Quantitative Trait Loci Differentiating the Outbreeding *Mimulus guttatus* From the Inbreeding *M. platycalyx*

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Manuscript received August 28, 1996
Accepted for publication March 24, 1997

ABSTRACT

Theoretical predictions about the evolution of selfing depend on the genetic architecture of loci controlling selfing (monogenic *vs.* polygenic determination, large *vs.* small effect of alleles, dominance *vs.* recessiveness), and studies of such architecture are lacking. We inferred the genetic basis of mating system differences between the outbreeding *Mimulus guttatus* and the inbreeding *M. platycalyx* by quantitative trait locus (QTL) mapping using random amplified polymorphic DNA and isozyme markers. One to three QTL were detected for each of five mating system characters, and each QTL explained 7.6–28.6% of the phenotypic variance. Taken together, QTL accounted for up to 38% of the variation in mating system characters, and a large proportion of variation was unaccounted for. Inferred QTL often affected more than one trait, contributing to the genetic correlation between those traits. These results are consistent with the hypothesis that quantitative variation in plant mating system characters is primarily controlled by loci with small effect.

THE evolution of selfing has been studied in a variety of theoretical models, each with specific assumptions about its inheritance. Most models assume selfing rate to be controlled by a single locus with two alleles (*e.g.*, FISHER 1941; CHARLESWORTH 1980; HOLSINGER *et al.* 1984; CAMBELL 1986; CHARLESWORTH *et al.* 1990). A purely polygenic basis for selfing was invoked by LANDE and SCHEMSKE (1985) in their models for the joint evolution of selfing and inbreeding depression. LATTA and RITLAND (1993) emphasized how facets of the genetic architecture (number of loci, average allelic effect, level of dominance) can affect the equilibrium selfing rate. However, empirical studies of the underlying genetic architecture of selfing are lacking.

Biometrical methods (those based on phenotypic variances of segregating generations, *cf.* WRIGHT 1968; LANDE 1981) have been used to estimate the number of loci controlling floral character differences for a few plant species. FENSTER and RITLAND (1994) estimated between five and 12 loci for the differences in several mating system characters between *M. guttatus* and two other inbreeding taxa, *M. micranthus* and *M. laciniatus*, but these estimates suffered from large standard errors. MACNAIR and CUMBES (1989) estimated between three and seven loci controlling the floral differences between the outbreeder *M. guttatus* and a copper-tolerant inbreeder *M. cupriphilus*. Variation in anther-stigma separation between populations was estimated to be controlled by at least seven loci in *Clarkia tembloriensis* (HOLTSFORD and ELLSTRAND 1992), and at least five loci in *Turnera ulmifolia* var. *angustifolia* (SHORE and BARRETT 1990). Anther-stigma separation has been found to be highly correlated with outcrossing rate in many self-compatible species (*e.g.*, RITLAND and RITLAND 1989; HOLTSFORD and ELLSTRAND 1992; BELAOUSSOFF and SHORE 1995), implying that selfing rate is controlled by several loci. However, biometrical methods have assumptions, such as additivity of gene effect, independence of loci and equality of allele effects, whose violation will bias estimates downward (ZENG *et al.* 1990). Furthermore, most biometrical techniques (save for those requiring generation of inbred lines) only infer average effects over all genes and do not identify individual gene effects.

Until recently, evolutionists have not exploited the potential for gene mapping with molecular markers to identify and characterize quantitative trait loci (QTL). The genus Mimulus (Scrophulariaceae) is an ideal experimental system for QTL mapping because species are often intercrossable (VICKERY 1978) yet morphologically distinct. In a seminal study, BRADSHAW *et al.* (1995) employed QTL mapping techniques to detect individual QTL of large effect responsible for reproductive isolation between the bird-pollinated *M. cardinalis* and the bee-pollinated *M. lewisii*. In this paper, we employ QTL mapping to detect QTL responsible for differences of selfing rate between the two yellow monkeyflowers, *M. guttatus* and *M. platycalyx*.

We first constructed a linkage map consisting of two isozyme and 99 random amplified polymorphic DNA (RAPD) markers, based on a backcross involving the

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outbreeding *M. guttatus* and the inbreeding *M. platycalyx* (LIN and RITLAND 1996a). This linkage map is used in this paper to infer the QTL map for each of six mating system characters differing between *M. guttatus* and *M. platycalyx*. We note that, as a quantitative trait, the actual rate of self-fertilization is difficult to measure, as it is inferred indirectly via marker segregation patterns among many progeny (HAUPTLI and JAIN 1985). An efficient alternative, employed here and by the aforementioned quantitative genetic studies, is to examine characters functionally related to selfing. We also use *selective genotyping* (the selective assay of genotypes at the extreme of the phenotypic distribution, *c.f.* LANDER and BOTSTEIN 1989; LIN and RITLAND 1996b) to increase the power of QTL detection. The inferred QTL map gives the minimum number of loci for mating system differences among taxa, as well as the distribution of QTL effects. In particular, we seek to identify QTL with large effect on mating system characters.

Finally, the shift from outcrossing to selfing is frequently associated with a change of an entire syndrome of selfing correlates, all related to male allocation and inbreeding avoidance (*e.g.*, reduced attraction to pollinators and reduction of the spatial and temporal separation of male and female reproductive organs within the flower, *cf.* ORNDUFF 1969; JAIN 1976; RITLAND and RITLAND 1989). This shift from outcrossing to selfing thus creates strong genetic correlations among mating system characters. Genetic correlations are caused either by genes with pleiotropic effects or by closely linked genes (FALCONER 1989, ch. 19). Linkage mapping can distinguish between these two causes of genetic correlations (JIANG and ZENG 1995) through QTL comparisons of maps for different characters. Thus, in this study we also compare the similarity of QTL locations for different traits to determine if pleiotropy underlies the genetic correlations of mating system characters.

MATERIALS AND METHODS

Plant materials and phenotypic measurements: *M. guttatus* is a predominantly outcrossing taxon (t = 0.6–0.9), while *M. platycalyx* is a predominantly selfing taxon (t = 0.1–0.2, RITLAND and RITLAND 1989). *M. guttatus* has larger flowers, more distinct anther-stigma separation and higher pollen-ovule ratio than *M. platycalyx* (RITLAND and RITLAND 1989). Both taxa, however, have the same haploid chromosome number of 14 and overlap in their natural distribution (VICKERY 1978).

Details of the source populations, crosses and growth room conditions were described in LIN and RITLAND (1996a). Two hundred and forty-seven individuals of a backcross between *M. guttatus* and *M. platycalyx* were grown under growth room conditions in a completely randomized design. Floral measurements were taken on the day after the flowers were fully open, and plants were grown to senescence. A Mimulus flower consists of a bilaterally symmetrical corolla, a pistil with one stigma on an exserted style, and four anthers with two on longer filaments and two on shorter filaments (see MACNAIR and CUMBES 1989; RITLAND and RITLAND 1989). Four flowers (on the fourth and fifth nodes) of each individual were measured for the following characters: flower width (FW, mm), flower length (FL, mm), pistil length (PL, mm), long-stamen length (LSL, mm) and short-stamen length (SSL, mm). A sixth character, anther-stigma-separation (SEP, mm), was derived by subtracting long-stamen length (LSL) from pistil length (PL). The mean of the four flowers was used as the trait value for each individual.

Selective genotyping: Before DNA isolation, the phenotypic correlations between characters were calculated (SAS INSTITUTE 1985). Except for SEP, all five characters were strongly correlated with each other (see RESULTS). Selective genotyping (LANDER and BOTSTEIN 1989) was employed to increase the power of detecting QTL based on the two uncorrelated traits SEP and FW. Ninety-six individuals with phenotypic values of at least 1 SD from the mean of FW or SEP were chosen for DNA isolation and genotyping. The rest of the 247 individuals were scored as missing data at the marker loci for interval mapping of QTL. DNA isolation procedures, and isozyme and RAPD genotyping protocols were described in LIN and RITLAND (1996a).

Linkage map and interval mapping of QTL: A linkage map consisting of 99 RAPD and two isozyme markers was constructed from the 96 selectively genotyped individuals (LIN and RITLAND 1996a). Linkage groups consisting of at least two markers were used for scanning of QTL for mating system characters. These linkage groups span 1437 contiguous cM, covering 58% of the genome [estimated by LIN and RITLAND (1996a) to be 2474 cM based on the maximum likelihood method of CHAKRAVARTI *et al.* (1991)]. We attempted to locate more markers to intervals larger than 30 cM using a bulked segregant analysis (MICHELMORE *et al.* 1991) but were unable to find any polymorphic loci for these intervals out of ~50 RAPD primers tested.

The frequency distributions of the six mating system characters in the backcross population of *M. platycalyx* × *M. guttatus* were approximately normal (Figure 1); thus no data transformations were performed. Scanning for QTL was carried out in MAPMAKER/QTL 1.1 (LINCOLN *et al.* 1992) with an approximate LOD threshold of 2.4. This value was determined using Figure 4 of LANDER and BOTSTEIN (1989), given the haploid chromosome number of 14 and an average distance between adjacent markers of 21.8 cM (LIN and RITLAND 1996a).

RESULTS

Gene effects: A total of 10 QTL were detected in five linkage groups (Table 1). This occurred despite the presence of limited phenotypic variation for most characters (c.v. range from 5.7 to 8.8%, except for anther-stigma separation with c.v. = 31.6%). Among species of the yellow monkeyflower complex, flower size is generally greater in outbreeders than in inbreeders (RITLAND and RITLAND 1989). However, flower size in the outbreeding *M. guttatus* did not substantially differ from that of the inbreeding *M. platycalyx*, despite great differences in their inferred outcrossing rates. In line with this, we found limited phenotypic variation for flower size in our backcross population, as indicated by the standard deviations (Table 1). Only one QTL for flower length differences was detected in linkage group 3, explaining 7.8% of the phenotypic variance, but no QTL was detected for flower width differences.

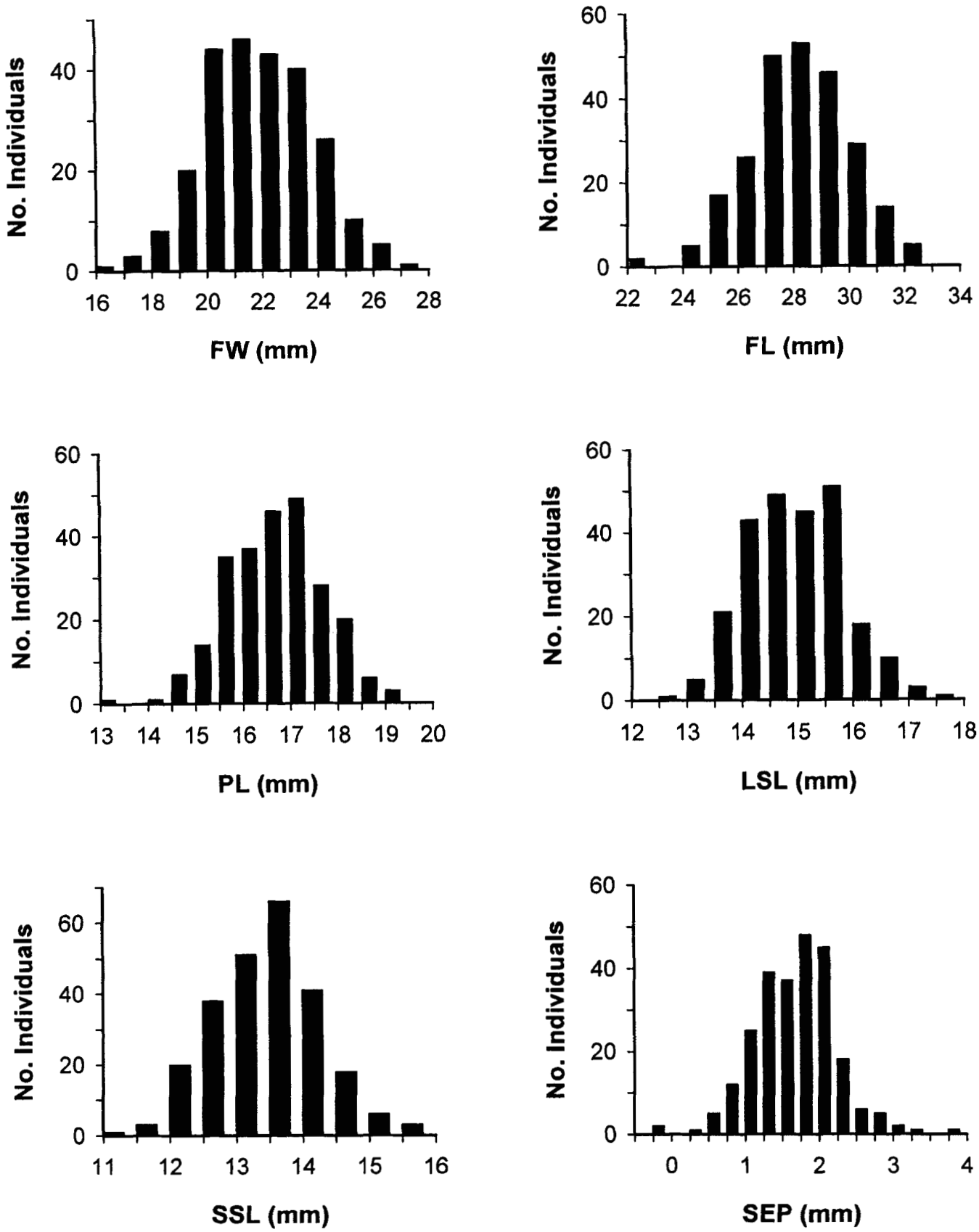

FIGURE 1.—Frequency distributions for six mating system characters in a backcross population of *M. platycalyx* × *M. guttatus*.

TABLE 1

Trait value, QTL location, LOD score and QTL effect for mating system characters in a backcross population of *M. guttatus* × *M. platycalyx*

Character	Mean ± SD (mm)	Var%[a]	LOD	Linkage group
FW	22.08 ± 1.93	—	—	—
FL	28.38 ± 1.80	7.8	2.13	3
PL	16.78 ± 1.00	28.6	4.68	1
LSL	15.06 ± 0.87	17.7	3.43	1
		7.6	2.04	2
		13.0	3.35	6
SSL	13.55 ± 0.77	12.9	3.40	1
		8.3	2.25	2
		12.4	2.97	6
SEP	1.72 ± 0.54	10.7	3.20	1
		10.5	2.47	5

[a] Var%, percentage of phenotypic variance explained by the QTL.

TABLE 2

Phenotypic correlation coefficients between mating system characters in a backcross population of *M. guttatus* × *M. platycalyx*

	FL	PL	LSL	SSL	SEP
FW	0.83	0.67	0.65	0.58	0.19
	(0.0001)	(0.0001)	(0.0001)	(0.0001)	(0.0027)
FL		0.83	0.85	0.82	0.18
		(0.0001)	(0.0001)	(0.0001)	(0.0052)
PL			0.84	0.78	0.50
			(0.0001)	(0.0001)	(0.0001)
LSL				0.97	−0.06
				(0.0001)	(0.3827)
SSL					−0.10
					(0.1141)

Probability $< |r|$ shown in parentheses.

The style in the *M. guttatus* flower is longer than the filaments, providing a distinct separation of stigma and anthers. By contrast, the style in the *M. platycalyx* flower is either of similar length, or even of shorter length, than the longer stamen. One QTL explaining 28.6% of the variation for pistil length between these two taxa was found in linkage group 1.

Three QTL were detected for long-stamen length in linkage groups 1, 2 and 6, explaining 17.7, 7.6 and 13.0% of the phenotypic variance, respectively. There are also three QTL found in linkage groups 1, 2 and 6 for short-stamen length, explaining 12.9, 8.3 and 12.4% of the phenotypic variance, respectively. The collective variation explained by these three loci (assuming additive effects among loci) is ~38 and 34%, for long-stamen length and short-stamen length, respectively.

Anther-stigma separation is the measure of the spatial separation of male and female reproductive organs. While *M. guttatus* has a distinct anther-stigma separation, *M. platycalyx* usually shows no or an indistinguishable separation of anther and stigma. The considerable variation in anther-stigma separation in our mapping population (Table 1) is due to at least two independent QTL, detected in linkage groups 1 and 5, respectively. These two QTL contributed 10.7 and 10.5%, respectively, to the variation in anther-stigma separation between the two taxa.

Pleiotropy: The phenotypic correlations between SEP and LSL and between SEP and SSL are nonsignificantly negative, whereas other correlations are all positive and highly significant (Table 2). QTL for long-stamen length in linkage groups 2 and 6 may have pleiotropic effect on short-stamen length, since the map locations of these QTL for different traits overlap (Figure 2). While the QTL for anther-stigma separation in linkage groups 1 and 5 and the QTL for flower length in linkage group 3 are clearly independent loci, it is hard to tell whether the three QTL for pistil length, long-stamen length and short-stamen length in linkage group 1 are one locus with pleiotropic effects on three characters or three tightly linked loci. Nevertheless, these results are in accordance with the phenotypic correlation coefficients. The highly significant phenotypic correlation between long- and short-stamen length is due at least to three loci with pleiotropic effects or tight linkage. While the independent QTL for anther-stigma separation result in weak or insignificant correlation between SEP and other characters, the strong correlation between pistil length and stamen length is due at least to one locus with pleiotropy or tight linkage.

DISCUSSION

This is the first study to use genetic markers to document QTL differences between taxa that differ for rates of self-fertilization. We found that between *M. guttatus* and *M. platycalyx*, individual QTL for mating system traits account for up to 28% of the backcross phenotypic variance, with most traits showing QTL effects of 10–20% (Table 1). Taken together, QTL accounted for up to 38% of the backcross variation, and a large proportion of the variation was unaccounted for. Given the ~100 progeny used in this study, only loci with relatively large effects can be detected (Beavis 1994; and see below). The unaccounted variation is likely due to loci with smaller effects detectable only with a larger mapping population. These results are thus consistent with the hypothesis that quantitative variation in mating system characters in Mimulus is primarily controlled by loci with small effects.

There are similarities and differences between the results of our mapping study and earlier biometrical studies in related Mimulus taxa (*M. Cupriphilus, M. micranthus* and *M. laciniatus*) (Macnair and Cumbes 1989; Fenster and Ritland 1994) and other plant species (*e.g.*, Turnera, Shore and Barrett 1990; Clarkia,

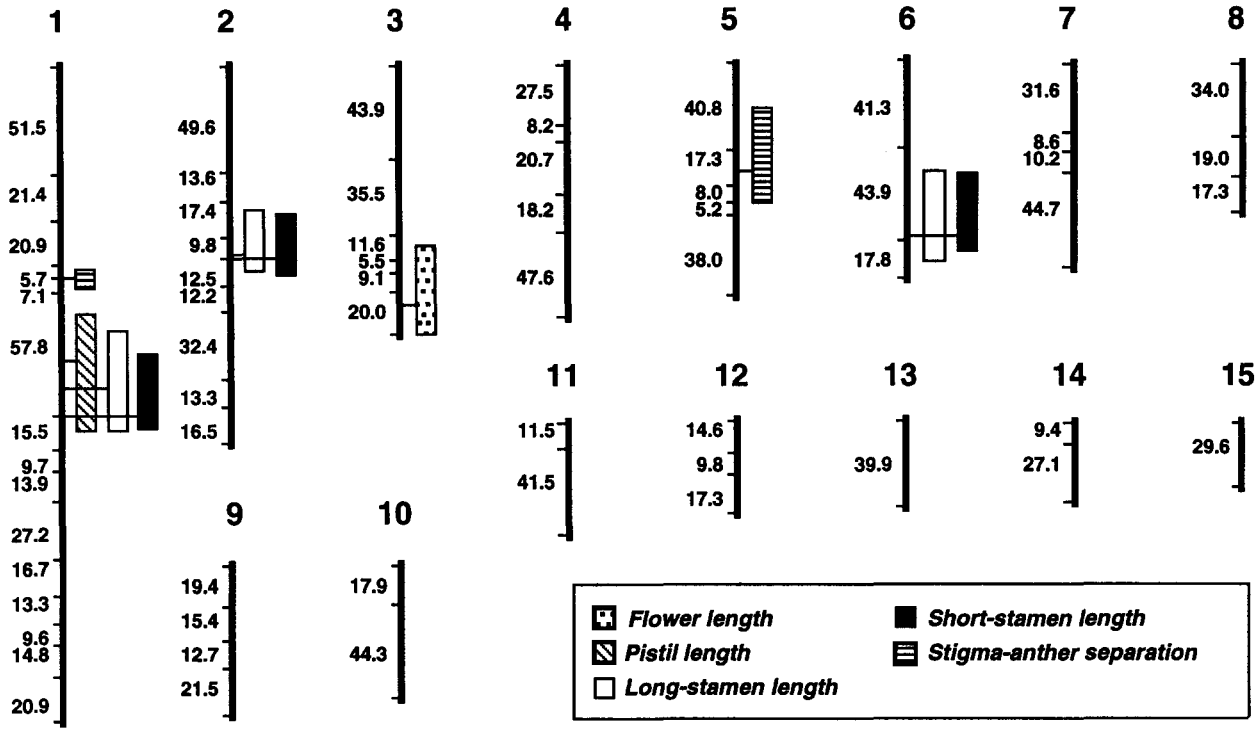

FIGURE 2.—Linkage map for the mapping of QTL for mating system differences between *M. guttatus* and *M. platycalyx*. The linkage groups are arranged according to their total length. Marker positions are shown by horizontal bars and map distances between markers by numerals to the left, and QTL positions by horizontal bars and their 1-LOD confidence intervals by vertical bars to the right of each linkage group.

HOLTSFORD and ELLSTRAND 1992). On one hand, both types of studies showed that several loci are involved in mating system divergence and at least some loci are of quite small effects. On the other hand, we also found loci with relatively large effects among those with small effects, which suggests that mating system evolution involves substitutions of alleles of a variety of effects. These are in contrast to the assumptions of many theoretical models (*e.g.*, FISHER 1941; LANDE and SCHEMSKE 1985), and new models for mating system evolution should incorporate a distribution of allele effects.

The number of loci and their relative effects for species differences of a quantitative trait have been the central issues of the controversy between "micro-" and "macromutationists" (FISHER 1958; GOULD 1980; CHARLESWORTH *et al.* 1982; GOTTLIEB 1984; COYNE and LANDE 1985). Although the neo-Darwinian view of evolution by small steps or genes of small effect has gained wide acceptance, genes of major effect may play important roles in speciation (ORR and COYNE 1992). In fact, some argue that there is a fundamental difference in the genetic basis of speciation between animals and plants, in that major morphological differences between plant species are often due to few genes (HILU 1983; GOTTLIEB 1984).

This view about the role of major genes is supported by a common finding of large single-gene effects for yield and structural characters in crop species (DOEBLEY and STEC 1991, 1993; DORWEILER *et al.* 1993; TANKSLEY 1993). Some evidence in support of this view also comes from genetic marker studies in wild plant species. For example, VLOT *et al.* (1992) found that most of the difference in the number of pappus parts between *Microseris douglasii* and *M. bigelovii* was explained by a single QTL. BRADSHAW *et al.* (1995) also detected at least one QTL accounting for more than 25% of the phenotypic variance in each of eight floral traits distinguishing the bird-pollinated *M. cardinalis* and the bee-pollinated *M. Lewisii*, and single QTL explaining >40% of the phenotypic variance are quite common.

Our results, however, argue for a predominant role of minor genes. Although some loci are of larger effect, there is little evidence for a significant number of loci with large effect. The majority of the variation in mating system characters is likely due to alleles of small effect. This implies that loci with very large effects are unlikely to be important in the speciation events in association with mating system divergence in these species. More studies with molecular markers in natural populations are needed before we can finally settle the long-standing controversy over the importance of major genes in speciation.

Qualifications: There are, however, two major qualifications to our findings. First, the true progenitor condition is unknown. In an evolutionary comparison of two taxa, one cannot infer the direction of evolution of phenotypic traits and their underlying QTL. However, the distribution of selfers in the inferred phylog-

eny of the yellow monkeyflower group (RITLAND and RITLAND 1989) suggests that outcrossing is ancestral, as do most models and theories for the evolution of mating systems (JAIN 1976). A related problem is that evolution since speciation has partially obscured the original QTL differences at speciation. The genetic differences we detect are due to evolution over twice the time interval since the species diverged from a common progenitor (LI and GRAUR 1991, p. 68), and fixation of new mutations will obscure the QTL responsible for speciation.

One plausible scenario for the putative evolution of self-fertilization in Mimulus may involve the initiation of selfing by a few genes of relatively large effects, followed by the subsequent build-up of minor modifier loci. Alternatively, self-fertilization may have evolved slowly, initiated by minor mutations, then followed by rapid changes in selfing rate as new mutations with larger effects accumulated. Examination of the dominance level of QTL responsible for mating system differences may provide valuable information to resolve this uncertainty (J-Z. LIN and K. RITLAND, unpublished data). Mutations that initiate selfing should be dominant or semi-dominant, because recessive mutations have a low probability of establishment due to "Haldane's sieve" effect in large outcrossing populations (MERRELL 1969; TURNER 1981; CHARLESWORTH 1992). If the QTL of large effects for mating system differences are largely recessive, then selfing is most likely initiated by dominant QTL with small effects followed by rapid changes due to subsequent recessive QTL of large effects. If these QTL are largely dominant, then initiation of selfing by QTL of large effect is also possible.

The second qualification of our results arises from the statistical biases of the interval mapping procedure. One bias is that at most one QTL is assumed to lie between adjacent markers (LANDER and BOTSTEIN 1989); the presence of more than one can cause biased estimates of map position. Improved methods for fine-scale mapping of multiple QTL have been developed (ZENG 1994). A second bias is that the interval mapping procedure can overestimate allele effects (BEAVIS 1994), particularly alleles with effect just above the level of statistical significance. To reduce this bias, BEAVIS (1994) advocated use of much larger sample sizes, on the order of 500, to provide less biased estimates of QTL effect for alleles of moderate (5–10%) effect. Our power of inference was limited by the relatively small number of progeny and low phenotypic variation in our mapping population for five of the characters (Table 1). Obviously taxa with more conspicuous differences might be used, but such cases are rare, and furthermore, focus on these cases for the purpose of statistical power may bias our picture of evolution. The statistical techniques for QTL mapping have been exclusively developed for the purpose of mapping (and cloning) QTL. Alternative statistical models for inferring the distribution of allele effects, irrespective of their specific locations, are needed.

Pleiotropy *vs.* linkage: Positive phenotypic correlations are observed between corolla width and corolla length, and between pistil length and stamen length, and negative correlations between stigma-anther separation and stamen length (Table 2). Such a pattern was also observed in previous studies (MACNAIR and CUMBES 1989; CARR and FENSTER 1994; FENSTER and RITLAND 1994). By contrast, we, and the latter two studies, found weak and inconsistent correlations between stigma-anther separation and other floral characters.

In line with the phenotypic correlations, QTL for stamen length and pistil length tended to occur in similar locations, while QTL for stigma-anther separation occurred at different locations than QTL for other traits (Figure 2). For example, the correlation between long- and short-stamen length is due to at least two QTL, located on linkage groups 2 and 6, each with pleiotropic effects on both characters. However, it is difficult to tell if QTL for stamen length and pistil length on linkage group 1 are the same gene with pleiotropic effects *vs.* separate but closely linked genes, each controlling a single character. More closely spaced markers and greater numbers of progeny are needed to exclude the possibility that a single inferred QTL is not a composite locus (ZENG 1994; JIANG and ZENG 1995).

We thank Drs. D. CHARLESWORTH, L. GOTTLIEB, J. DOEBLEY, J. SHORE, Y-B. FU, J. DOLE and two anonymous reviewers for comments on an earlier version of this article. This work was supported by funds from Natural Sciences and Engineering Research Council of Canada as postgraduate scholarships to J.Z.L. and operating grants to K.R.

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Communicating editor: D. CHARLESWORTH