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MINOR QUANTITATIVE TRAIT LOCI UNDERLIE FLORAL TRAITS ASSOCIATED WITH MATING SYSTEM DIVERGENCE IN *MIMULUS*

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Abstract.—The genetic basis of species differences provides insight into the mode and tempo of phenotypic divergence. We investigate the genetic basis of floral differences between two closely related plant taxa with highly divergent mating systems, *Mimulus guttatus* (large-flowered outcrosser) and *M. nasutus* (small-flowered selfer). We had previously constructed a framework genetic linkage map of the hybrid genome containing 174 markers spanning approximately 1800 cM on 14 linkage groups. In this study, we analyze the genetics of 16 floral, reproductive, and vegetative characters measured in a large segregating *M. nasutus* × *M. guttatus* F_2 population ($N = 526$) and in replicates of the parental lines and F_1 hybrids. Phenotypic analyses reveal strong genetic correlations among floral traits and epistatic breakdown of male and female fertility traits in the F_2 hybrids. We use multitrait composite interval mapping to jointly locate and characterize quantitative trait loci (QTLs) underlying interspecific differences in seven floral traits. We identified 24 floral QTLs, most of which affected multiple traits. The large number of QTLs affecting each trait (mean = 13, range = 11–15) indicates a strikingly polygenic basis for floral divergence in this system. In general, QTL effects are small relative to both interspecific differences and environmental variation within genotypes, ruling out QTLs of major effect as contributors to floral divergence between *M. guttatus* and *M. nasutus*. QTLs show no pattern of directional dominance. Floral characters associated with pollinator attraction (corolla width) and self-pollen deposition (stigma-anther distance) share several pleiotropic or linked QTLs, but unshared QTLs may have allowed selfing to evolve independently from flower size. We discuss the polygenic nature of divergence between *M. nasutus* and *M. guttatus* in light of theoretical work on the evolution of selfing, genetics of adaptation, and maintenance of variation within populations.

Key words.—Adaptation, floral evolution, genetic architecture, *Mimulus*, quantitative trait locus, selfing, speciation.

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The genetic basis of species differences provides insight into past evolutionary change and has long been a subject of contention among evolutionary biologists. Darwin (1859) posited a fundamental continuity between the variation among individuals and the differences between species. Integrated with modern genetics, this view has become a central tenet of the evolutionary synthesis: Natural selection acts on extremely slight phenotypic differences and adaptation results from the fixation of alleles with individually small effects at many loci (Fisher 1930). The micromutationist view has been challenged almost since its inception, however, with detractors arguing that adaptation and speciation often entail the sorts of phenotypic jumps that Darwin ruled out (e.g., Huxley 1860; Morgan 1932; Gould 1980; Gottleib 1984). Although it is now theoretically clear that gradual microevolutionary processes can explain abrupt macroevolutionary patterns (Charlesworth et al. 1982; Lande 1983), the empirical problem remains largely unresolved (Orr and Coyne 1992). We still know little about the evolutionary processes and genetic materials that underlie phenotypic divergence between populations and species.

Most traits that differentiate populations or species do not show simple Mendelian inheritance, but vary continuously in segregating hybrid populations. This pattern alone demonstrates that many traits are polygenic (Lande 1981), but biometric approaches to estimating gene number can do little beyond confirming the existence of multiple quantitative trait loci (QTLs) underlying divergence (Zeng et al. 1990). In the last decade, the development of genetic mapping techniques and the increasing accessibility of molecular markers have allowed direct investigation of the genetic architecture of polygenic traits (Lander and Botstein 1989; Tanksley 1993; Zeng 1994). QTL mapping studies of crop plants have found that domestication often involves major alleles at genes with pleiotropic effects and epistatic interactions (e.g., Doebley and Stec 1993; Dorweiler et al. 1993; Tanksley 1993; Doebley et al. 1995). Comparative QTL mapping has further indicated that the same loci (but independent mutations) have been involved in convergent adaptations to cultivation in some domesticated plant groups (e.g., cereals: Paterson et al. 1995). These studies, and others in crop systems, demonstrate the power of QTL mapping to genetically dissect complex traits and indicate that strong artificial selection can produce adaptation by large steps.

QTL studies of wild systems have suggested that genes of large effect also play an important role in the phenotypic differences between species (Bradshaw et al. 1995, 1998; Voss and Schafer 1997). However, other studies have identified large numbers of relatively minor QTLs underlying divergence in morphological traits between sister taxa (True et al. 1997; MacDonald and Goldstein 1999; Zeng et al. 2000; Westerbergh and Doebley 2002; for review see Orr 2001). Because the power to detect QTLs and the degree of bias toward overestimation of their effects is highly sensitive to sample size (Beavis 1994) and the characteristics of the genetic map (e.g., Jiang and Zeng 1997; Noor et al. 2001), variation between systems in genetic architecture may be due in part to differences in experimental design. However, var-

iation in the number and magnitude of QTL effects may also reflect variation in the nature of selection during phenotypic divergence, as well as the nature of standing genetic variation and the frequency and effects of new mutations. Recent theoretical work has generated clear predictions about the distribution and directionality of QTL effects underlying adaptation (Orr 1998a,b), but we still have strikingly little theoretical or empirical information on the magnitude, pleiotropic effects, and interactions of genetic factors recruited by natural selection.

In this study, we investigate the genetic architecture of phenotypic differences between two flowering plant species with highly divergent mating systems (selfing vs. outcrossing). The shift from outcrossing to self-fertilization is perhaps the most common evolutionary transition in flowering plants (Stebbins 1970; Barrett et al. 1996) and has important implications for the ecology, genetics, and long-term evolutionary potential of populations. Natural selection may favor the evolution of selfing for a number of reasons, including reproductive assurance, energetic efficiency, and the preservation of coadapted gene complexes (for review see Jarne and Charlesworth 1993). Selfing may also be favored by the inherent transmission advantage of an allele causing self-fertilization with no concomitant loss of outcross male fitness (Fisher 1941; Nagylaki 1976). Inbreeding depression, which is generally high in outcrossing populations (Husband and Schemske 1996), will oppose the spread of selfing alleles under both transmission selection and ecological selection (e.g., Lloyd 1979; Lande and Schemske 1985). However, theory also shows that a mutation causing complete or near complete selfing will spread to fixation even in the presence of high inbreeding depression (Lande and Schemske 1985; Holsinger 1988; Charlesworth et al. 1990; Schultz and Willis 1995). In that case, the evolution of selfing would necessarily involve very major genetic effects. Because we have clear predictions about the magnitude and pleiotropic effects of selfing rate modifiers fixed under different modes of selection, understanding the genetic architecture of traits associated with mating system evolution may be particularly useful in untangling the complex dynamics of this transition.

Our QTL analyses focus on floral characters associated with mating system divergence between *Mimulus guttatus* (outcrossing) and *M. nasutus* (selfing), closely related members of the *M. guttatus* species complex (yellow monkeyflowers, Scrophulariaceae). *Mimulus guttatus* is the most common species and presumed ancestral type in an interfertile group containing several primarily selfing taxa. The species complex has become a model system for the study of mating system evolution and reproductive isolation (e.g., Vickery 1964; Kiang and Hamrick 1978; MacNair and Cumbes 1989; Ritland 1991; Dole 1992; Willis 1992; Fenster and Ritland 1994b; Dudash et al. 1997; Fenster and Carr 1997; Lin and Ritland 1997; Fishman and Willis 2001). *Mimulus guttatus* exhibits characteristic adaptations for outcrossed bee-pollination, including a showy corolla with a broad throat and prominent landing pad and a touch-sensitive stigma exerted beyond the anthers. Population-level outcrossing rates are variable but generally high (0.66–1.0; e.g., Willis 1993b; Latta and Ritland 1994). In contrast, *M. nasutus* flowers exhibit little or no stigma-anther separation, have very reduced corollas, and generally self-pollinate prior to anthesis. Although the functional relationships between particular floral characters and either autogamous selfing rates or individual outcrossing rates have not yet been determined in yellow monkeyflowers, floral characters such as corolla width or stigma-anther separation clearly influence mating system through pollinator attraction and/or self-pollen deposition (e.g., Karron et al. 1997; Chang and Rausher 1998). By reducing the probability of pollen transfer between diverging populations, changes in such floral characters may also act as prezygotic barriers to introgression and thus play a direct role in the speciation process.

Prior to mapping QTLs underlying floral divergence, we constructed a linkage map of a large F_2 hybrid population generated by crossing inbred lines of *M. nasutus* and *M. guttatus* (Fishman et al. 2001). This framework map covers $>$ 85% of the genome, contains codominant as well as dominant markers, and meets high criteria for marker inclusion and placement, providing a strong foundation for the accurate localization of QTLs. For the phenotypic analyses, we measured 16 divergent floral, reproductive, and vegetative characters in a large F_2 mapping population ($N > 500$). We present biometric analyses of all characters, but because nonfloral characters exhibit substantial epistatic breakdown in hybrids (Fishman and Willis 2001), the QTL analyses focus on seven floral characters associated with between-species differences in mating system. Our large sample size provides power to confidently detect and accurately estimate the effects of QTLs explaining as little as 5% of the genetic variance segregating in such an F_2 population (based on 0.6 trait heritability, high linkage map density, and composite interval mapping approach; Z.-B. Zeng, unpubl. ms). We use multitrait composite interval mapping to locate floral QTLs, estimate their effects on individual characters, and assess the extent of pleiotropy (Jiang and Zeng 1995). This multitrait approach acknowledges the developmental integration of floral morphology and provides greater power to detect and estimate small QTL effects. Our analyses generate a detailed picture of polygenic adaptation in a flowering plant and provide insight into the evolution of selfing and the nature of adaptive divergence in the wild.

Materials and Methods

Study System

The yellow monkeyflowers of the *M. guttatus* species complex (section *Simiolus*) are common wildflowers with their center of diversity in western North America (Pennell 1951; Vickery 1978). Extensive morphological variation and the potential for hybridization has complicated taxonomic assignments within *Simiolus*, and the members of the *M. guttatus* complex have been grouped into a few highly variable species and also divided among as many as 20 distinct species (e.g., Pennell 1951). *Mimulus guttatus* ($2n = 28$), the most common species in the complex, is predominantly outcrossing (Willis 1993b; Latta and Ritland 1994). However, routine self-fertilization appears to have evolved at least several times within the species complex (Pennell 1951; Vickery 1978; Fenster and Ritland 1994a). *Mimulus nasutus* Greene ($2n = 28$) is the most widespread and most distinct of the

selfing taxa, producing cleistogamous or nearly cleistogamous flowers (Diaz and MacNair 1998). *Mimulus nasutus* is generally thought to be derived from a *M. guttatus*–like ancestor, but phylogenetic relationships among members of the complex have not been fully resolved (Fenster and Ritland 1994a; note that the taxon we refer to as *M. nasutus* probably corresponds to *M. micranthus* as identified by these authors).

Partial pre- and postmating reproductive barriers isolate *M. guttatus* and *M. nasutus* in the wild. Allopatric populations are more common, but the two species often coexist in seasonally wet areas such as road cuts and ephemeral streambeds. At sympatric sites, potential premating barriers to hybridization include differences in microhabitat and flowering time (Kiang and Hamrick 1978), as well as differences in floral morphology (Ritland and Ritland 1989; Dole 1992), pollen production (Ritland and Ritland 1989; Fenster and Carr 1997), and pollen tube growth (Diaz and MacNair 1999) associated with their divergent mating systems. Despite these isolating mechanisms, hybrids are frequently observed in the wild (e.g., Ritland 1991). Experimental hybridizations indicate that partial postzygotic barriers have developed between *M. nasutus* and *M. guttatus* (Vickery 1964, 1978). In addition, analysis of the partial male and female sterility of the F_1 and F_2 hybrids from the same cross studied here has implicated negative epistatic interactions between heterospecific genomes as the primary source of hybrid breakdown (Fishman and Willis 2001).

Generation of F_2 Mapping Population

To simplify the interpretation of segregating genotypic and phenotypic variation, we crossed a single inbred line of *M. guttatus* with a single inbred *M. nasutus* genotype. The *M. guttatus* parental line (IM62) was derived from an annual, highly outcrossing population from the Oregon Cascades (Iron Mountain: Willis 1993a; Sweigart et al. 1999). This parental line was formed by more than five generations of selfing with single seed descent (Willis 1993b) and is near the outcrossed population mean for floral characters and pollen fertility (J. Willis and A. Kelly, unpubl. data). The *M. nasutus* parental line was derived from a population in north-central Oregon (Sherar's Falls) and maintained for several generations in the greenhouse through autonomous self-fertilization. As expected from the cleistogamous floral morphology of *M. nasutus*, both the Sherar's Falls population and the particular parental line used in this study (SF5.4) are highly inbred (i.e., homozygous at marker loci highly variable in *M. guttatus* populations; Kelly and Willis 1998). The F_2 mapping population was generated by crossing the *M. nasutus* and *M. guttatus* inbred lines (IM62 as pollen parent) to generate F_1 hybrids, then self-pollinating a single F_1 individual.

In March 1997, we grew the F_2 mapping population (N = 600 initially) and F_1 hybrids and parental lines (N = 100 each) in a common garden at the University of Oregon Department of Biology greenhouse. Greenhouse and plant culture conditions were similar to those used during parental line formation and in previous experiments with these populations (Willis 1999a,b). The plants were grown in 2.25-inch (5.6-cm) pots filled with a soil-less potting mix (Sunshine Mix # 2 Sun Gro Horticulture, Bellevue, WA) and placed in a fully randomized design. We planted about five seeds per pot and thinned to the centermost individual after most seeds had germinated (14 days), but did not explicitly measure germination rates or subsequent mortality.

Phenotypic Analyses

We measured 16 floral, vegetative, and reproductive characters on plants that flowered. As an overall estimate of plant size and vigor, we measured the lengths of the first two leaves on each plant at the time of its first flower. For the first four flowers on each plant, we recorded the date of anthesis and measured seven floral size characters associated with shifts in mating system (Fig. 1). The *M. nasutus* plants produced both cleistogamous (generally the first four) and chasmogamous flowers, whereas all *M. guttatus* and hybrid flowers were chasmogamous. Phenotypic and QTL analyses were conducted on plant means for the seven floral size characters. Three components of male fertility—the number of viable pollen grains per flower, the total number of pollen grains per flower, and the fraction of viable pollen grains per flower—were obtained from counts of aniline blue-stained pollen collected from first two flowers on each plant (for methodological details see Fishman and Willis 2001). We quantified autonomous self-fertilization by counting the seeds of the unmanipulated third flower on each plant in the insect-free greenhouse. We estimated maximum female fertility per flower by counting the seeds produced after supplemental pollination of the fourth flower on each plant with *M. guttatus* (IM62) pollen. We then calculated the ratio of autonomous seed production to supplemented seed production (autofertility), which provides a measure of autonomous selfing that is independent of variation in ovule number or maternal resources.

For each character, we calculated the mean and variance of each class (IM62 parent, SF parent, F_1, F_2) and tested the class distributions for normality (Shapiro-Wilks W-test; SAS Institute 1994). Because the F_1 hybrids and the two parental lines are each genetically homogeneous, the phenotypic variances of these classes reflect only environmental variance, whereas the F_2 phenotypic variance reflects both environmental variance and the segregation of alleles at loci differentiating the parental lines. We calculated the environmental variance (V_E) as a weighted average of the parental and F_1 phenotypic variances,

$$V_E = \frac{2\ \mathrm{Var}(F_1) + \mathrm{Var}(IM) + \mathrm{Var}(SF)}{4}. \qquad (1)$$

The environmental standard deviation (ESD) for each character was calculated as the square root of V_E. We calculated the genotypic variance as $V_G = \mathrm{Var}(F_2) - V_E$, then estimated broadsense heritability for each character as $H^2 = V_G/\mathrm{Var}(F_2)$. The environmental and genotypic covariance matrices were estimated with parallel calculations (cov_E = average phenotypic covariance within the parental and F_1 classes and $cov_G = \mathrm{cov}[F_2] - cov_E$). Genetic correlations (r_G) among characters were estimated as $cov_G(i, j)/s_i s_j$, where $cov_G(i, j)$ is the genetic covariance between traits i and j and s_i and s_j are the respective square roots of the genotypic variances of the two traits. Environmental correlations were calculated as

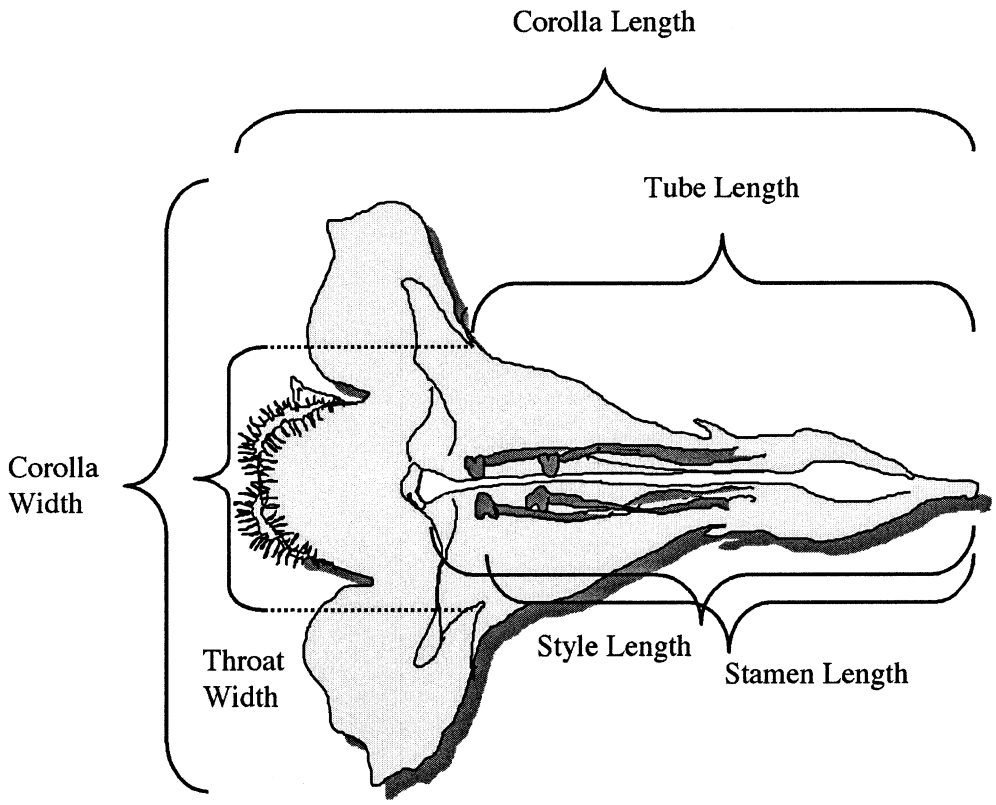

FIG. 1. Cutaway view of a *Mimulus guttatus* flower showing the floral traits measured for quantitative genetic and quantitative trait locus analyses. In addition to the six traits illustrated, we also analyzed stigma-anther separation (style length minus stamen length).

$cov_E(i, j)/s_i s_j$, where $cov_E(i, j)$ is the environmental covariance between traits i and j and s_i and s_j are the respective square roots of the environmental variances of the two traits. These calculations assume no changes in the variance-covariance matrix between generations in the common greenhouse environment. Genetic correlations were not calculated for characters with negative estimates of H^2.

Linkage Map

Previously, we constructed a linkage map by assessing the genotypes of the F_2 population ($N = 526$) at 255 amplified fragment length polymorphism (AFLP), microsatellite, and gene-based markers (for full details of map construction see Fishman et al. 2001). All marker loci mapped to 14 linkage groups, which presumably correspond to the 14 pairs of chromosomes in these *Mimulus* species. We used several rounds of ordering and evaluation in Mapmaker 3.0 (Lander et al. 1987) to construct a framework map consisting of 174 markers that met additional reliability criteria (e.g., low apparent error rates, additivity of intermarker distances) as well as our statistical thresholds for linkage (LOD $\geq$ 6) and order (LOD $\geq$ 2). We also made an effort to alternate dominant markers from both phases with codominant markers, which provides power for QTL detection and estimation comparable to maps consisting entirely of codominant markers (Jiang and Zeng 1997). The linkage map spans 1780 cM Kosambi, has an average interval length of 12 cM, and covers more than 85% of the estimated genome length (Fishman et al. 2001). Although there is significant transmission ratio distortion in localized regions of the linkage map, it does not affect the placement of mapped markers (Fishman et al. 2001). In addition, the distortion is probably not severe enough to diminish our power to detect and accurately estimate QTL effects. In all but one of the distorted regions (the exception being LG11; Fishman et al. 2001), the sample size of even the rarest homozygous marker genotype is more than 75 F_2 individuals.

Quantitative Trait Locus Analyses

We mapped QTLs underlying the seven floral traits with composite interval mapping (CIM; Zeng 1993, 1994) and multitrait composite interval mapping (MCIM; Jiang and Zeng 1995) using QTL Cartographer software (Basten et al. 2002). For each trait, the CIM procedure tests the hypothesis that an interval between adjacent markers contains a QTL affecting the trait, while using multiple regression on additional markers (cofactors) to statistically account for the effects of segregating QTLs elsewhere in the genome. The cofactors included in each CIM model were determined with forward-backward stepwise regression, with the critical P-values set at 0.05. Tests were performed at 2-cM intervals with a flanking window size of 10 cM used to exclude potential cofactors tightly linked to the test interval. The likelihood ratio (LR) test statistic for each interval is $-2 \ln(L_0/L_1)$, where L_0/L_1 is the ratio of the likelihood under the null hypothesis of no QTL to the likelihood under the hypothesis that there is a QTL in the interval. The LR statistic at a genomic position is distributed as χ^2 with 3 df under the null

TABLE 1. Phenotypic data for 16 *Mimulus* traits measured on parental lines and hybrids in a common garden. Floral length and width values are in millimeters. Means, standard errors, and sample sizes (in parentheses) are given for each class. The difference in species means for each character was standardized by its environmental standard deviation (ESD). Broad-sense heritabilities were calculated from the segregational variance in the F_2 hybrid population (see Materials and Methods).

Character	Class				Mean species difference/ESD	Broad-sense heritability (H^2)
	M. guttatus (IM62)	F_1 hybrids	F_2 hybrids	*M. nasutus* (SF 5.4)		
Leaf length	138.41 ± 3.81 (96)	155.35 ± 4.12 (95)	119.13 ± 2.20 (543)	179.04 ± 4.53 (88)	1.01	0.39
Days to flower	30.29 ± 0.35 (97)	27.22 ± 0.47 (97)	36.24 ± 0.26 (563)	30.21 ± 0.51 (90)	0.02	0.49
Tube length	12.05 ± 0.08 (97)	12.05 ± 0.09 (97)	11.09 ± 0.06 (564)	7.03 ± 0.10 (94)	5.50	0.54
Throat width	8.42 ± 0.07 (97)	6.61 ± 0.06 (97)	6.14 ± 0.04 (564)	1.89 ± 0.07 (94)	10.19	0.62
Corolla width	22.29 ± 0.22 (97)	16.86 ± 0.20 (97)	15.76 ± 0.13 (564)	3.35 ± 0.18 (94)	9.74	0.61
Style length	13.67 ± 0.08 (97)	12.90 ± 0.09 (97)	12.04 ± 0.06 (564)	6.75 ± 0.10 (94)	7.94	0.62
Stamen length	11.90 ± 0.08 (97)	11.93 ± 0.07 (97)	10.92 ± 0.05 (564)	7.41 ± 0.09 (94)	5.67	0.60
Stigma-anther distance	1.76 ± 0.08 (97)	0.97 ± 0.05 (97)	1.13 ± 0.03 (564)	−0.66 ± 0.05 (94)	4.42	0.34
Corolla length	24.85 ± 0.17 (97)	21.90 ± 0.19 (97)	20.22 ± 0.12 (564)	9.17 ± 0.19 (94)	8.68	0.62
Viable pollen grains	128.02 ± 8.42 (61)	73.73 ± 4.96 (79)	71.18 ± 3.12 (560)	73.98 ± 7.37 (53)	1.03	0.49
Nonviable pollen grains	51.61 ± 4.00 (61)	99.54 ± 3.86 (79)	96.65 ± 3.11 (560)	24.11 ± 1.97 (53)	0.92	0.84
Fraction viable pollen	0.69 ± 0.02 (61)	0.41 ± 0.02 (79)	0.39 ± 0.01 (560)	0.68 ± 0.03 (53)	0.04	0.39
Total pollen grains	179.62 ± 8.32 (61)	173.28 ± 6.28 (79)	167.83 ± 4.65 (560)	98.09 ± 7.11 (53)	1.42	0.73
Autogamous seed set	20.15 ± 6.07 (60)	117.50 ± 7.90 (80)	43.82 ± 2.34 (530)	365.4 ± 10.4 (51)	5.19	—
Supplemented seed set	159.53 ± 11.0 (60)	162.92 ± 8.54 (77)	88.05 ± 2.84 (541)	384.8 ± 16.0 (51)	2.54	—
Percent autogamy	0.14 ± 0.05 (56)	0.85 ± 0.09 (75)	0.57 ± 0.03 (510)	1.00 ± 0.03 (49)	1.48	0.37

hypothesis (Jiang and Zeng 1995). As an initial screen for QTLs with CIM, we used a rough LR threshold corresponding to a Type I error rate of $\alpha = 0.05$ corrected for multiple tests ($\chi^2_{0.05/M}$, where M is the number of mapped intervals; Zeng 1994).

Because the floral traits were highly genetically correlated and the single-trait CIM analyses identified QTLs for multiple characters in the same mapping interval, we then used MCIM to jointly map QTLs affecting the seven floral size traits. The MCIM procedure is similar to single trait CIM, but the LR test statistic is $-2 \ln(L_0/L_a)$, where L_a is the likelihood under the alternative hypothesis that the interval contains a QTL affecting any of the included traits. MCIM provides additional power and accuracy for mapping QTLs because it takes into account the correlational structure of the phenotypic data (Jiang and Zeng 1995). We used permutation tests (Churchill and Doerge 1994; Doerge and Churchill 1996) to select a LR threshold corresponding to an experimentwise Type I error rate of $\alpha = 0.05$ for the joint mapping ($n = 1000$ permutations). We permuted the phenotypic data across genotypes while maintaining their correlations (i.e., the seven trait values for each individual were permuted as a block).

To determine whether the QTL positions identified by MCIM significantly influenced a particular floral trait, we used the test for pleiotropy proposed by Jiang and Zeng (1995). Given a QTL position, pleiotropy is indicated by the rejection of the null hypothesis of no QTL for more than one trait each tested with no restriction on the others, but with the model parameters estimated jointly by MCIM. The LR test statistics under the null hypotheses for each trait at each position will be χ^2 distributed with 2 df and, because each position is fixed prior to the test, there is no correction for multiple tests along the genome (Jiang and Zeng 1995). We performed this test for each QTL identified by MCIM, defining the test position as the interval between the markers flanking the QTL peak. We used the single trait output from the MCIM analysis to assess $LR = -2 \ln(L_0/L_1)$, where L_1 is the likelihood of a QTL affecting a particular trait, with effects on other traits unconstrained. A LR threshold of 5.99 ($\chi^2_{0.05,2}$) was used to define significant effects on a given trait. Additive (a) and dominance (d) effects of the QTL were recorded at the LR peak in the significant test intervals.

RESULTS

Phenotypic Differences among Species and Hybrids

The *M. guttatus* and *M. nasutus* parental lines were highly differentiated for all floral characters (Table 1), with the mean of the *M. guttatus* line 4–10 environmental standard deviations (ESDs) higher than the *M. nasutus* line. Broadsense heritabilities (H^2) for these characters were moderate, ranging from 0.34 to 0.62 (average 0.56). The corolla width data (Fig. 2) exemplifies the pattern of distribution for the floral characters. Generally, the F_1 and F_2 hybrids had trait means greater than the midparent value (equal to the IM62 parent mean in several cases), suggesting, on average, partial dominance of *M. guttatus* alleles for flower size. Within each class, the individual values for these characters were distributed approximately normally (Shapiro-Wilks W-test, $P > 0.05$), with no evidence of multimodality. Although the F_2 population showed an increase in variance relative to the parental and F_1 classes, both parental extremes were not reconstituted. This suggests the segregation of many genes of small to moderate effect on floral characters.

The parental lines were also differentiated for most male and female reproductive characters, but genetic analysis of these traits is complicated by partial sterility in the F_1 and F_2 generations (Table 1). As discussed in detail elsewhere, low pollen viability and reduced ovule numbers/seed maturation in hybrids appears to result from epistatic incompatibilities between the parental genomes (Fishman and Willis 2001). Pollen viability (viable grains/total grains), the num-

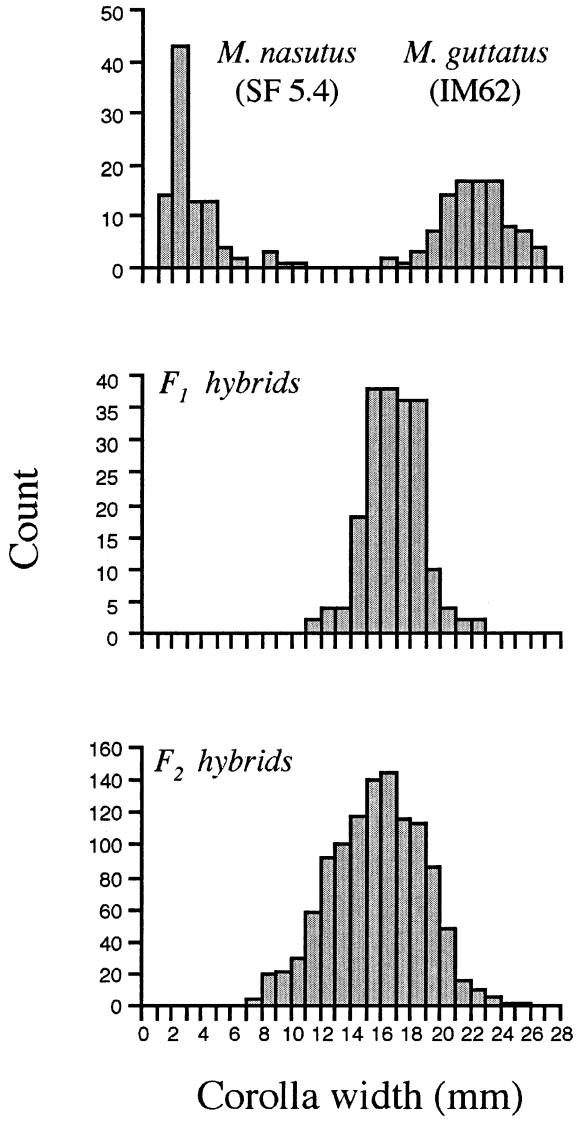

FIG. 2. Phenotypic distributions of corolla width in parental, F_1 hybrid, and F_2 hybrid *Mimulus* populations.

ber of viable grains, and the number of nonviable grains all had F_2 means and distributions inconsistent with an additive-dominance model of inheritance (Fishman and Willis 2001). Total pollen production (viable grains + nonviable grains), in contrast, showed a pattern of inheritance similar to the floral traits (Table 1). The IM62 parent produces twice as many pollen grains as the *M. nasutus* line and, on average, *M. guttatus* alleles appear dominant. However, the negative epistatic interactions affecting male fertility characters in the F_2 generation makes them all poor candidates for QTL analysis.

The parental lines were also widely differentiated for female reproductive characters, with the *M. nasutus* (SF) line producing 2.5 times as many seeds as the *M. guttatus* line after supplemental hand-pollination and almost 20 times as many by autogamous selfing. However, like pollen viability, our measure of maximum female fertility (supplemented seed set) shows strong hybrid breakdown. The F_1 hybrids had intermediate fecundities, with the average near the *M. guttatus* (lower) mean. In contrast, the F_2 hybrids matured half as many seeds as the *M. guttatus* parent and the distribution of individual seed numbers was strongly skewed toward zero. The resultant reduction in segregational variance leads to a negative estimate of H^2 for this character. Because both the parental classes and the F_1 hybrids were approximately normally distributed around their means (Shapiro-Wilks W; $P > 0.05$), simple transformations of the data are unlikely to find a more appropriate underlying scale. Autogamous seed set and percent autofertility (selfed seeds/maximum seeds) showed a similarly epistatic pattern, with unexpectedly low hybrid means relative to an additive-dominance model and skewed distributions in the F_2 generation. The two measures of overall plant performance (leaf length and days to first flower) also showed epistatic breakdown, with the F_2 hybrids flowering later (and with smaller leaves) than either parent (Table 1). In contrast, the F_1 hybrids flowered significantly earlier than either parental class. The epistatic breakdown of these female fertility and vegetative characters also precludes CIM analysis in an F_2 hybrid population.

Genotypic and Environmental Correlations

Strong genotypic correlations indicate pleiotropy or linkage of genes affecting the different floral size characters (Table 2). With the exception of pairs involving stigma-anther separation (SA), which had relatively low heritability ($H^2 = 0.34$), all pairs of floral traits had r_G-values > 0.87. SA is moderately correlated with style length ($r_G = 0.56$) and least correlated with stamen length ($r_G = 0.18$), with r_G-values of 0.21–0.36 for associations with the other floral size measures. Environmental correlations (r_E) showed a parallel pattern but lower values (Table 2). The first six floral characters were highly correlated with one another ($r_E > 0.65$), and style length is the character most correlated with SA ($r_E = 0.44$). The environmental correlation between SA and stamen length was negative (-0.20), consistent with the calculation of SA as style length minus stamen length.

Male fertility measures (e.g., viable pollen, total pollen) were also genetically correlated with the six floral size characters with high heritability ($r_G > 0.58$). Percent autofertility was only weakly correlated with the floral size characters ($r_G = 0.18$–0.28). However, a weak negative genetic correlation with stigma-anther separation ($r_G = -0.14$) and strong correlations with viable pollen grains and total pollen grains ($r_G > 0.5$) suggest that the functional contribution of these traits to self-pollination may be detectable despite hybrid breakdown in female fitness. Environmental correlations between male or female fertility traits and floral characters were generally very low (r_E range: -0.12 to 0.27).

The two vigor characters showed strong and consistent patterns of genotypic correlation with floral and male fertility characters, suggesting loci or interacting sets of loci with broad pleiotropic effects. Genotypic correlations of leaf length (LL) with the flower size measures and male fertility were positive and very high (r_G range: 0.94 to 1.1). Genotypic correlations of flowering time (days to first flower; FT) with floral size characters, male fertility, and leaf length were strongly negative (i.e., early flowering genotypes tended to

TABLE 2. Genotypic (above the diagonal) and environmental (below) correlations between characters in interspecific F_2 hybrids.

Character	LL	FT	TL	TW	WW	SL	AL	SA	FL	VI	NV	PV	TP	SS	FS	PA
Leaf length (LL)		−0.64	1.06	1.01	0.94	0.93	1.05	0.06	0.99	0.98	0.56	0.75	0.94	—	—	0.25
Days to flower (FT)	−0.20		−0.61	−0.57	−0.51	−0.53	−0.50	−0.26	−0.50	−0.73	−0.38	−0.50	−0.68	—	—	0.08
Tube length (TL)	0.45	0.13		0.91	0.93	0.89	0.94	0.21	0.98	0.67	0.48	0.51	0.71	—	—	0.20
Throat width (TW)	0.40	0.23	0.68		0.96	0.93	0.92	0.36	0.94	0.65	0.39	0.58	0.64	—	—	0.18
Corolla width (WW)	0.47	0.14	0.71	0.76		0.93	0.96	0.28	0.97	0.68	0.39	0.56	0.65	—	—	0.15
Style length (SL)	0.47	0.11	0.85	0.62	0.69		0.93	0.53	0.90	0.65	0.39	0.57	0.64	—	—	0.15
Stamen length (AL)	0.38	0.11	0.83	0.67	0.63	0.79		0.20	0.95	0.68	0.29	0.65	0.59	—	—	0.23
Stigma-anther distance (SA)	0.21	0.01	0.13	0.01	0.18	0.44	−0.20		0.23	0.17	0.39	0.00	0.37	—	—	−0.15
Corolla length (FL)	0.51	0.13	0.88	0.74	0.85	0.84	0.79	0.18		0.68	0.47	0.51	0.71	—	—	0.15
Viable pollen grains (VI)	0.10	0.13	0.08	0.18	0.19	0.15	0.19	−0.04	0.15		0.23	0.69	0.72	—	—	0.50
Nonviable pollen grains (NV)	0.21	−0.11	0.18	0.15	0.18	0.18	0.13	0.09	0.14	−0.12		−0.29	0.84	—	—	−0.13
Fraction viable pollen (PV)	0.00	0.16	−0.03	0.03	0.03	0.01	0.06	−0.07	0.03	0.74	−0.57		0.17	—	—	0.49
Total pollen grains (TP)	0.20	0.07	0.17	0.25	0.26	0.22	0.24	0.01	0.21	0.86	0.41	0.39		—	—	0.18
Autogamous seed set (SS)	0.07	0.05	0.02	0.01	0.14	0.02	0.01	0.02	0.09	0.21	−0.22	0.25	0.08		—	—
Supplemented seed set (FS)	0.04	−0.07	0.06	0.05	0.08	0.00	0.04	−0.06	0.02	0.13	0.01	0.11	0.13	0.34		—
Percent autogamy (PA)	0.00	0.09	−0.10	−0.04	0.03	−0.07	0.00	−0.11	0.02	0.07	−0.22	0.17	−0.05	0.45	−0.37	

have relatively large leaves and flowers). Leaf length showed moderate environmental correlations with flower size characters (r_E range: 0.4 to 0.5), but environmental correlations involving flowering time were generally low (r_E range: −0.2 to 0.2).

Quantitative Trait Locus Analyses

Identification of floral quantitative trait loci.—As a first step, we used composite interval mapping (CIM) to map QTLs underlying individual floral traits. We then used multitrait CIM to map QTLs for all floral traits in a single joint analysis. Given the strong genotypic and environmental correlations among traits, the joint mapping (MCIM) approach is more appropriate (Jiang and Zeng 1995). Because the two methods identified overlapping sets of QTLs, but MCIM has more power to detect and estimate the effects of those QTLs on all traits, we focus on the joint mapping results here.

Based on the likelihood-ratio statistic (LR) profile of the joint MCIM model (Fig. 3), we identified 24 putative QTLs affecting one or more floral traits. QTLs were located on all 14 linkage groups. Twenty-three LR peaks exceeded the permutation-based detection threshold of 41.17, but we excluded two of those and also accepted several lower peaks based on the single trait LR profiles produced by CIM and MCIM. On LG1, we identified only a single QTL (~50–60 cM) because the slight dip between adjacent peaks was associated with a single codominant (more constrained) marker and the phenotypic effects of putative QTLs at the two peaks were indistinguishable. On LG2, we accepted a QTL at 116 cM with a marginally significant LR statistic of 40. In the CIM analyses, two flower width traits both had LR peaks greater than our standard single-trait LR threshold ($\chi^2_{2,0.0003} = 18.54$; see Materials and Methods) at this position. The three adjacent peaks on LG3 were accepted as separate QTLs because each had distinct effects on corolla width and other characters. We accepted a QTL on LG4 that had a marginally significant joint LR of 36 but significant QTL peaks for two flower length traits in the single trait CIM. On LG5, two peaks exceeded the joint LR threshold, but the one at about 85 cM appears to be a spurious ghost peak between the peak at 110 cM and an insignificant peak at about 64 cM. Both the single-trait LR profiles from MCIM and the CIM of individual traits identified a QTL peak at 64 cM for corolla width but no 85 cM peak in LR for any trait, so we place the putative QTL at the former location. Finally, we accepted a marginally significant peak (LR = 39.5) at about 36 cM on LG11. Significant QTL peaks at this location were independently identified in the single trait CIM analyses of both stigma length and stigma-anther separation.

Pleiotropy of quantitative trait loci.—We used Jiang and Zeng's (1995) test for pleiotropy to determine which traits each floral QTL affected. The additive and dominance effects of the significant QTLs underlying each trait are given in Table 3 (many of these QTLs also had nonsignificant but above-background peaks for the other traits). Almost all (21 of 24) of the floral QTLs identified by MCIM had significant effects on multiple traits. Five QTLs had very broad pleiotropic effects, significantly influencing all of the measured floral traits, and two more affected all traits other than stigma-

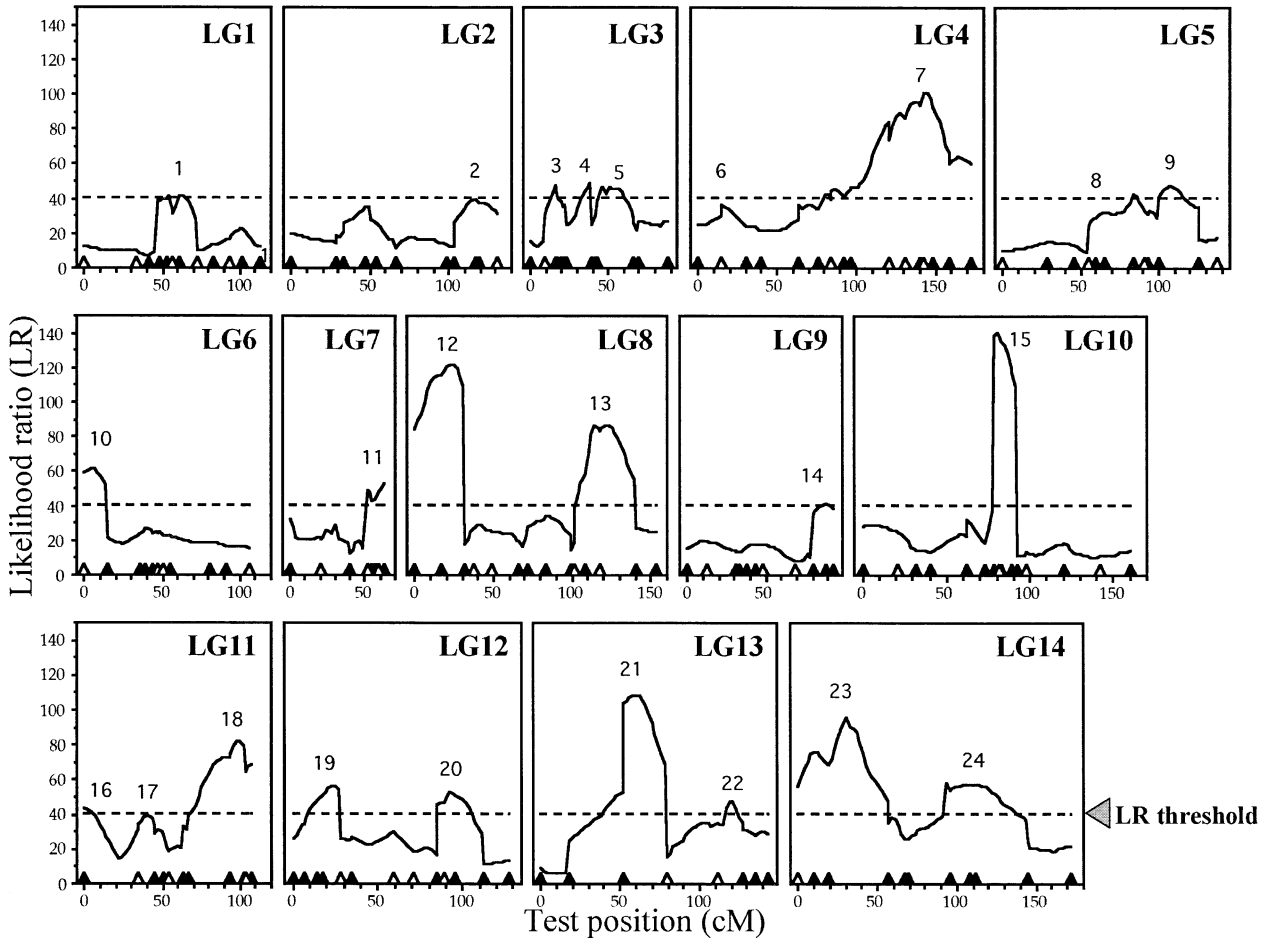

FIG. 3. Likelihood-ratio (LR) test statistic profile from multitrait composite interval mapping of seven floral traits in the *Mimulus nasutus* × *M. guttatus* F_2 population. The dashed line indicates the LR significance threshold for joint mapping generated by permutation analysis ($\alpha < 0.05$, experimentwide). We identified 24 quantitative trait loci across the 14 linkage groups, primarily based on peak height relative to the LR threshold (see text for explanation of individual peaks). The positions of mapped markers (Δ, codominant; ▲, dominant) are shown along each linkage group.

anther separation. Other QTLs had effects restricted to particular aspects of floral size (e.g., QTL2 and QTL4 significantly affected only the two floral width characters). Although several of the highly pleiotropic QTLs affected stigma-anther separation, this character also accounted for two of the three QTLs with effects on only a single trait and a disproportionate number of those with effects on two traits. The large number of floral QTLs with pleiotropic effects translates into a similarly polygenic basis for each individual trait (mean number of QTLs = 13, range = 11–15).

Direction of quantitative trait locus effects.—In general, the direction of QTL allelic effects was consistent with the phenotypic differences between the parental species (i.e., the smaller-flowered *M. nasutus* carried the minus allele). However, there were a few highly pleiotropic QTLs (e.g., QTL9 and QTL21) where the *M. nasutus* allele had opposite effects on all characters (positive values in Fig. 4). At two other highly pleiotropic QTLs (QTL15 and QTL23), the *M. nasutus* allele reduced all length and width characters, but had opposite effects on stigma-anther separation (by reducing stamen length to a greater degree than style length).

Dominance relationships.—Many of the floral QTLs showed at least partial dominance of one parental allele, but no overall pattern of directional dominance was evident (Table 3). For example, the *M. nasutus* allele at QTL8 has partially to completely recessive effects on five floral characters, whereas the *M. nasutus* allele at QTL17 has completely dominant effects on style length (Table 3). Two QTLs (QTL16 and QTL21) displayed strong overdominance, as indicated by positive dominance effects much greater than the absolute magnitudes of the corresponding additive effects. This could reflect true overdominance, but is more likely to result from the unusually low density of markers (particularly codominant markers) in these regions of the linkage map. Low marker resolution may reduce power to estimate heterozygous effects accurately and may also increase the probability of associative overdominance due to multiple QTLs linked in repulsion.

Magnitude of quantitative trait locus effects.—To assess the magnitude of QTLs, we scaled their additive effects in two ways (Fig. 4). For studies of divergence, one biologically relevant measure of QTL size is the proportion of the species difference explained by the substitution of alternative alleles. For QTLs affecting each floral trait, we first standardized 2*a*

TABLE 3. Additive (*a*, above) and dominance (*d*, below) effects of the 24 quantitative trait loci (QTLs) on each floral character. QTL effects are only shown if the single-trait likelihood ratio at a QTL located by joint mapping (MCIM) exceeded the significance threshold of 5.99 ($\chi^2_{0.05,2}$). *Mimulus guttatus* homozygous genotypes were scaled to zero and *M. nasutus* homozygotes to 2*a*. Thus, negative values of *a* indicate that *M. nasutus* carries the minus allele.

QTL	Position (LG, marker, cM)	Throat width	Corolla width	Tube length	Corolla length	Style length	Stamen length	Stigma-anther
1	1, 7, 61							−0.115
								−0.057
2	2, 10, 114	−0.195	−0.672					
		−0.024	−0.046					
3	3, 2, 9		−0.762			−0.244	−0.169	−0.109
			0.203			0.050	0.133	−0.102
4	3, 5, 19	0.442	1.184					
		−0.003	0.060					
5	3, 7, 38	−0.221	−0.841			−0.223		−0.178
		−0.047	−0.183			−0.182		−0.237
6	4, 2, 17		−0.395	−0.275	−0.540			
			−0.139	−0.094	−0.151			
7	4, 13, 148			0.231				0.031
				−0.031				0.159
8	5, 5, 64	−0.155	−0.823	−0.245	−0.688	−0.245	−0.182	
		0.110	0.433	0.156	0.262	0.231	0.234	
9	5, 10, 103	0.170	0.684	0.408	0.759	0.530	0.402	0.225
		−0.192	−0.787	−0.343	−0.577	−0.444	−0.233	−0.096
10	6, 1, 4						0.186	−0.190
							0.090	0.153
11	7, 8, 63	−0.182	−0.694	−0.255	−0.782	−0.463	−0.248	−0.219
		0.130	0.061	−0.002	−0.040	−0.055	0.015	−0.072
12	8, 2, 25	−0.146				−0.331	−0.148	−0.185
		0.160				0.257	0.162	0.084
13	8, 13, 116	−0.246						0.122
		0.170						0.011
14	9, 12, 89	−0.085	−0.317	−0.231	−0.448	−0.269	−0.138	−0.150
		−0.185	−0.473	−0.360	−0.603	−0.165	−0.301	0.149
15	10, 8, 81	−0.369	−1.377	−0.268	−0.915	−0.213	−0.361	0.172
		0.076	0.104	0.128	0.250	0.157	0.106	0.027
16	11, 1, 10			−0.632	−1.467	−0.623	−0.625	
				1.367	2.95	1.367	1.192	
17	11, 2, 34					−0.246		−0.190
						−0.252		−0.120
18	11, 9, 100		−0.611	−0.382	−0.719		−0.265	
			−0.294	−0.071	−0.056		0.011	
19	12, 1, 14			0.199				
				−0.095				
20	12, 11, 96							−0.151
								−0.141
21	13, 3, 66	0.157	0.440	0.550	0.667	0.259	0.167	
		0.550	1.427	0.514	1.294	0.557	0.609	
22	13, 7, 127	−0.112						−0.114
		−0.229						0.019
23	14, 2, 16	−0.495	−0.736	−0.650	−1.366	−0.643	−1.146	0.115
		0.342	0.717	0.589	1.248	0.655	1.027	0.084
24	14, 9, 112	−0.254	−0.272	−0.222	−0.313		−0.091	
		0.019	0.481	0.233	0.493		0.214	

by the difference in the parental means (Fig. 4a). For floral width traits, the leading QTLs each explained only 15% of the species difference and most QTLs (>75%) had homozygous effects smaller than 10% of the species difference. In total, the QTLs affecting these characters account for only about half of the species difference, suggesting that many smaller (undetected) QTLs or epistatic interactions may also contribute to divergence in flower width. The leading QTLs for flower length characters (QTL16 and QTL23) were larger, together spanning one-half to one-third of the species difference (tube length, 51%; total flower length, 36%). QTL effects on style length were more moderate, but all together accounted for a similar total amount of the species difference (~75%; Fig. 3a). The leading length QTLs had disproportionate effects on stamen length, with the substitution of *M. nasutus* alleles at QTL23 and QTL16 generating reductions

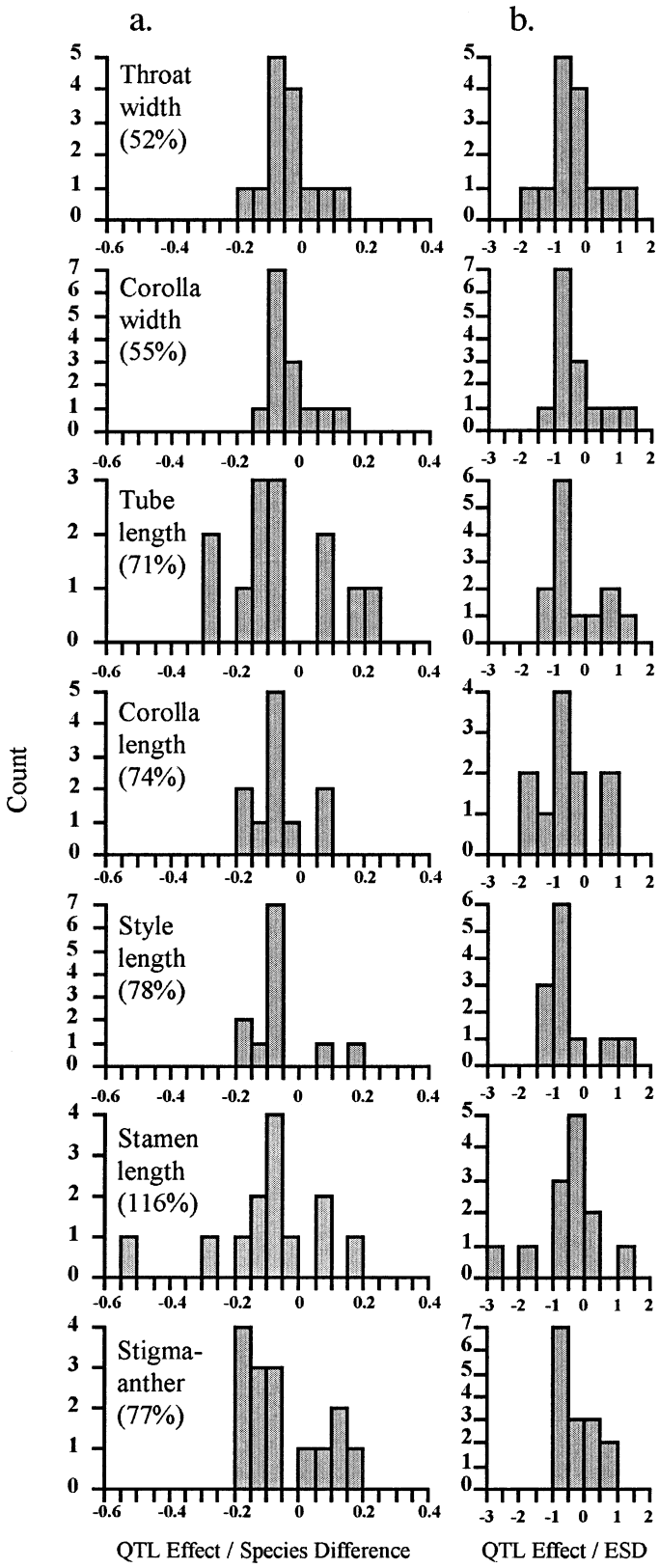

FIG. 4. The distributions of homozygous effects (2*a*) at individual quantitative trait loci (QTLs) scaled to (a) the difference in parental line means and (b) the environmental standard deviation (ESD) for each trait. QTLs at which *M. nasutus* alleles decrease the trait value have negative values of 2*a*. The total percentage of the species difference explained by all QTLs detected for each trait is given in parentheses.

in stamen length equivalent to 51% and 27%, respectively, of the difference between the parental species. Not surprisingly, stamen length was the only character for which the detected QTLs explained all of the difference in the species means. Stigma-anther distance was not as strongly affected by any single QTL, but seven QTLs with effects in the expected direction (*M. nasutus* minus) each accounted for more than 10% of the species difference in this character. The relatively large number of moderately sized QTLs affecting stigma-anther separation may reflect some aspect of this character's genetics or development, but the relatively low measurement resolution (and low heritability) of this character may also constrain the estimation of QTL effects.

A second measure of the evolutionary impact of a novel QTL allele is the magnitude of its effects relative to variation within the background population. If the phenotypic distance between species is large, even QTL alleles with quite small effects relative to the current species difference may have appeared as major mutations in an ancestral population. One way to assess this is to consider QTL effects relative to the environmental component of phenotypic variance. In Figure 4b, we show the distribution of QTL homozygous effects (2*a*) for each floral character standardized by the environmental standard deviation (ESD) for that character. At the majority of QTLs, the substitution of one parental genotype for the other caused a change in phenotype equivalent to 0.5–1.0 ESDs. Only a single QTL had a homozygous effect greater than 2 ESDs (QTL23 on stamen length).

DISCUSSION

The Genetic Architecture of Floral Traits Associated with Selfing in Mimulus

Like many sister taxa with divergent mating systems, *M. guttatus* and *M. nasutus* are widely differentiated for multiple floral and reproductive characters. Autogamous self-fertilization in *M. nasutus* is associated with striking reductions in corolla size and stigma-anther separation, as well as with changes in the production of male and female gametes (Table 1). Because the selfing and outcrossing members of the *M. guttatus* complex are generally interfertile at the level of hybrid seed production, the genetic architecture of their differences has been well characterized with traditional quantitative genetic (biometric) methods (MacNair and Cumbes 1989; Fenster and Ritland 1994). Our phenotypic data on hybrids between *M. nasutus* and *M. guttatus* corroborate the general patterns observed in these previous studies: interspecific variation in floral traits is moderately heritable (Table 1), relatively low segregational variance in the F_2 generation rules out single gene control (Fig. 2), and phenotypic associations among traits often reflect strong genetic correlations (Table 3). We also observe average dominance of the *M. guttatus* (larger) phenotype for most floral traits, similar to the pattern observed in crosses between *M. guttatus* and selfer *M. cupriphilus* (MacNair and Cumbes 1989), but opposite to that reported for several other crosses between selfers and *M. guttatus* (Fenster and Ritland 1994b). While illustrating the complexity of mating system as a trait, this aggregate view of the genetic architecture of divergence leaves several important issues unresolved. How polygenic are floral traits?

Do major QTLs play a role in the evolution of floral traits associated with selfing? Are the observed genetic correlations among floral traits due to pleiotropy? What are the dominance relationships of alleles at individual QTLs? By mapping and characterizing QTLs underlying floral divergence between *M. guttatus* and *M. nasutus*, we explicitly address these questions and can begin to infer past evolutionary processes.

Quantitative trait locus number.—The changes in floral morphology associated with the evolution of selfing in *M. nasutus* are highly polygenic. We identified 24 QTLs underlying the differences between *M. nasutus* and outcrosser *M. guttatus* in seven floral traits (Table 3), and almost all of these QTLs significantly affected more than one floral character (for a total of 91 significant QTL effects). We detected at least 11 QTLs underlying each floral trait and, with the exception of stamen length, the QTL effects on each trait summed to less than 80% of the difference between the parental species. This unexplained difference suggests either the action of additional QTLs of individually undetectable effect or genetic background effects and/or epistatic interactions among detected QTLs. The high-coverage of our linkage map and large F_2 mapping population provides power for composite interval mapping of QTLs explaining as little as 3% of the phenotypic variance in a given floral trait. It follows that any undetected QTLs have even smaller effects and are probably very numerous. For example, in the absence of epistasis, at least 18 additional QTLs with effects on corolla width just smaller than the smallest detected QTL (2.5% of species difference) would be required to explain the divergence in this character. Because all methods of estimating gene number are inherently biased toward underdetection of QTLs and overestimation of QTL effects (Beavis 1994; Zeng 1994) and each QTL region may contain multiple linked genes, the large number of QTLs we detected is an absolute minimum estimate of gene number.

The number of QTLs identified here is similar to or greater than previous biometric estimates of the effective number of factors controlling floral characters in the *M. guttatus* species complex. For example, Fenster and Ritland (1994b) calculated a minimum of 13.7 factors (range 7.5–42.5) underlying corolla width divergence between an annual, outcrossing *M. guttatus* similar to our Iron Mountain population (which they termed *M. nasutus*; for illustration of this identification see Ritland and Ritland 1989) and selfer *M. micranthus* (probably the same species as our *M. nasutus*). In contrast, MacNair and Cumbes (1989) estimated that a minimum of three to seven factors were involved in the reductions in flower traits that cause autogamous selfing in *M. cupriphilus*, although other traits associated with its adaptation to heavy-metal sites appeared to be under the control of single or major loci. An early QTL study of floral divergence between *M. guttatus* and *M. platycalyx*, a selfer with relatively large flowers but no stigma-anther separation, found only six QTLs (with a total of 10 significant effects) affecting the five floral characters measured (Lin and Ritland 1997). It is conceivable that divergence between *M. guttatus* and *M. platycalyx* has involved far fewer genes than the wholesale reductions in floral morphology we see in *M. nasutus*. However, although both *M. platycalyx* and *M. nasutus* are equally divergent from *M. guttatus* for stigma-anther separation, we found far more QTLs (15 vs. two) underlying this trait in our mapping population. In addition to any differences in the evolutionary history of these two selfers, this discrepancy illustrates the improved power of a high-coverage linkage map, large F_2 mapping population and composite interval mapping approach. Investigation of the orthology of QTLs involved in the evolution of selfing in these two species awaits comparative mapping studies.

Evidence for major quantitative trait loci?.—The highly polygenic nature of floral divergence between *M. guttatus* and *M. nasutus* revealed by our mapping does not automatically rule out an important evolutionary role for QTLs of large effect. In addition, previous biometric analyses of selfer $\times$ outcrosser hybrids have suggested that 32–45% of the F_2 variance in stigma-anther separation could be explained by a major QTL despite a large number of segregating factors (Fenster and Ritland 1994b). The QTLs that we detected had a broad range of effects on individual traits, ranging from quite large (QTL23; 51% of the species difference in stamen length) to very small (QTL14; 2.6% of the divergence in corolla tube width). Every floral trait had at least one QTL explaining more than 14% of the species difference, but the vast majority of effects were small relative to the total divergence (Table 3, Fig. 4). In general, different QTLs had the leading effects on different characters. However, because most QTLs affected multiple characters it is difficult to rank their total effects on floral morphology.

The debate over the genetic architecture of adaptation and speciation has often been couched in terms of the existence of major genes (Orr and Coyne 1992). Although QTLs that explain nearly all of the divergence in a particular trait are clearly major factors in the recent evolutionary history of that trait, the definition of QTLs of more moderate effect depends on one's perspective. In reviewing early QTL studies, Tanksley (1993) characterized QTLs as potentially major if they explained $>10\%$ of the phenotypic variance in the mapping population and found that such QTLs were quite common in crop plants. Because the model estimation in composite interval mapping includes cofactors controlling for genetic variation at unlinked loci, we cannot directly determine the proportion of the F_2 variance explained by an individual QTL detected with CIM or MCIM. To assess QTL effects in terms of the phenotypic variance, we reanalyzed each trait with interval mapping and examined the QTL positions previously identified by MCIM. In the interval mapping results, QTLs had r^2-values ranging from 0.001 to 0.32. QTL16, which appears overdominant, explained over 25% of the variance in each of four floral length traits and QTL23 explained 24% of the variance in stamen length. No other QTL accounted for more than 10% of the F_2 variance of any trait. Because interval mapping detected fewer QTLs and may be more likely to overestimate effects (Zeng 1994), this is a liberal test for major genes. By this criterion, little if any of the F_2 variance is explained by QTLs with major effects.

For those interested in the building blocks of adaptive divergence, a more intuitive measure of QTL size is the effect of the substitution of alternative alleles relative to the total divergence between populations or species (e.g., Orr 2001). Considered in this way, substituting *M. nasutus* alleles at a few of our QTLs (e.g., QTL23, QTL15) would certainly ap-

pear to generate substantial steps (>20%) toward the reduced selfer floral morphology. However, because models of adaptive evolution provide no clear criterion for defining major versus minor QTLs (Barton and Keightley 2002), it is difficult to differentiate these leading QTLs from the majority with smaller effects on this scale.

Perhaps the most relevant criterion for identifying potential major genes is the magnitude of the phenotypic change caused by the substitution of alternative QTL alleles relative to the phenotypic variation within populations (Mackay 1996; True et al. 1997; Zeng et al. 2000). The debate about the role of major genes in adaptation contrasts phenotypic discontinuities (of whatever absolute size) versus gradual transitions (Orr and Coyne 1992). Thus, major QTL alleles should generate qualitative differences in phenotype, such that a gradation of forms does not occur. Using this criterion, True et al. (1997) defined a major QTL as one at which alternative homozygous genotypes differ in phenotype by more than 3.28 ESDs. Although our *Mimulus* species are separated by as many as 10 ESDs for some floral characters (Table 1), even the largest of the floral QTLs in this study does not qualify as a major factor by this criterion (Fig. 4b). To generate statistically nonoverlapping homozygous classes, a corolla width QTL would need to have an effect greater than 32% of the species difference, but the largest QTL for this character is about half that size. For characters with less divergence and/or lower heritability, such as stigma-anther distance, a major QTL would need to explain >60% of the difference between the parental lines. Considered relative to the standing variation in an ancestral population, our scaling of QTL effects on each trait to ESDs calculated from homogeneous lines in a controlled greenhouse environment is actually very generous. For example, the coefficient of variation (CV) of corolla width at the Iron Mountain *M. guttatus* population during a single season was at least twice the corolla width CV of the IM62 parental line grown in the greenhouse (CV = 0.20, n = 462 and CV = 0.098, n = 97, respectively; L. Fishman and J. H. Willis, unpubl. data). Thus, it is extremely unlikely that substitution of a *M. nasutus* genotype at any of the QTLs we identified could have had the kind of saltational effects characteristic of major genes. The improbability of major genes underlying floral trait divergence between *M. nasutus* and *M. guttatus* is further underlined by the fact that the QTLs identified here span large genomic regions (>5 cM) and multiple genes may contribute to the estimates of QTL effect.

Because the floral QTLs identified in this study generally affect multiple characters, it is also important to consider their effects on the multivariate distribution of phenotypes when evaluating their potential as major genes. Theoretically, a QTL with minor effects on each of several correlated traits could appear as a major factor in its total effect on the floral phenotype. For this to occur, alternative genotypes at the QTL would need to be distinguishable in multidimensional phenotypic space. In this study, the environmental correlations among floral size traits are uniformly positive (Table 2), as are the phenotypic correlations among floral traits in the wild parent population of *M. guttatus* (L. Fishman and J. H. Willis, unpubl. data). Thus, a novel QTL allele that decreased corolla width but increased corolla length, for example, might generate a distribution of phenotypes distinguishable from the alternative genotype or background population. In our study, however, none of the detected QTLs have effects strikingly counter to the axes of covariance among floral size traits (Tables 2, 3). The *M. nasutus* alleles at several QTLs increase stigma-anther separation (SA) while decreasing overall floral size, but this effect does not oppose the (generally low) environmental correlations between SA and other traits (Table 2). Although we cannot rule out the possibility that floral QTLs have major effects on other characters such as selfing rate or fitness (see below), it seems clear that major genes do not account for the overall divergence in flower size between *M. nasutus* and *M. guttatus*.

Pleiotropy.—Multiple floral characters have diverged in parallel between *M. guttatus* and *M. nasutus*. Strong genotypic correlations among corolla size characters in the segregating F_2 population suggest either pleiotropy or linkage of genes affecting different aspects of floral morphology (Table 2). The location of significant QTL peaks for multiple characters in regions identified by joint mapping (MCIM) points to a pleiotropic basis for the observed associations, but we cannot rule out close linkage (within marker intervals).

Although moderately genetically correlated with other floral traits (Table 2), stigma-anther separation appears to be the floral trait most able to evolve independently. We identified at least five QTLs that affect this trait, but do not have substantial effects on other floral characters (Table 3). We discuss the consequences of this genetic architecture for mating system evolution in a later section.

Dominance and epistasis.—In this study, we found that the floral size characters show a pattern of inheritance consistent with partial dominance, on average, of the *M. guttatus* (larger) genotype. Dominance of presumed ancestral genotypes has been observed in other quantitative genetic analyses of traits associated with the evolution of selfing in *Mimulus* (MacNair and Cumbes 1989). These observations suggest that the alleles recruited during the evolution of the small, selfing flowers in the inbreeding species are recessive on average. In contrast, Fenster and Ritland (1994) found little or no average dominance in several crosses among selfing and outcrossing species of the *M. guttatus* complex and suggested that this could reflect the evolution of dominance relationships during the transition to selfing. This mapping study provides the opportunity to understand patterns of dominance in terms of the effects of individual QTLs. Contrary to the pattern of inheritance inferred from the phenotypic distributions of parents and hybrids, we find no bias in dominance toward either parent's alleles. Many of the QTLs found in this study (nine of 24) show strong directional dominance, but the direction of dominance is split more or less evenly between the two parental species (Table 3). However, *M. nasutus* alleles appear recessive at the two directionally dominant QTLs with the largest overall effects of flower size (QTL23 and QTL9), which may partly account for the phenotypic pattern. The diversity of the dominance estimates among our many QTLs illustrates that individual genetic factors may have effects quite different than the aggregate estimate for polygenic traits.

Our finding of no pattern of directional dominance at individual QTLs is consistent with the partially to completely

inbreeding mating systems of our study species. In a large outcrossing population, the probability of fixation of completely recessive advantageous new mutations is much less than that for favorable mutations with some expression in heterozygotes, a phenomenon known as Haldane's sieve (Haldane 1927; Turner 1981). In contrast, advantageous recessive mutations enjoy increased rates of fixation in partially selfing populations. With high selfing, the probability of fixation is roughly independent of the degree of dominance and the entire spectrum of advantageous mutations may be recruited during adaptive evolution (Charlesworth 1992). Therefore, although Haldane's sieve may have hindered the fixation of recessive alleles very early in the transition to selfing, later fixations would be unconstrained and the QTL effects may be a fairly random sampling of the dominance spectrum of advantageous mutations. In addition, Haldane's sieve predictions may not hold if newly favorable alleles are recruited from standing genetic variation (Orr and Betancourt 2001), and this source of adaptive variation may also contribute to the broad distribution of dominance effects that we observe.

The phenotypic distributions of all floral size traits show significant deviations from an additive-dominance model of gene action, but to much less a degree than fertility and vegetative characters (Table 1; Fishman and Willis 2001). On average, F_2 hybrids have slightly larger flowers than predicted from the F_1 and parental phenotypic distributions. However, the epistatic effects on flower size in the segregating F_2 population appear unrelated to the epistatic breakdown of hybrid seed production and pollen fertility caused by Dobzhansky-Muller incompatibilities (Fishman and Willis 2001), because most floral characters show positive rather than negative genetic correlations with fertility (Table 2). Nonadditive interactions among QTLs may account for the portion of the total difference between species left unexplained by summing the effects of individual QTLs. Epistasis or threshold expression may be particularly important for corolla width. Plants of *M. nasutus* often make cleistogamous flowers with no corolla flare, especially at the first two flowering nodes (Diaz and MacNair 1998). In contrast, all F_2 hybrids in our mapping population made *M. guttatus*–like open flowers of varying dimensions (Fig. 2). This discontinuity in corolla shape raises the possibility that cleistogamy may result from the synergistic effects of *M. nasutus* alleles at many different floral QTLs or from interactions between genotypes at floral QTLs and the *M. nasutus* genetic background or developmental environment.

We do not explicitly estimate epistatic interactions among floral QTLs in the analyses presented here, but the use of unlinked cofactors in composite interval mapping accounts for genetic background effects to some degree (Zeng 1994). One method for quantifying nonadditive interactions among QTLs is multiple interval mapping (MIM), which includes epistatic parameters in QTL model construction and selection (Kao et al. 1999; Zeng et al. 1999). A variant of MIM that allows joint mapping of multiple traits is being developed (Z.-B. Zeng, pers. comm.) and could be used to search for interactions among the floral QTLs identified in this study.

Inferences about the Evolution of Selfing

In this study, we focused on two kinds of floral traits that frequently change during the evolution of selfing—those that directly affect autogamous self-pollen deposition (e.g., stigma-anther separation) and those that may affect both self-pollination and pollinator attraction (e.g., corolla width). Stigma-anther separation has been shown to be negatively correlated with autofertility and selfing rate both among individuals (e.g., Kohn and Barrett 1994; Karron et al. 1997; Chang and Rausher 1999) and among populations or species (Barrett et al. 1996). In the *M. guttatus* species complex, the selfing taxa have reduced stigma-anther separation and increased autofertility rates relative to *M. guttatus* (Ritland and Ritland 1989). A similar negative relationship between SA and self-fertility is seen among populations of *M. guttatus* (Dole 1992; Carr and Fenster 1994) and, despite substantial male and female sterility, in our *M. nasutus* × *M. guttatus* F_2 mapping population (Table 2). Therefore, QTLs that affect stigma-anther separation are candidate mating-system modifiers and their genetic properties may provide clues to how and why selfing has evolved. Reductions in overall flower size also often accompany the evolution of selfing, but the functional relationships between corolla size characters and either autofertility or selfing rates in natural populations are less clear. In an obligate selfer such as *M. nasutus*, reduced corollas may have evolved as a direct consequence of selection for self-fertilization prior to anthesis (e.g., Fishman and Wyatt 1999), a correlated response to selection on other floral characters affecting selfing rate, or a response to selection on resource allocation once population selfing rates were high. Because corolla size can affect pollinator visitation in *Mimulus* (Leclerc-Potvin and Ritland 1994), mating system modifiers that increase selfing rate by reducing flower size may also reduce outcross pollen donation.

Inbreeding depression is high in *M. guttatus* populations (>0.6; Dole and Ritland 1993; Willis 1993b; Latta and Ritland 1994; Dudash et al. 1997), primarily as a result of high rates of mutation to mildly deleterious, partially recessive alleles (Dudash and Carr 1998; Willis 1999a,b). Because such inbreeding depression is not due to readily purged mutations of large effect (e.g., recessive lethals or steriles), it should persist even if a population inbreeds during periodic bouts of pollinator failure or population bottlenecks. Thus, it would be a formidable barrier to the evolution of selfing in an ancestral *M. guttatus*–like population. A mutant allele causing nearly complete selfing can spread rapidly to fixation even in the face of such a barrier (Lande and Schemske 1985; Holsinger 1988; Charlesworth et al. 1990; Schultz and Willis 1995), but our finding of no major QTLs suggests that the evolution of selfing in *M. nasutus* was a process of gradual adaptation rather than a single mutational step. The serial fixation of mating system modifiers of relatively small effect implies consistent directional selection for increased self-fertilization sufficient to overcome the costs of inbreeding. Ecological conditions favoring self-pollination to assure reproduction may provide such selection and are more viable explanations for the evolution of selfing in this system than the purging of genetic load alone.

The directionality of allelic effects at QTLs underlying

floral divergence also suggests an adaptive explanation for the evolution of selfing. Only five of the 24 QTLs have alleles whose effects on floral morphology are completely opposite to the expectation from the parental phenotypes, and a sign test indicates that this proportion deviates significantly from the neutrality ($P < 0.005$; Orr 1998b, eq. 9). Because pleiotropy produces nonindependence, we cannot assess the overall deviation from neutrality of all 91 QTL effects. However, the QTL data generally support directional selection for decreased flower size in the *M. nasutus* lineage.

The pleiotropic effects of potential mating system modifier loci are particularly important in considering alternative scenarios for the evolution of selfing. All else being equal, a novel allele that increases an individual's rate of self-fertilization relative to the outcrossing background population will rapidly spread to fixation unless inbreeding depression is greater than 0.5 (Fisher 1941; Lloyd 1979; Lande and Schemske 1985). Because this selective advantage derives from extra allelic transmission via the male gametes used in selfing, however, it is diminished or eliminated by trade-offs between self and outcross male fitness (pollen discounting; Nagylaki 1976; Holsinger et al. 1984). The reduced floral morphologies of extant selfer species often entail complete pollen discounting (e.g., Fishman 2000), suggesting that the evolution of selfing may have involved ecological selection. However, it is still not clear whether trade-offs in male fitness are an intrinsic feature of the individual genetic steps toward complete selfing. For self-compatible species, the best (but by no means perfect; see Kohn and Barrett 1994; Rausher and Chang 1998) candidate for a mating system modifier allele with no negative effects on outcross pollen donation would be one that reduces stigma-anther separation but does not affect other floral traits. Although many of the QTLs with *M. nasutus* alleles that reduce SA also have pleiotropic effects on corolla length and/or width (e.g., QTL3, QTL11, QTL15), others appear to act on SA independently of overall corolla size. Six QTLs (QTL1, QTL10, QTL12, QTL17, QTL20, QTL22) affect SA but have negligible effects on corolla size, and the fixation of *M. nasutus* alleles at all of these QTLs would reduce SA from the *M. guttatus* mean (1.76 mm) to near zero. One possible scenario for the evolution of complete selfing would involve the serial fixation of these alleles by automatic selection (given initial inbreeding depression $<$ 0.5), followed by selection for reduced allocation to corollas and the fixation (at other QTLs) of alleles with broad pleiotropic effects.

If we had found only QTLs with pleiotropic effects on both SA and corolla size characters, we could have ruled out an automatic transmission advantage as the primary driving force in the evolution of selfing in *M. nasutus*. The opposite is not true; the existence of QTLs with effects on SA alone does not rule out either pollen discounting or an important role for ecological selection for self-pollination. Even slight changes in SA may generate trade-offs between self and outcross male fitness (e.g., Chang and Rausher 1998), and selection for selfing as a reproductive assurance mechanism could fix alleles with a range of effects of floral morphology. Unfortunately, we cannot yet make functional connections between floral QTLs and components of reproductive fitness because epistatic breakdown of fertility in the F_2 hybrids confounds any associations between floral traits and either autofertility rates or pollen production. However, now that we have mapped and characterized floral QTLs associated with the evolution of complete selfing, we are planning to isolate these potential mating system modifiers in isogenic backgrounds through backcrossing and marker selection. Such QTL introgression lines are a powerful tool for testing the effects of evolutionarily realistic changes in floral morphology on reproductive fitness under a range of natural and experimental conditions.

Inferences about the Nature of Adaptive Evolution

The historical controversy over the genetic basis of adaptation was reinvigorated by the early discovery of QTLs of large effect underlying both crop plant domestication (see Tanksley 1993) and divergence between wild plant species (Bradshaw et al. 1995). Following this ground-breaking work, QTLs underlying adaptive divergence have been mapped and characterized in a handful of wild systems (*Drosophila*: e.g., True et al. 1997; Jones 1998, 2001; MacDonald and Goldstein 1999; Zeng et al. 2000; salamanders: Voss and Schaffer 1997; pea aphids: Hawthorne and Via 2001; sunflowers: Kim and Rieseberg 1999; *Mimulus*: Lin and Ritland 1997; Bradshaw et al. 1998; teosinte: Westerbergh and Doebley 2002). It is too early to derive general conclusions about the genetics of adaptation, but one thing is clear: The narrow sampling of taxonomic diversity has not restricted the diversity of genetic architectures observed. Indeed, the highly polygenic basis of floral divergence between *M. guttatus* and *M. nasutus* contrasts most sharply with the oligogenic basis of floral divergence between two other species of *Mimulus* (*M. cardinalis* and *M. lewisii*; Bradshaw et al. 1998). Can such comparisons of genetic architecture tell us anything about past evolutionary processes? Chance, and the pervasive statistical issues of QTL underdetection and QTL effect overestimation (Beavis 1994; Barton and Keightley 2002), may simply overwhelm any biological signal. However, we argue here that the genetic architecture of divergence may, in part, reflect the nature of the genetic variation available for selection within populations and the nature of the adaptive landscape.

Theoretical treatments of adaptive evolution generally model the fixation of spontaneous mutations during a population's approach to a distant fixed optimum in a monotonic fitness landscape (for review see Barton and Keightley 2002). In his initial formulation, Fisher (1930) considered the probability that a mutation of given effect would be favorable in multivariate phenotypic space. Because mutations of large effect are more likely to overshoot the optimum, Fisher argued that small steps must be the stuff of adaptation. However, Kimura (1983) noted that although mutations with subtle effects may more often be favorable, advantageous mutations of larger effect are more likely to escape stochastic loss and ultimately go to fixation. Thus, mutations of intermediate effect are more likely to contribute to adaptive evolution, when the first step alone is considered (Kimura 1983). Recently, Orr (1998a, 1999, 2000) extended Kimura's approach to models to consider the distribution of a series of mutations fixed during a population's adaptive walk toward

a fixed optimum. Because the maximum advantageous step becomes progressively smaller as the population nears the optimum, these new models generally predict an exponential distribution of fixed mutational effects (Orr 1998a, 1999). However, it is important to note that these models make no prediction about the absolute size of the initial step. Instead, the distribution of mutational effects on fitness sets limits on the size of the maximum adaptive step. Thus, the spectrum of advantageous mutations in different taxa or affecting different kinds of traits could theoretically explain differences between systems in the role of major genes. Indeed, under these classic models, the supply of mutational variation is a primary source of differences in genetic architecture. Unfortunately, there is simply too little empirical data on the magnitude and distribution of mutational effects in nature to make predictions about any particular instance of adaptive evolution.

These classic theoretical models of adaptive evolution focus on the recruitment of new mutations, but the availability and nature of standing variation may be a more important determinant of the size distribution of alleles fixed during adaptation. Consider a population that suddenly finds itself at some distance from a new optimum phenotype. With the exception of unconditionally deleterious mutations kept at very low frequency, alleles responsible for the standing genetic variation in that population are now both potentially advantageous and at high frequency relative to new mutations. For a given positive selective effect, alleles already segregating within the population should fix before rare spontaneously arising mutations. Thus, standing genetic variation may often be recruited in initial adaptive steps and, by rapidly moving the population closer to the optimum, could also limit the maximum size of later adaptive steps due to novel mutations. The distribution of allelic effects fixed from the standing variation will depend on the contribution of potentially advantageous alleles to the genetic variance of the initial population, which in turn depends on the both the distribution of mutational effects and past evolutionary process that affect allele frequency. We do not yet have formal predictions about the genetics of adaptive evolution via the fixation of standing variation, but it seems reasonable that such adaptation might involve relatively small steps.

The nature and maintenance of standing genetic variation remains poorly understood, but substantial heritable variation for quantitative traits divergent between populations or species is often found within populations, and studies are beginning to suggest that the same QTLs underlie variation at both scales (e.g., Nuzhdin and Reiwitch 2000). In outcrosser *M. guttatus*, we know that there is substantial within-population genetic variation for the floral characters differentiating *M. guttatus* and *M. nasutus* (Carr and Fenster 1994; Robertson et al. 1994; Kelly and Willis 2001). Moreover, because this variation does not appear to be caused by deleterious alleles maintained at very low frequency by mutation-selection balance (Kelly and Willis 2001), it may be readily available for adaptive evolution. Given a change in the environment that favored increased self-pollination (e.g., loss of pollinators), such standing variation could contribute to rapid evolution toward the new adaptive peak. In general, however, it is not yet clear whether the quantitative genetic variance within populations is maintained by mutation-selection-drift balance of deleterious alleles or by some form of balancing selection (Barton and Keightley 2002). More empirical and theoretical work is necessary to connect the within-population variation maintained by these each of these processes to that involved in responses to divergent natural selection.

Deviations of the monotonic adaptive landscape envisioned by Fisher (1930) could also generate systematic variation in the genetic basis of adaptive divergence. Although little theory has been developed for adaptation under such conditions, at least two types of alternatives seem biologically plausible: A population may shift between adaptive phenotypic peaks separated by valleys of low fitness or may track a moving optimum phenotype.

Models considering an adaptive walk up a single monotonic fitness peak generally predict an exponential distribution of step sizes, given no standing variation. However, if the fitness landscape contains multiple peaks, such that small to intermediate steps are usually maladaptive but large ones could be favorable, the genetics of adaptation would necessarily involve very major genes. This reasoning has been formalized in theoretical work on the genetics of mimicry, which suggest that resemblances to new unpalatable model species evolve through substitutions at major genes followed by the fixation of minor modifier alleles (e.g., Sheppard et al. 1985). Recently, an analogy between mimicry and shifts in pollination syndrome has been proposed to explain the oligogenic basis of floral divergence between *M. lewisii* and *M. cardinalis* (Bleiweiss 2001). *Mimulus cardinalis* exhibits classic features of a hummingbird pollination syndrome (tubular red corolla, exserted stigma, copious nectar) and is thought to be recently derived from the primarily bumblebee-pollinated *M. lewisii* (broad pink corolla, inserted stigma, low nectar volume). It is clear that the species differences in petal color (carotenoid concentration) and nectar volume both involve major QTLs (Bradshaw et al. 1995, 1998) and that these leading QTLs also strongly influence pollinator preferences (Schemske and Bradshaw 1999). Whether or not the evolution of hummingbird pollination in *M. cardinalis* qualifies as mimicry, the syndrome's existence as an alternative strategy with no adaptive intermediates (increases in carotenoid content reduce visitation by bees; Schemske and Bradshaw 1999) could exert very strong positive selection on major mutations causing the full peak shift. In contrast, relatively small steps may be adaptive during the evolution of selfing because the production of even one additional selfed seed is advantageous when ovules are otherwise unfertilized (e.g., when pollinator service is limiting).

The distribution of QTLs effects underlying divergence may also involve relatively minor effects if one or both populations has been tracking a moving optimum. Imagine a situation in which a slight increase in size would be advantageous, but a larger increase less so. Once the population has evolved toward the new phenotypic optimum, however, additional size increases might become adaptive. Thus, at the arbitrary point of divergence when the genetic basis of adaptation is evaluated, the step sizes will look very small relative to the total distance traveled. Sexual selection, which often involves rapid coevolution of male and female traits, may best be described in terms of moving optima and may

involve the serial fixation of mutations of apparently small effect. It is interesting to note that QTL studies of divergence between species often find the most polygenic genetic basis for sexual characters (Orr 2001). For example, divergence between *Drosophila simulans* and *D. mauritiana* in male posterior lobe shape is highly polygenic, with more than 19 QTLs each explaining fewer than three of the 32 ESDs differentiating these sister taxa (True et al. 1999; Zeng et al. 2000). Because a major mutation causing the full species difference would probably never have been advantageous, classic models of the distribution of selected effects may not apply. Similarly, the evolution of obligate autogamous self-fertilization in flowering plants may often involve moving optima. Because selfing rates (and their fitness consequences) depend on the local population context as well as on individual floral morphology (e.g., Chang and Rausher 1998; Fishman 2000), the fixation of one allele affecting mating system may alter the selective environment at other QTLs affecting the same traits. In particular, the fixation of alleles that increase competing or delayed self-pollination may pave the way for alleles promoting self-fertilization prior to anthesis. Theoretical models of how moving optima affect the predicted distribution and magnitude of QTL effects should allow further inference.

QTL analyses provide historically unprecedented insight into the genetic basis of adaptation and speciation, but raise new questions about the evolutionary forces and genetic variation contributing to divergence. We have drawn attention here to some factors that may pattern QTL effects, with particular emphasis on understanding the polygenic nature of floral characters associated with mating system evolution. However, far more theoretical and empirical work is necessary to draw firm connections between variation within populations, processes of adaptive divergence, and the genetic basis of species differences. As the state of the art of QTL mapping continues to improve, so too will the opportunities to confidently compare and interpret genetic architectures.

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