-cross and F_2 segregation variances, using a model that incorporates dominance. The number of factors, averaged over taxa pairs, ranged from 5.3 (for stigma–anther separation) to 12.8 (for pistil length). Dominance was slight to moderate for most characters, with a slight bias for inbreeding characteristics to be dominant over outbreeding traits. F_2 segregation patterns indicate strong genetic correlations among floral size characters but variable correlation of these characters with flowering time and little correlation with stigma–anther separation. Crosses between the two selfing and two outcrossing taxa did not lead to the recovery of extreme transgressive segregants in the F_2. These results suggest that either homologous loci are involved in repeated shifts to selfing or that the likelihood of appearance of novel recombinations is limited by the large number of loci, each with small effect. The implications of these results are discussed in the context of recent models of mating system evolution.

Keywords: gene number, mating system, *Mimulus*, quantitative genetics.

Introduction

Predominant self-fertilization is a common and widespread condition in angiosperms, especially among annual herbs (Stebbins, 1950; Grant, 1981). The shift from outcrossing to selfing frequently involves a suite of features (Ornduff, 1969), including reduced size of attractive structures such as the corolla and a reduction in the spatial and temporal separation of anthers and stigmas within the flower. Selfing has evolved repeatedly at both the within-genus (*Arenaria*, Wyatt, 1988; *Leavenworthia*, Rollins, 1963; Lloyd, 1965; Solbrig & Rollins, 1977) and within-species levels (*Eichhornia paniculata*, Husband & Barrett, 1993; Fenster & Barrett, 1994), with similar but non-homologous suites of characters appearing in each taxon.

The genetic basis underlying these changes of morphology associated with mating system evolution has received little investigation. These few studies, based on genetic analysis of segregation in crosses

between divergent populations within species or between related species, have shown that many genes are responsible (*Turnera*, Shore & Barrett, 1990; *Clarkia*, Holtsford & Ellstrand, 1992; *Mimulus*, Macnair & Cumbes, 1989). In accord, mating system variation within populations is usually found to be polygenic (Breese, 1959; Shore & Barrett, 1990; Holtsford & Ellstrand, 1992; Carr & Fenster, 1994), but occasionally mating system variation is associated with the segregation of major genes (*Senecio*, Marshall & Abbott, 1982, 1984; *Ipomea*, Clegg & Epperson, 1988).

Recent models have demonstrated that the evolutionary fate of genes for selfing depends on their level of dominance and the number of loci. Mixed-mating systems are likely to be derived from the fixation of additive or dominant genes, as opposed to genes with recessive effects, as recessive inheritance shifts equilibria towards higher selfing (Latta & Ritland, 1993, 1994). Evolution towards complete selfing is likely with few loci because associations easily develop between loci affecting inbreeding depression and loci controlling selfing (Holsinger, 1988, 1991; Uyenoyama & Waller,

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1991a, b, c). In contrast, polygenic control of the mating system favours a partial selfing equilibrium (Charlesworth *et al.*, 1990; Latta & Ritland, 1993).

Section *Simiolus* of *Mimulus* (Scrophulariaceae) consists of both predominantly selfing and predominantly outcrossing species (Ritland & Ritland, 1989) whose specific status remains uncertain (see below). Many species are cross-compatible (Vickery, 1978), allowing for the formal genetic analysis of trait differentiation. Here, we report on the genetic basis of mating system differences between four closely related *Mimulus* species. Two of the species are selfers. In genera that have undergone repeated evolution of selfing, the independent evolutions of different selfing species act as 'replicates' of the evolutionary process. Comparisons of outcrossing–selfing pairs within the genus are less influenced by confounding differences of life history than are comparisons of outcrossing–selfing pairs among genera or plant families.

In this study, we performed all pairwise hybridizations between the four taxa and analysed segregation patterns in backcross and F_2 generations for gene number and levels of dominance at loci differentiating these taxa. In this process, we incorporate dominance into the estimators of gene number using a model following Serebrovsky (1928, cited in Wright, 1968). The major questions we then address are as follows. Firstly, are phenotypic differences among taxa for the mating system governed by many or few loci? Secondly, are alleles at these loci recessive in the selfing taxa? Thirdly, do changes in position of stigmas and anthers result in pleiotropic changes in other floral characters? Fourthly, are the same processes evident along independent phylogenetic lineages? Elsewhere, we report on the role of natural selection in mating system evolution (Fenster & Ritland, 1994) and estimate genetic variation within populations for traits associated with the mating system (Carr & Fenster, 1994).

Materials and methods

Study species

Populations of section *Simiolus* species occur in stream edges and wet meadows. *Mimulus guttatus* DC is polytypic and has an extensive distribution in western N. America whereas *M. micranthus* Heller and *M. laciniatus* Gray are monotypic and endemic to the Coast Range foothills and to the Sierra foothills of California, respectively (Grant, 1924). *Mimulus nasutus* Greene is intermediate in flower size and occurs in serpentine soils of northeastern California. Taxa are intercrossable (Vickery, 1978) and all have a haploid chromosome number of $n = 14$ (Campbell, 1950). *Mimulus*

guttatus is facultatively perennial whereas the other taxa are 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* by some (e.g. Campbell, 1950) but as distinct species by others (e.g. Grant, 1924). The precise identity of *M. nasutus* is also in dispute (F. Ganders, personal communication). In keeping with some previous taxonomic treatments and with Ritland & Ritland (1989), as well for notational brevity, we refer to each of these entities as distinct 'taxa', which implies no specific status to any.

The larger-flowered *M. guttatus* and *M. nasutus* are herkogamous and largely outcrossing ($t = 0.6$–0.9) compared with the smaller-flowered and predominantly selfing *M. micranthus* ($t = 0.1$–0.2) and *M. laciniatus* ($t = 0.2$–0.4) (Ritland & Ritland, 1989). Selfing taxa show reduced allocation to a number of traits that contribute to male function including corolla size and pollen number (Ritland & Ritland, 1989). Stigma–anther separation is correlated between populations to levels of outcrossing and autogamy (Dole, 1992) and within populations to levels of autogamy (Carr & Fenster, 1994). Patterns of isozyme and cpDNA variation support the hypothesis that *M. micranthus* and *M. laciniatus* are independently derived from *M. guttatus* (Ritland & Ritland, 1989; Fenster & Ritland, 1992, 1994).

One population from each taxon was chosen for study and collected for seed: (1) a perennial, very large-flowered population of *M. guttatus* near the tip of Pt Reyes, Marin Co., (2) an annual, smaller-flowered population of *M. nasutus* individuals along Hwy 20 at Lake Co./Calousa Co. line, (3) a population of *M. micranthus* 10 km west of Bartlett Springs, Lake Co., and (4) a population of *M. laciniatus* 8 km west of Hetch-Hetchy dam, Tuloumne Co.

Crosses among taxa

All 12 pairwise reciprocal crosses among the four taxa were conducted. Authenticity of F_1 hybrids was verified by heterozygotes at isozyme loci fixed for alternative alleles in the parents. F_2 progeny were produced by intercrossing F_1 individuals. Backcrosses were performed in both directions (BC_1 and BC_2), except that due to difficulties in performing the crosses, backcrosses were not performed using the two selfing taxa. *Mimulus guttatus* and *M. nasutus* parental stocks were maintained by random crossing of individuals within each species and *M. micranthus* and *M. laciniatus* parental stocks were maintained by collecting selfed seed.

For each pair of taxa, parental, F_1, BC and F_2 generations were grown simultaneously. The crosses *M. guttatus* × *M. micranthus* and *M. nasutus* × *M. micranthus* were grown in early 1989 and the crosses *M. guttatus* × *M. laciniatus* and *M. guttatus* × *M. nasutus* grown in mid-1989, both in 5 cm pots, at 18°C/14°C day/night with 18 h days in a growth chamber at the University of Toronto. The crosses *M. nasutus* × *M. laciniatus* and *M. micranthus* × *M. laciniatus* were grown in the glasshouse at College Park, MD, in early 1990 under 18 h days in 5 cm pots but with slightly warmer temperatures. Sample sizes for all crosses are indicated in Table 1.

The following characters were measured on individuals in all generations: (1) day to first flower, (2) widest corolla width, (3) average stamen height (averaged over low and high anthers), (4) pistil length, (5) stigma–anther separation (pistil length minus high

Table 1 Generation means for each character

Cross	Sample size	ln(Flower time, days)	Corolla width	Corolla length	Stamen level	Pistil length	Stigma–anther separation
M. micranthus × *M. guttatus*							
P_1	33	3.78 (0.01)	7.0 (0.2)	8.5 (0.1)	7.0 (0.1)	5.4 (0.1)	−2.09 (0.13)
P_2	56	3.92 (0.01)	29.9 (0.4)	27.3 (0.3)	18.9 (0.1)	23.4 (0.2)	2.91 (0.16)
F_1	103	3.68 (0.01)	18.7 (0.2)	18.0 (0.1)	12.7 (0.1)	14.1 (0.1)	0.47 (0.08)
BC_2	88	3.82 (0.01)	23.7 (0.2)	22.8 (0.2)	15.6 (0.1)	18.1 (0.1)	1.38 (0.07)
F_2	292	3.75 (0.01)	17.4 (0.2)	17.7 (0.1)	12.4 (0.1)	13.8 (0.1)	0.61 (0.06)
M. micranthus × *M. nasutus*							
P_1	33	3.78 (0.01)	7.0 (0.2)	8.5 (0.1)	7.0 (0.1)	5.4 (0.1)	−2.09 (0.13)
P_2	31	3.72 (0.01)	25.6 (0.5)	21.7 (0.4)	14.5 (0.2)	18.1 (0.3)	2.67 (0.20)
F_1	46	3.84 (0.02)	16.7 (0.4)	15.1 (0.2)	10.7 (0.2)	11.4 (0.2)	0.20 (0.12)
BC_2	189	3.73 (0.01)	19.8 (0.2)	17.6 (0.1)	12.4 (0.1)	14.3 (0.1)	1.10 (0.06)
F_2	303	3.71 (0.01)	15.4 (0.2)	15.2 (0.1)	10.9 (0.1)	11.8 (0.1)	0.26 (0.06)
M. laciniatus × *M. guttatus*							
P_1	50	3.40 (0.01)	5.9 (0.2)	9.1 (0.1)	7.1 (0.1)	7.5 (0.1)	0.06 (0.08)
P_2	35	3.89 (0.01)	28.8 (0.5)	26.2 (0.3)	18.4 (0.2)	24.0 (0.2)	4.08 (0.18)
F_1	100	3.66 (0.01)	19.3 (0.2)	18.3 (0.1)	12.7 (0.1)	15.6 (0.1)	1.98 (0.06)
BC_2	93	3.84 (0.01)	25.0 (0.4)	23.3 (0.2)	15.9 (0.2)	20.3 (0.2)	3.01 (0.09)
F_2	210	3.76 (0.01)	17.8 (0.2)	17.6 (0.2)	12.4 (0.1)	14.6 (0.1)	1.33 (0.07)
M. laciniatus × *M. nasutus*							
P_1	36	4.45 (0.02)	10.6 (0.3)	11.9 (0.2)	8.8 (0.1)	8.9 (0.2)	−0.41 (0.12)
P_2	32	4.16 (0.02)	26.8 (0.4)	23.0 (0.3)	16.1 (0.2)	19.0 (0.2)	2.02 (0.18)
F_1	49	4.10 (0.02)	20.5 (0.5)	18.0 (0.4)	12.7 (0.2)	14.9 (0.2)	1.49 (0.11)
BC_2	99	4.23 (0.04)	20.3 (0.3)	19.5 (0.2)	13.8 (0.2)	15.8 (0.2)	1.12 (0.09)
F_2	255	4.11 (0.01)	19.1 (0.3)	17.0 (0.2)	11.6 (0.1)	14.0 (0.1)	1.57 (0.07)
M. nasutus × *M. guttatus*							
P_1	35	3.68 (0.02)	22.7 (0.4)	18.7 (0.3)	13.0 (0.2)	16.7 (0.2)	2.80 (0.14)
P_2	35	3.89 (0.01)	28.8 (0.5)	26.2 (0.3)	18.4 (0.2)	24.0 (0.3)	4.08 (0.19)
F_1	68	3.71 (0.01)	24.8 (0.3)	22.2 (0.2)	15.4 (0.1)	19.6 (0.2)	3.03 (0.12)
BC_1	102	3.77 (0.01)	22.8 (0.3)	19.8 (0.2)	13.7 (0.1)	17.8 (0.2)	2.95 (0.08)
BC_2	103	3.80 (0.01)	26.0 (0.3)	23.3 (0.2)	16.5 (0.1)	21.3 (0.2)	3.51 (0.10)
F_2	238	3.73 (0.01)	23.3 (0.2)	20.8 (0.1)	14.8 (0.1)	18.9 (0.1)	2.98 (0.07)
M. micranthus × *M. laciniatus*							
P_1	38	3.96 (0.02)	6.6 (0.2)	8.7 (0.2)	6.6 (0.1)	5.9 (0.1)	−0.98 (0.14)
P_2	36	4.45 (0.02)	10.6 (0.3)	11.9 (0.2)	8.8 (0.1)	8.9 (0.2)	−0.41 (0.12)
F_2	187	4.29 (0.01)	9.3 (0.2)	11.3 (0.1)	8.2 (0.1)	8.0 (0.1)	−0.60 (0.07)

Values in parentheses are SE.
The first parent listed is the smaller-flowered of the two.
All measurements (except flower time) are in mm.

anther height), and (6) corolla tube length. Measurements were made using digital calipers to within 0.1 mm on the first opened flower of each plant. These characters all correlate with mating system evolution in *Mimulus* (Ritland & Ritland, 1989; Dole, 1992; Carr & Fenster, 1994).

Characters were left untransformed except for date of first flower. From the means and variances of parental and segregating generations, dominance and gene number were estimated with the formulae derived below. Genetic correlations (equivalently, the proportion of genetic factors in common between a pair of traits) were estimated as the segregation covariance in the F_2 (Humphreys & Nicholls, 1984), divided by the geometric mean of the segregational variance of the two traits. The bootstrap procedure (see below) was used to calculate standard errors of estimates.

Estimation of gene number with dominance of gene effects

The well-known procedure of Wright (1968) and Lande (1981) estimates the minimum number of factors (gene loci) differentiating the two taxa as:

$$N_E = \frac{(U_{P1} - U_{P2})^2 - C}{8S}, \tag{1}$$

where S is an estimate of the 'segregational variance' (increased phenotypic variance due to segregating alleles in the F_2), the Us are estimated means in the two parental taxa and C is a correction factor equal to the statistical error variance of $U_{P1} - U_{P2}$ (Cockerham, 1986).

This estimator has a number of assumptions and potential biases. These include the assumptions of additivity of gene effects within and among loci, no linkage and equality of effects among loci (Wright, 1968). Non-additive gene effects among loci may either increase or decrease the estimate of gene numbers (Mather & Jinks, 1982) but are probably small compared with the effects of linkage, which can cause a serious underestimate of the number of gene loci controlling a trait (Zeng *et al.*, 1990). Variation of allelic effects decreases the estimate of the number of genetic factors (Zeng *et al.*, 1990).

An assumption we will relax in this paper is that of no dominance, e.g. additivity of factors within loci. Classically, when factors appear to deviate from additivity as revealed by the means of F_1 and segregating generations, the appropriate data transformations are applied to yield additive means. However, it is often difficult to be sure that the correct transformation has been found as there are only three or four points

(parental, F_1 and F_2 means) to gauge the fit of the transformation. A model for the estimation of gene number with dominance was first proposed by Serebrovsky (1928, cited in Wright, 1968), who considered the case of complete dominance. Wright (1968, pp. 383–384) later extended it to arbitrary dominance and gave a formula for estimating dominance using the parental and F_1 means. In this section we briefly describe a reparameterized version of this estimator incorporating estimates of dominance based on the segregating generations (as opposed to the F_1). These procedures assume uniform dominance among loci.

We first consider estimation of gene number in a segregating F_2. Let two diploid taxa be fixed for alternative alleles A_1 and A_2 at each of n loci controlling a character. A given locus causes a deviation from the mean phenotype of $-2a$, $2d$, and $2a$ for genotypes A_1A_1, A_1A_2 and A_2A_2, respectively. If $d = 0$, this model becomes that of additive effects only, and if $d = -a$ or $d = a$, it portrays complete dominance. Assuming that alleles conferring an increase of the character occur in the same taxon, the means of the P_1, F_1 and P_2 are $u - 2an$, $u + 2dn$ and $u + 2an$, respectively. The expected segregational variance in the F_2 is $s = (2 + d^2)(u_2 - u_1)^2/(16n)$, so that the estimator for gene number, obtained by solving for n, is:

$$N_{E(d)} = \frac{(2 + (D/A)^2)(U_{P1} - U_{P2})^2 - C}{16S}, \tag{2}$$

where U_{P1} and U_{P2} are the parental means and D/A, the ratio of dominance to additive effects, is estimated as:

$$(D/A) = \frac{2(U_{F2} - E_{F2})}{(U_{P1} - U_{P2})}. \tag{3}$$

In these expressions, estimated quantities are denoted by capital letters and their true values by lower case. Also, C is a correction factor equal to the statistical variance of $(2 + (D/A))(U_{P1} - U_{P2})$ and $E_{F2} = (U_{P1} + U_{P2})/2$ is the average of the two parental means or that expected in the absence of dominance.

In the above estimator, the ratio of dominance to additive effects is sufficient to estimate gene number (this ratio equals -1 or $+1$ for complete dominance). The use of U_{F2} to estimate d/a is somewhat arbitrary, as the mean of the F_1 might alternatively be used. However, as the degree of effective dominance can change among segregating generations due to epistasis, it is best to base the estimate of d/a upon the current generation of study.

We next consider a segregating backcross taxon. This cross also yields information about dominance

and gene number, but less efficiently. In a backcross to the first parent, the expected segregational variance is $s_1 = (1 + d^2)(u_2 - u_1)^2/(16n)$, while a backcross to the second parent has expected segregation variance $s_2 = (1 - d^2)(u_2 - u_1)^2/(16n)$. In a backcross to the first parent, let $E_{B1} = (3/4)U_{P1} + (1/4)U_{P2}$. The estimators are now:

$$N_{E(d)} = \frac{(1 + (D/A)^2)(U_{P1} - U_{P2})^2 - C}{16S}.$$ (4)

$$(D/A) = \frac{4(U_{B1} - E_{B1})}{(U_{P1} - U_{P2})}.$$ (5)

The estimators for backcrosses to the second parent are the same except that B1 is replaced by B2 and P1 and P2 are interchanged, resulting in a degree of the sign of D.

Estimation of segregational variances via regression

To find the segregational variance, the environmental variance in the segregating generation must be determined. Segregation variances are normally found by subtraction of the F_1 variance (purely environmental) from the variance of the segregating generation (cf. Lande, 1981). To average biases due to dependence of environmental variance on heterozygosity, Wright (1968) suggests using as the estimator of environmental variance the quantity $(1/4)V_{P1} + (1/2)V_{F1} + (1/4)V_{P2}$. To remove biases due to dependencies of variances on the means, one avenue is again to find an appropriate transformation (Wright, 1968).

If transformations cannot be found or are deemed unsuitable, another method is to use linear regression to estimate environmental variances in the F_2 and backcross generations. We adopted this procedure as follows. Firstly, the means for the P_1, F_1 and P_2 data were calculated. The data were then pooled and the squared deviations of individual observations from their means (y-axis) were regressed on their means (P_1, F_1 and P_2, respectively, plotted on the x-axis). The fitted regression was then used to predict expected environmental variance at the observed F_2 and BC mean values. Even if there is no dependence of variance on the mean, this procedure is most efficient at extracting information about variance but assumes linearity of dependence of the variance on the mean.

Determination of statistical error

Formulae for the variances of estimates derived by Taylor series approximations, such as those given for gene number by Lande (1981) and for genetic corre-

lations by Falconer (1981), substantially underestimate the actual variance (Zeng et al., 1990). Error terms are neglected and, in addition, the skewness of the error distribution is approximated as symmetrical. To include these additional features of error, we adopted the bootstrap method (Efron & Gong, 1983) to estimate error. In this procedure, individuals are sampled with replacement to form replicate data sets and the distribution of estimates among replicates approximates the true error distribution. In our study, we based 95 per cent confidence intervals upon the 2.5 and 97.5 percentiles of the distribution of 1000 bootstrap estimates.

Results

Parental, F_1, F_2 and backcross means for each character and each cross are presented in Table 1. The inbreeders, *M. micranthus* and *M. laciniatus*, generally had floral characters one-quarter to one-third the size of those of outbreeders. Between the inbreeders, *M. micranthus* exhibited significantly more extreme inbreeding features. Stamen and pistil lengths were correspondingly shorter and stigma–anther separation was negative in these inbreeders. Between the two outbreeders, *M. guttatus* exhibited more extreme outbreeding features than *M. nasutus*. However, time to flowering showed no relationship with degree of inbreeding.

Estimates of the dominance ratio, based on eqns 3 and 5, are given in Table 2. Except for days to flower, dominance effects were small and often less than 20 per cent of additive effects. Days to first flower demonstrated marked overdominance towards earlier flowering in the F_1. Among crosses, the highest level of dominance was observed between the two predominant outbreeders *M. nasutus* × *M. guttatus*, with the smaller-flowered *M. nasutus* showing dominance. Between the two inbreeders, the larger-flowered *M. laciniatus* showed dominance over the smaller-flowered *M. micranthus*. In the crosses *M. laciniatus* × *M. guttatus* and *M. laciniatus* × *M. nasutus*, the direction of dominance changed among generations. There are no general trends in the direction of dominance based on the F_1. However, traits associated with smaller flower size demonstrate partial dominance over larger flower size in the BC and F_2 generations. Among floral characters, stigma–anther separation showed the largest deviations from additivity.

A typical distribution of the characters across generations is presented for corolla width for the cross *M. micranthus* × *M. guttatus* (Fig. 1). The observation of variation in the parental and F_1 generations and little overlap of the F_2 with either parental, suggests that the segregation of many loci of small effects is responsible

Table 2 Estimates of degree of dominance in F_1, backcross and F_2 generations

	ln(Flower time, days)	Corolla width	Corolla length	Stamen level	Pistil length	Stigma–anther separation
M. micranthus × *M. guttatus*						
F_1	−2.41 (0.33)	0.02 (0.03)	0.01 (0.02)	−0.04 (0.03)	−0.04 (0.02)	0.02 (0.05)
BC_2	−1.79 (0.35)	−0.08 (0.06)	0.04 (0.05)	−0.11 (0.05)	−0.18 (0.04)	−0.22 (0.11)
F_2	−1.42 (0.21)	−0.09 (0.02)	−0.03 (0.02)	−0.09 (0.02)	−0.07 (0.02)	0.08 (0.05)
M. micranthus × *M. nasutus*						
F_1	−2.78 (1.02)	0.04 (0.05)	0.00 (0.05)	−0.02 (0.06)	−0.05 (0.03)	−0.04 (0.07)
BC_2	0.33 (1.13)	−0.24 (0.08)	−0.25 (0.08)	−0.15 (0.09)	−0.21 (0.06)	−0.32 (0.14)
F_2	1.13 (0.78)	−0.10 (0.03)	0.01 (0.03)	0.04 (0.04)	0.01 (0.02)	−0.01 (0.06)
M. laciniatus × *M. guttatus*						
F_1	0.10 (0.07)	0.17 (0.03)	0.07 (0.02)	−0.01 (0.02)	−0.02 (0.02)	−0.04 (0.05)
BC_2	0.59 (0.16)	0.32 (0.09)	0.32 (0.08)	0.13 (0.08)	0.09 (0.06)	−0.06 (0.15)
F_2	0.47 (0.07)	0.03 (0.03)	−0.01 (0.03)	−0.06 (0.03)	−0.13 (0.02)	−0.37 (0.05)
M. laciniatus × *M. nasutus*						
F_1	1.46 (0.25)	0.23 (0.06)	0.09 (0.08)	0.07 (0.07)	0.19 (0.05)	0.57 (0.16)
BC_2	0.10 (0.58)	−0.59 (0.10)	−0.26 (0.11)	−0.28 (0.12)	−0.28 (0.08)	−0.48 (0.26)
F_2	1.37 (0.19)	0.06 (0.04)	−0.09 (0.04)	−0.22 (0.04)	0.01 (0.04)	0.63 (0.13)
M. nasutus × *M. guttatus*						
F_1	−0.69 (0.18)	−0.31 (0.13)	−0.06 (0.06)	−0.12 (0.06)	−0.19 (0.06)	−0.64 (0.29)
BC_1	−0.76 (0.28)	−0.89 (0.25)	−0.54 (0.16)	−0.44 (0.14)	−0.47 (0.14)	−0.77 (0.52)
BC_2	0.80 (0.34)	−0.95 (0.30)	−0.41 (0.15)	−0.47 (0.16)	−0.43 (0.13)	−0.51 (0.61)
F_2	−0.48 (0.14)	−0.80 (0.12)	−0.45 (0.05)	−0.34 (0.06)	−0.41 (0.05)	−0.72 (0.26)
M. micranthus × *M. laciniatus*						
F_2	0.32 (0.07)	0.34 (0.15)	0.62 (0.13)	0.44 (0.12)	0.39 (0.10)	0.34 (0.44)

Values in parentheses are SE.
The first parent listed for a cross is the smaller-flowered parent.

for increased variation in the F_2. This is supported by our estimates of the number of factors differentiating pairs of taxa, based on eqns 2 and 4. The averages over all crosses ranged from 5 to 12 factors (Table 3). The lowest number of genes was exhibited by stigma–anther separation, while pistil length showed the highest numbers of factors. Because days to first flower showed overdominance, estimates for this character were very low and should be regarded as artifacts of overdominance.

Individuals in the F_2 generation more extreme than either parent were only observed for the trait corolla width for the crosses *M. micranthus* × *M. laciniatus* (Fig. 2a) and *M. nasutus* × *M. guttatus* (Fig. 2b). However, there were no significant departures of the frequencies in the tails of the F_2 distributions from those expected, based on the means and standard deviations of the parental generations.

The 95 per cent confidence intervals given in Table 3, obtained by the bootstrap procedure, show substantial errors of estimates. Figure 3 illustrates how the error is markedly skewed in the positive direction, in

the case of corolla width in the cross *M. micranthus* × *M. guttatus* based on segregation observed in the F_2 generation (eqn 2). This skewness lengthens the upward bound considerably above that expected from standard errors.

Because of the large errors in the positive direction, few estimates of gene number were significantly less than 10 and many were extremely large. Significantly fewer than 10 loci controlled differences between *M. laciniatus* and *M. guttatus* for two characters (stamen level, stigma–anther separation) and between *M. laciniatus* and *M. nasutus* for three characters (corolla width, stamen level, stigma–anther separation). Because of the skewed error, the lower bounds for estimates are more informative. Lower bounds ranged from zero (indicating no segregational variance) to 10 (shown by pistil length).

Table 4 gives the estimates of genetic correlations between characters shown in the F_2 generation. All size characters (corolla width, corolla length, anther height, pistil length) have consistently high positive genetic correlations with each other. In contrast, date of first
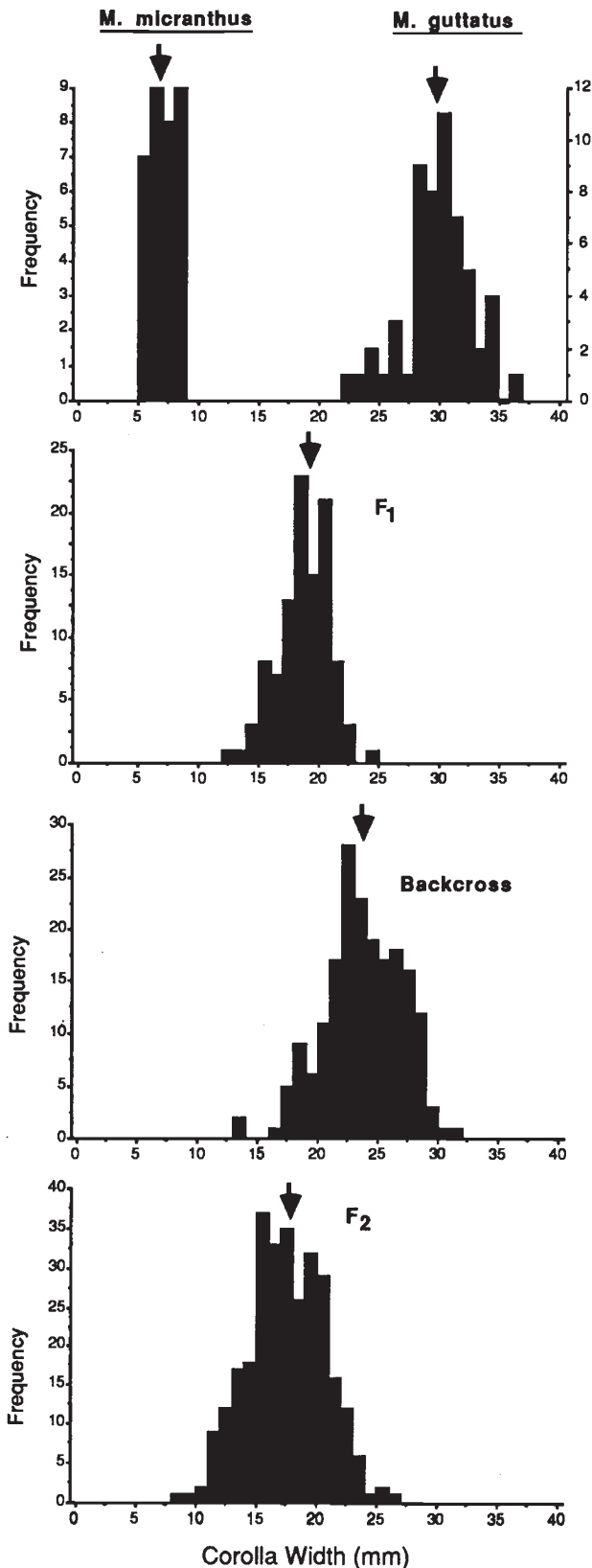
Fig. 1 Distribution of corolla widths for parental, F_1, BC_2 and F_2 generations in the cross *M. micranthus* × *M. guttatus* (arrows denote means).

flower showed a variable correlation with other characters among pairs of taxa. Crosses with *M. micranthus* suggest a weak relationship between date of first flower and flower size, while crosses involving either *M. guttatus* or *M. nasutus* with *M. laciniatus* suggest earlier flowering plants have reduced flower size. Date of first flower was not consistently correlated with stigma–anther separation.

Stigma–anther separation was weakly and erratically correlated with corolla width, corolla length and anther height but showed a consistent positive correlation with pistil length (Table 4). Partial correlation analysis of the segregation covariance reveals a much stronger negative association between stigma–anther separation and anther height (Table 5).

No maternal effects were observed in crosses between *M. guttatus* and *M. laciniatus* or *M. nasutus* with either *M. micranthus* or *M. laciniatus* (results not shown). In the cross *M. micranthus* × *M. guttatus*, all traits except stigma–anther separation showed small maternal effects, about 5–20 per cent of their maximum values. The effects largely disappeared in the F_2 for all traits except corolla width and stigma–anther separation. They are expected to increase estimates of the F_1 and F_2 variances and hence to lower the estimates of the number of factors differentiating the taxa.

Discussion

Gene number

In general, we found that many genes are responsible for the evolution of inbreeding in *Mimulus*, section *Simiolus*. Estimates of the number of genetic factors were close to the number of chromosomal segments segregating (14 for *Mimulus*, Vickery, 1978). Macnair & Cumbes (1989) also estimated gene number between another small-flowered selfer, *M. cupriphilus* (presumably independently derived) and the larger-flowered *M. guttatus*. Their results were similar to our own, i.e. many genes appear to be responsible for the differentiation of floral characters among *Mimulus* taxa. The exception observed in the cross of *M. laciniatus* × *M. nasutus* may be associated with a greater role of epistasis differentiating these taxa (see below). In addition, the low estimates of number of loci differentiating *M. macranthus* from *M. laciniatus* are likely to be biased downward by transgressive segregation. Although the two species differ significantly for the traits, the gene counting procedure employed here assumes all loci are fixed in the same direction and is best used when parental strains differ greatly (Wright, 1968). Note that our estimates of gene number were

Table 3 Estimates of the minimum number of genetic factors separating taxa for floral characters, obtained with the procedure that allows for uniform dominance

	ln (Flower time, days)	Corolla width	Corolla length	Stamen level	Pistil length	Stigma–anther separation
M. micranthus × *M. guttatus*						
BC$_2$	14.5 (0.0–116)	8.9 (5.0–30.2)	8.4 (4.9–20.6)	6.5 (4.2–11.8)	19.1 (12.7–36.9)	9.4 (0.0–111)
F$_2$	1.1 (0.5–6.3)	9.4 (6.9–14.4)	12.1 (9.1–18.6)	11.6 (8.4–17.6)	15.0 (10.0–20.4)	6.1 (3.6–16.0)
M. micranthus × *M. nasutus*						
BC$_2$	0.1 (0.0–0.3)	9.1 (2.9–60.0)	16.3 (0.0–177)	26.6 (0.0–239)	27.2 (0.0–249)	Undefined
F$_2$	0.1 (0.0–0.3)	13.7 (7.5–45.2)	14.1 (8.2–55.0)	8.1 (4.4–21.7)	15.6 (10.0–26.7)	9.2 (3.3–131)
M. laciniatus × *M. guttatus*						
BC$_2$	5.1 (0.0–44.9)	7.9 (4.5–18.6)	13.0 (7.6–41.1)	5.7 (3.2–13.4)	9.3 (5.5–21.2)	Undefined
F$_2$	1.3 (0.9–2.4)	11.8 (7.9–19.3)	9.8 (7.3–13.9)	6.8 (5.4–9.1)	13.2 (9.9–18.5)	4.1 (2.7–6.8)
M. laciniatus × *M. nasutus*						
BC$_2$	0.1 (0.0–1.4)	0.9 (0.0–41.7)	3.6 (0.0–85.9)	2.3 (0.0–38.5)	3.5 (0.0–40.8)	1.1 (0.0–18.2)
F$_2$	0.7 (0.4–1.4)	3.1 (2.1–5.9)	5.2 (0.0–35.8)	4.4 (2.7–8.8)	7.3(4.6–14.6)	2.0 (0.8–8.6)
M. nasutus × *M. guttatus*						
BC$_1$	0.5 (0.4–16.6)	0.1 (0.0–1.3)	1.3 (0.0–24.9)	0.9 (0.0–15.8)	1.9 (0.0–36.3)	Undefined
BC$_2$	0.1 (0.0–1.1)	0.0 (0.0–5.8)	0.3 (0.0–2.1)	0.7 (0.0–5.8)	0.7 (0.1–2.8)	Undefined
F$_2$	1.3 (0.6–7.3)	3.7 (1.3–92.3)	7.8 (3.5–66.4)	9.9 (3.8–90.8)	11.3 (5.2–165)	2.6 (0.5–50.2)
M. micranthus × *M. laciniatus*						
F$_2$	5.1 (2.3–27.4)	0.7 (0.4–1.7)	0.8 (0.4–2.4)	1.0 (0.5–2.4)	1.4 (0.9–6.9)	0.2 (0.1–2.4)
Mean of selfers × outcrossers (first four crosses above)						
F$_2$	0.8 (0.6–2.1)	9.5 (7.5–17.4)	10.3 (8.0–22.1)	7.7 (6.3–11.3)	12.8 (10.0–16.2)	5.3 (3.4–35.9)

Values in parentheses are 95% confidence intervals.

changed by less than five per cent when we incorporated dominance and are consistent with Wright's (1968) observation that unless dominance is strong the gene counting methods are relatively insensitive to dominance.

The isozyme and cpDNA evidence for the independent origin of *M. laciniatus* and *M. micranthus* (Ritland & Ritland, 1989; Fenster & Ritland, 1992, 1994) suggests that the evolution of selfing in these taxa should involve different genes. However, the lack of extreme transgressive phenotypes in the F$_2$ of crosses between the two inbreeding and two outbreeding taxa suggests that similar genetic and developmental mechanisms are shared by the closely related taxa. Alternatively, the lack of novel recombinants may reflect the segregation of large numbers of loci of small effect involved with the reduction of flower size. In contrast, controlled crosses among selfing variants from different parts of the range of *Eichhornia paniculata* in Brazil indicate that different recessive genes are responsible for changes in filament height associated with the evolution of selfing and suggest that one or a few loci may control filament length (Fenster & Barrett, 1994).

The method we have used to estimate gene number provides a *minimum* estimate of the number of loci differentiating two species. A single gene (or leading factor) can be responsible for as much as $1/\sqrt{n}$ of the genetic variation segregating in the F$_2$, where *n* is the number of factors differentiating the two taxa (Wright, 1968; Lande, 1981). Major genes, if present, probably contribute proportionally less to the differences between taxa because of linkage and departures from additivity. With estimates of 5–10 linkage groups segregating alleles for stigma–anther separation in the F$_1$ of the wide crosses of *M. guttatus* with either *M. micranthus* or *M. laciniatus*, roughly 32–45 per cent of the F$_2$ variance can be explained by a major gene. This corresponds to a major gene causing a decrease in stigma–anther separation of approximately 0.4–0.7 mm.

Some studies of variation within species have found single gene mutations of large phenotypic effect (Gottlieb, 1984; Ambros, 1988; Sawhney, 1992). One cannot determine, however, if these mutations will eventually be responsible for the evolution of differences among new taxa (the 'mutational fallacy', cf. Coyne & Lande, 1985). However, a retrospective

study such as our own cannot reconstruct the sequence of genetic events responsible for mating system evolution. For example, we cannot ascertain whether a single major gene initiated the evolution of selfing, with the subsequent build-up of minor modifier genes, or whether polygenes were responsible from the beginning. Studies of extant genetic variation in outcrossing populations of *M. guttatus* do not provide any evidence
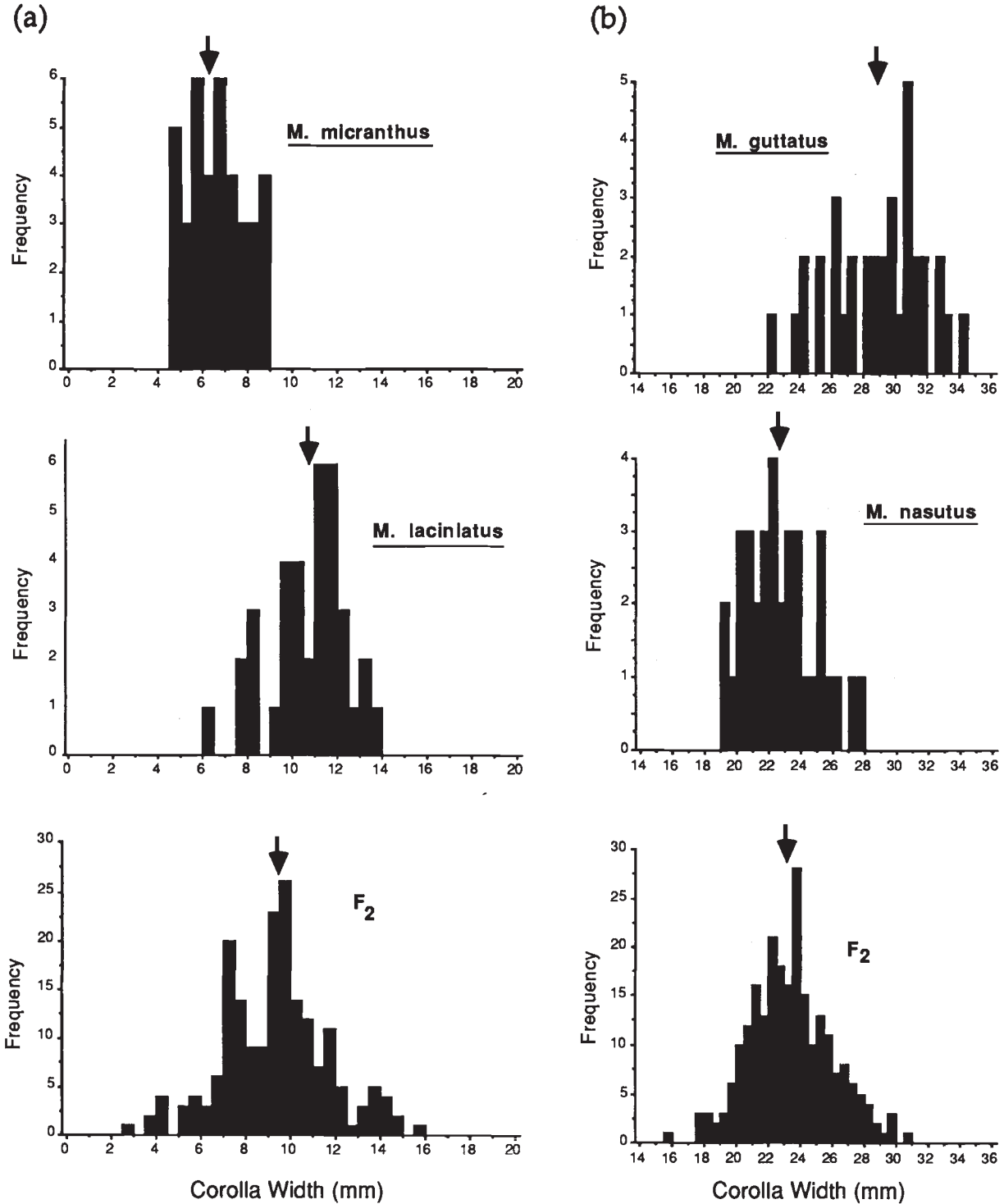

Fig. 2 Distribution of corolla widths for parental and F$_2$ generations in the crosses: (a) *M. micranthus* × *M. laciniatus*, (b) *M. nasutus* × *M. guttatus* (arrows denote means). The appearance of F$_2$ segregants that exceed the parental ranges is an artifact of the limited sample sizes of the parental generations.
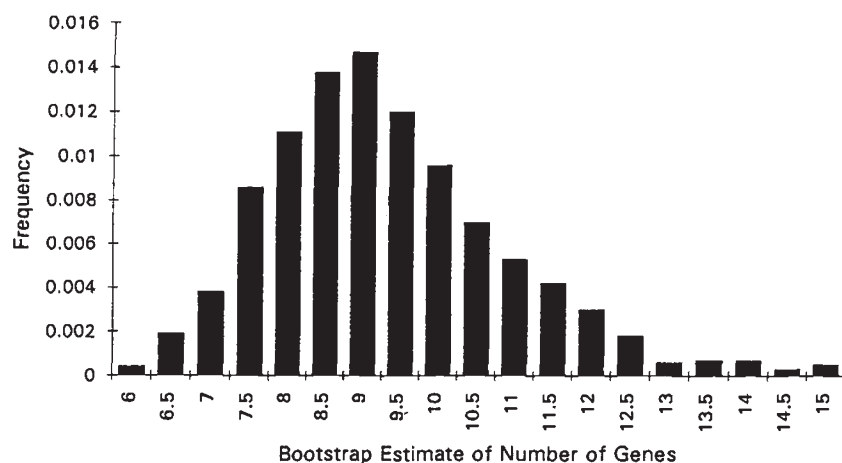
Fig. 3 Distribution of bootstrap estimates for the number of genes controlling corolla width for the cross *M. micranthus* × *M. guttatus*.

of major gene control of any of the floral characters associated with mating system evolution (Carr & Fenster, 1994). We suggest that molecular methods, if they can determine the ages of all alleles responsible for mating system differentiation, may offer a definitive picture of this historical process.

The magnitude of trait differences between taxa showed little relationship with the estimated number of genes causing these differences. For example, despite their sharing of a number of outcrossing features, the number of genetic factors differentiating the larger-flowered *M. guttatus* and *M. nasutus* was usually large, and on average, did not differ from the number of factors differentiating inbreeders from outbreeders. In contrast, stigma–anther separation showed less differentiation between *M. nasutus* and *M. laciniatus* and the estimate of gene number was likewise low. Thus, differences in trait values among taxa are not associated with the number of factors differentiating them.

We have also emphasized in this paper the nature of the error distribution of gene number estimates and the utility of using the bootstrap method (Efron & Gong, 1983) to estimate errors of gene number estimates, as well as genetic correlation estimates. Estimates of gene number have very uncertain upper limits due to the positive skewness of the error distribution (Fig. 3). By contrast, the lower bound is much more certain. Because most violations of the Wright estimator (variation of gene effect among loci, linkage) result in downward bias of estimates, in the light of the positive skewness of error, we conclude that the lower error bound is quite robust as a minimum estimate, while the upper error bound is neither a maximum estimate nor a very certain estimate. Indeed the standard errors based on the formula given in Lande (1981) (not shown here) generally correspond to the lower error bound and differ more from the upper bound.

Gene action

Most traits demonstrated limited dominance, especially for the crosses between selfers and outcrossers. Dominance should increase the probability and rate of evolution for selfing (Haldane, 1927), and the partial dominance towards inbreeding characters suggests that the rate of evolution of selfing from outcrossing would be accelerated. If outcrossing had evolved from selfing, the level of dominance should have no effect upon the spread of alleles increasing outcrossing and there should not be any trends in the level of dominance. Thus, our results support the scenario that selfing has evolved from outcrossing through the spread of mildly dominant mutations. The evolution of selfing from outcrossing is also supported by reduced species-wide levels of isozyme and cpDNA variation in *M. micranthus* vs. *M. guttatus* which are indicative of a progenitor-derived species pair (Fenster & Ritland, 1992).

Different dominance relationships were quantified by Macnair and Cumbes (1989) in the cross between *M. guttatus* and the smaller-flowered *M. cupriphilus*. Generally, they observed dominance towards the *M. guttatus* phenotype for many of the same floral traits measured here. Macnair (1989) provides evidence that *M. cupriphilus* is very recently derived. The limited dominance in our crosses may reflect either the evolution of loss of dominance in less recently derived selfers or different sets of loci responsible for differentiation across taxa.

Dominance, however, is not a necessary precondition for the evolution of selfing as recessive adaptations can readily evolve in a mixed-mating system (Charlesworth, 1992). Limited dominance is often observed for many quantitative traits (Falconer, 1981) and is further suggestive that many loci of small effect are responsible

Table 4 Estimates of genetic correlations among characters in the F$_2$ generation

	Corolla width	Corolla length	Anther height	Pistil length	Stigma–anther separation
Flower time					
gut. × mic.	−0.56(−1.0, 0.07)	−0.20(−1.0, 0.66)	−0.18(−1.0, 1.0)	0.10(−1.0, 1.0)	0.27(−1.0, 1.0)
nas. × mic.	0.00(−0.37, 0.40)	0.04(−0.30, 0.47)	0.01(−0.35, 0.39)	−0.13(−0.39, 0.15)	−0.65(−1.0, −0.05)
gut. × lac.	0.60(0.20, 0.97)	0.54(0.03, 0.89)	0.60(0.12, 0.91)	0.56(0.07, 0.86)	−0.08(−0.37, 0.21)
nas. × lac.	0.71(0.44, 1.0)	0.66(0.32, 1.0)	0.65(0.34, 0.9)	0.54(0.15, 0.88)	−0.09(−0.54, 0.60)
gut. × nas.	0.37(−1.0, 1.0)	0.05(−1.0, 1.0)	0.41(−1.0, 1.0)	0.52(−1.0, 1.0)	0.79(−0.68, 1.0)
mic. × lac.	0.16(−0.42, 0.64)	0.19(−0.42, 0.61)	−0.09(−1.0, 0.47)	0.37(−0.12, 1.0)	0.17(−0.81, 1.0)
Corolla width					
gut. × mic.		0.96(0.82, 1.0)	0.90(0.75, 1.0)	0.79(0.66, 0.90)	0.27(−0.01, 0.62)
nas. × mic.		0.98(0.79, 1.0)	0.65(0.27, 0.90)	0.86(0.59, 1.0)	0.52(−0.17, 1.0)
gut. × lac.		0.89(0.81, 0.97)	0.83(0.72, 0.93)	0.87(0.78, 0.95)	0.05(−0.24, 0.30)
nas. × lac.		1.10(0.89, 1.0)	0.82(0.50, 1.0)	0.80(0.46, 0.97)	−0.02(−0.41, 0.39)
gut. × nas.		0.73(−0.06, 1.0)	0.67(−0.33, 1.0)	0.82(0.12, 1.0)	0.52(−1.0, 1.0)
mic. × lac.		0.79(0.53, 1.0)	0.74(0.35, 1.0)	0.62(0.09, 0.99)	−0.30(−1.0, 1.0)
Corolla length					
gut. × mic.			1.01(0.92, 1.0)	0.98(0.89, 1.0)	0.40(0.12, 0.76)
nas. × mic.			0.92(0.75, 1.0)	0.85(0.62, 1.0)	0.16(−0.56, 1.0)
gut. × lac.			0.97(0.92, 1.0)	0.99(0.96, 1.0)	0.03(−0.21, 0.28)
nas. × lac.			0.88(0.56, 1.0)	0.82(0.45, 1.0)	−0.09(−0.57, 0.62)
gut. × nas.			0.99(0.72, 1.0)	1.08(0.83, 1.0)	0.50(−1.0, 1.0)
mic. × lac.			0.85(0.58, 1.0)	0.63(0.20, 1.0)	−0.27(−1.0, 1.0)
Anther height					
gut. × mic.				0.97(0.86, 1.0)	0.29(0.01, 0.76)
nas. × mic.				0.99(0.79, 1.0)	0.31(−0.34, 1.0)
gut. × lac.				0.93(0.85, 0.99)	−0.23(−0.46, 0.02)
nas. × lac.				0.82(0.63, 0.93)	−0.23(−0.63, 0.31)
gut. × nas.				0.96(0.69, 1.0)	0.12(−1.0, 1.0)
mic. × lac.				0.84(0.26, 1.0)	−0.53(−1.0, 1.0)
Pistil length					
gut. × mic.					0.61(0.40, 0.84)
nas. × mic.					0.66(0.23, 1.0)
gut. × lac.					0.16(−0.11, 0.37)
nas. × lac.					0.36(−0.08, 0.85)
gut. × nas.					0.36(−1.0, 1.0)
mic. × lac.					0.35(−1.0, 1.0)

Values in parentheses are 95% confidence intervals.
Crosses are listed in decreasing order of absolute difference in flower size among parents (gut. = *M. guttatus*, nas. = *M. nasutus*, lac. = *M. laciniatus*, mic. = *M. micranthus*).

Table 5 Partial genetic correlations of stigma–anther separation with pistil length and stamen level

Cross	Pistil length	Anther height
M. guttatus × M. micranthus	0.72	−0.43
M. guttatus × M. laciniatus	0.99	−0.99
M. nasutus × M. laciniatus	0.86	−0.79
M. micranthus × M. laciniatus	0.57	−0.54

All values are significant at $P < 0.001$.

for trait differentiation (Kacser & Burns, 1981). Furthermore, intermediate F_1 values may reflect directional selection acting on inherent variation within a population rather than the occurrence of new mutations (Charlesworth, 1992). Investigation of within-population patterns of genetic variation in *M. guttatus* reveals the presence of significant amounts of additive genetic variation for floral characters associated with mating system and implies a limited contribution of dominance effects to intrapopulation levels of genetic variation (Carr & Fenster, 1994). A very significant exception to the pattern of dominance was observed for days to first flower where overdominance was consistently observed. Indeed, characters more closely associated with fitness are expected to be more often controlled by genes with nonadditive effects (Falconer, 1981).

Changes in the direction of dominance from F_1 and F_2 to BC_2 generations is indicative of epistatic gene interaction. A joint scaling test was used to determine the relative contribution of additive and nonadditive gene action (Mather & Jinks, 1982, analysis not shown here). Lack of backcrosses to the selfing species allowed us to test only a subset of the genetic components. Estimates of the genetic components associated with epistatic gene action were almost always much smaller than additive effects and, with few exceptions, smaller than dominance effects. The exception involved the cross of *M. laciniatus × M. nasutus*. The greatest reversals of dominance effects from F_1 to BC_2 were also observed in this cross. These results suggest that the lower estimates of gene number differentiating *M. laciniatus* from *M. nasutus* may in part be an artifact of increased epistasis. However, we conclude that the evolution of mating system in *Mimulus* is predominantly dependent on the fixation of alleles with mostly additive effects.

Genetic correlations

We found strong positive genetic correlations among characters associated with floral size in *Mimulus*. If the genetic correlations based on the F_2 segregation patterns reflect population patterns of covariation during the evolution of selfing, then selection on one floral character may have resulted in a correlated response in other characters. Similar genetic correlations are also observed within populations of *M. guttatus* and *M. micranthus* (Carr & Fenster, 1994), supporting our inferences from this current study.

The genetic correlations between the time of first flower and other floral characters were inconsistent in sign and varied depending on the parental species. Crosses of *M. micranthus* with either *M. guttatus* or *M. nasutus* revealed that floral morphology was either negatively or weakly genetically correlated to time of first flower. Macnair & Cumbes (1989) also observed a lack of correlation between flowering time and degree of herkogamy. In contrast, crosses of *M. laciniatus* with the two outcrossing taxa demonstrated consistent strong positive genetic correlations of time to first flower with other floral characters. Parent–offspring regressions and correlations among family means for two populations of *M. guttatus* and *M. micranthus*, respectively, also revealed that the genetic correlation of time of first flower with other floral traits varies at the population level (Carr & Fenster, 1994). Taken together, these results suggest that mating system may or may not be a correlated response to selection for rapid development (Lloyd, 1965; Moore & Lewis, 1965; Solbrig & Rollins, 1977). The trajectory of trait evolution will ultimately depend on the genetic architecture particular to the evolutionary lineage.

Stigma–anther separation showed no consistent genetic correlations with the other characters. This may reflect the relatively late developmental processes responsible for positioning of stigma and anther (Lord & Hill, 1987; Richards & Barrett, 1992). Consequently, the loss of herkogamy may not reduce male fertility because of correlated changes in floral features associated with pollinator attraction (Epperson & Clegg, 1987; Ritland, 1991). Closer proximity of stigma and anthers actually promoted pollen export to females in *Ipomopsis aggregata* (Campbell, 1989), presumably because pollen was placed on a location of the pollinator which increased its transfer efficiency. Therefore, shifts in the mating system in *Mimulus*, or in other groups, may not be constrained by pleiotropic action of loci associated with decreased stigma–anther separation.

Evolutionary consequences of polygenic control

Polygenic control of mating systems has consequences for the dynamics of the evolution of selfing in *Mimulus*. Simulations have demonstrated that the likely outcome of major gene control is the frequent association of the mating system modifying locus with individuals with low genetic load (Holsinger, 1988; Charlesworth *et al.*, 1990). Thus the evolution of selfing is more likely with major genes. This is a result of Fisher's (1941) observation of the 50 per cent transmission advantage of selfers over outcrossers in the absence of inbreeding depression or pollen discounting (Holsinger *et al.*, 1984). The polygenic control of mating system in *Mimulus* implies that such associations are unlikely and that factors other than the 50 per cent transmission advantage are likely to be responsible for the evolution of selfing. Polygenic inheritance, intermediate heritabilities and additivity or slight dominance for selfing characters favour the maintenance of a stable mixed-mating system (Latta & Ritland, 1993, 1994). Thus the genetic architecture for mating system variation in *Mimulus* documented here corresponds to the observation of mixed-mating sytems in *Mimulus* which span the range from predominant selfing to predominant outcrossing but do not include either complete outcrossing or complete selfing (Ritland & Ritland, 1989).

Acknowledgements

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