THE GENETIC BASIS OF FLORAL TRAITS ASSOCIATED WITH MATING SYSTEM EVOLUTION IN LEPTOSIPHON (POLEMONIACEAE): AN ANALYSIS OF QUANTITATIVE TRAIT LOCI

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Abstract.—Mapping of quantitative trait loci (QTL) was used to investigate the genetic architecture of divergence in floral characters associated with the mating system, an important adaptive trait in angiosperms. Two species of Leptosiphon (Polemoniaceae), one strongly self-fertilizing (L. bicolor) and the other partially outcrossing (L. jepsonii), were crossed to produce F2 and both backcross progenies. For each crossing population, a linkage map was created using amplified fragment length polymorphism markers, and QTL were identified for several dimensions of floral size. For each of the five traits examined, three to seven QTL were detected, with independent datasets yielding congruent results in some but not all cases. The phenotypic effect of individual QTL was generally moderate. We estimated that many of the QTL were additive or showed dominance toward L. bicolor, whereas comparison of mean trait values for parental and cross progenies showed apparent overall dominance of L. jepsonii traits. Colocalization of QTL for different dimensions of floral size was consistent with high phenotypic correlations between floral traits. Substantial segregation distortion was observed in marker loci, the majority favoring alleles from the large-flowered parent. A low frequency of male sterility in the F2 population is consistent with the Dobzhansky-Müller model for the evolution of reproductive isolation.

Key words.—Genetics of adaptation, Linanthus, pleiotropy, quantitative trait loci, self-fertilization, self-incompatibility.

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The past decade has seen renewed interest in the process and genetic architecture of adaptation. Throughout much of the 20th century, Fisher’s (1930) theoretical work dominated views of adaptation. Because random mutations with minor phenotypic effects are more likely to be favorable than those of large effect, Fisher argued that adaptation generally occurs by the fixation of many genes of small effect. An alternative hypothesis, that adaptation often involves one or a few major genes, has had its supporters, particularly with respect to the evolution of plant species (Gottlieb 1984). Until recently, however, empirical evidence for testing these competing hypotheses was scarce (Orr and Coyne 1992). A renewal of interest has been spurred both by theoretical work (reviewed in Orr 2005a) and the development of molecular methods such as mapping of quantitative trait loci (QTL; Orr 2005b; reviewed in Erickson et al. 2004). In QTL mapping, statistical associations between molecular marker genotypes and phenotypic traits in segregating progeny can be used to detect and map loci contributing to adaptive traits.

QTL mapping has been widely used for genetic analyses of economically important traits in agricultural and horticultural plant species. The results of over a decade of plant QTL mapping studies, mostly focused on cultivated species, provide evidence that genes of major effect often play a large role in trait evolution (reviewed in Remington and Purugganan 2003). For a variety of reasons, however, the genetic architecture of traits in artificially selected species may not be representative of evolutionary processes in natural populations (Remington and Purugganan 2003). The genetic basis of adaptation can be understood only from studies of wild populations. Relatively few QTL studies have been carried out for natural plant populations, but several of these have addressed the genetic basis of floral traits associated with different modes of reproduction (Lin and Ritland 1997; Bradshaw et al. 1998; Fishman et al. 2002; Georigiades et al. 2002; Hodges et al. 2002), laying the groundwork for understanding adaptation in these traits.

Floral traits associated with the mating system are prominent adaptive features of higher plants. The transition from cross-fertilization (outcrossing) to self-fertilization (selfing) is thought to be a common trend in plant evolution (Stebbins 1950, 1974). This transition is of particular interest because it affects a number of evolutionary parameters. Theoretical and empirical studies have shown a relationship between the mating system, levels of genetic variation, and how that variation is distributed within and among populations (Allard 1975; Hamrick and Godt 1989). Selfing is expected to have an intrinsic genetic transmission advantage when it does not reduce the amount of pollen contributed to cross-pollination (Fisher 1941) and can provide reproductive assurance when pollinators or mates are limited (Baker 1955; Stebbins 1957). Opposing these factors is inbreeding depression, the reduction in viability of selfed relative to outcrossed progeny (Charlesworth and Charlesworth 1987).

Diverse genetic and morphological mechanisms influence the frequency of self- versus cross-fertilization in plants (Richards 1997). Floral features that determine the mating system of a species may include both traits that prevent self-pollen deposition or self-fertilization and those that influence the rate of cross-pollination by biotic or abiotic vectors. Selfing is prevented in many plant species by self-incompatibility (SI), a genetic mechanism by which plants recognize and reject self-pollen (de Nettancourt 1977). Plants that lack such mechanisms may nevertheless be largely outcrossing. The degree of stigma-anther separation and relative timing of male and female function are associated with variation within...
and among populations in the outcrossing rate (Schoen 1982; Holtsford and Ellstrand 1992; Motten and Antonovics 1992; Karron et al. 1997). Traits that influence the mating system through pollinator visitation include flowering phenology (Schmitt 1983; Cruzan et al. 1994) and flower size (Lyons and Antonovics 1991). Flower size is also thought to evolve in response to increases in the selving rate as allocation of resources to structures for pollinator attraction and reward is reduced. This trend has been observed in a wide range of selving taxa (Cruden 1977; Ritland and Ritland 1989).

The genetic basis of the mating system—that is, the number of loci and levels of dominance—has been shown to affect the evolutionary outcome of selection in some models of mating system evolution (Charlesworth and Charlesworth 1990; Latta and Ritland 1993). For instance, Latta and Ritland (1993) showed that, under some conditions, mixed mating systems can be evolutionarily stable and that this outcome is more likely when many genes control the rate of selving. Therefore, empirical knowledge of the genetic architecture of mating system traits is critical for understanding their evolution. Studies of the genus *Mimulus* have explored the genetic basis of mating system evolution using both QTL mapping (Lin and Ritland 1997; Fishman et al. 2002) and biometric approaches (Macnair and Cumbes 1989; Fenster and Ritland 1994). The growing consensus of these studies is that traits distinguishing selving and outcrossing species within the genus are generally due to many genes of small to moderate effect. This work provides a foundation for studies of other genera, including data reported here for species of *Leptosiphon* (Polemoniaceae).

This study addresses the genetics of floral traits associated with mating system divergence in *Leptosiphon* (formerly *Linanthus* until systematic revision; Porter and Johnson 2000), a genus of annual plants of western North America. The transition from SI to self-fertilization has occurred multiple times within the genus (Goodwillie 1999), which suggests that evolution in the mating system is adaptive. Two closely related species of *Leptosiphon* that differ in their rate of selfing provide an opportunity to study a part of this transition. Both species are capable of fertilization when self pollen is applied. Therefore, divergence in the mating system is likely conferred by floral traits that affect pollinator visitation or self-pollen deposition and by pollen-pistil interactions that affect the timing of self-fertilization.

Here we present the results of a QTL mapping study using amplified fragment length polymorphism (AFLP) markers to investigate the genetic architecture of floral morphological traits that distinguish a highly selving (*Leptosiphon bicolor*) and a partially outcrossing (*L. jepsonii*) species. While AFLP markers are abundant and relatively easy to develop, they act generally as dominant markers and therefore are not a powerful tool for estimating dominance effects. For loci with dominance, the effect of an allelic substitution will differ in reciprocal backcrosses; with complete dominance, QTL will be detectable only in a backcross to the parent with the recessive allele. We develop an approach for estimating dominance effects by comparing QTL effects in backcrosses to both parental species, as well as in F2 progeny. In addition, we consider possible explanations for significant segregation distortion observed in marker loci. Finally, we report a low frequency of male sterility in the F2 progeny and discuss its implications in the evolutionary divergence of *L. bicolor* and *L. jepsonii*.

**Materials and Methods**

**Study Species**

*Leptosiphon bicolor* is widely distributed from southern California to the Puget Sound region, whereas *L. jepsonii* grows in only two counties in the North Coast Ranges of California. Cytological studies of *L. bicolor* (n = 9; Patterson 1979) and allozyme and flow cytometry results for both species (Goodwillie 2000; C. Goodwillie, unpubl. data) indicate that they are diploid. The two are sister taxa and appear to be recently diverged (Goodwillie 1999). Despite their genetic similarity, the species differ in their mating systems and pollination biology. Allozyme analyses of progeny arrays in two populations of *L. bicolor* in Napa County yielded outcrossing rate (t) estimates of 1% and 7% (Goodwillie 2000). The *L. bicolor* population used for this study lacks polymorphic loci required for outcrossing rate estimation, but the absence of genetic variation suggests a high selving rate. *Leptosiphon jepsonii* is less strongly self-fertilizing, with outcrossing rate estimates ranging from 10% to 71% (Goodwillie 2000; Goodwillie and Ness 2005). The population in this study showed 14% outcrossing in 1995.

Although both *L. bicolor* and *L. jepsonii* can produce seeds by autonomous self-fertilization (Goodwillie 2000), they differ in the timing of self-compatibility. *Leptosiphon bicolor* can self-fertilize as soon as flowers open, a mating system referred to as "competing selving" because simultaneous self and outcross pollinations compete for ovules (Lloyd 1979). In contrast, selving is usually delayed in *L. jepsonii* by a transient pollen-pistil interaction, in which flowers are largely SI when they open and become self-compatible after one to two days (Goodwillie et al. 2004). Both modes of selving can assure reproduction in the absence of pollinators. Delayed selving is thought to be particularly advantageous with variable pollination because it allows outcrossing when pollinators are present, whereas competing selving can incur costs (Lloyd 1979, 1992). If inbreeding depression is purged, however, the costs of selving are minimized. In fact, inbreeding depression is negligible (9%) in *L. bicolor* and substantially higher in *L. jepsonii* (23%) for the populations used in this study (Goodwillie 2000).

In overall floral morphology, the species are quite similar, with five-lobed salverform corollas arranged in head-like inflorescences. However, they are distinguished by morphological traits that are associated with the mating system. Compared to *L. jepsonii*, the more selving *L. bicolor* has smaller corolla lobes, corolla tubes, stigma lobes, and anthers (Schemske and Goodwillie 1996). In addition, *L. bicolor* has reduced stigma-anther separation, which is typical of highly selving species. *Leptosiphon jepsonii* is visited and pollinated frequently by bees (C. Goodwillie, unpubl. data). In contrast, we have observed only rare pollinator visits to *L. bicolor* in several years of research at study sites in Napa County. When crossed, the species produce fertile F1 offspring. In natural populations, however, they appear to be reproductively isolated for the most part; morphologically distinct
populations of the two species have been found to occur less than 100 m apart. Reproductive isolation is likely conferred by the primarily self-fertilizing mating system of *L. bicolor*.

**Experimental Crosses**

Plants of both species were raised in the greenhouse from seed collected in populations in Napa, California. For crosses, flowers were emasculated as buds to prevent self-fertilization and hand-pollinated after anthesis. Two unrelated plants of each species were selected to generate two F1 sibships. From each F1 sibship several plants were raised and used for F2 crosses and backcrosses to each parental species (BCb, backcross to *L. bicolor*; BCj, backcross to *L. jepsonii*). In the initial crosses, *L. bicolor* was used as the paternal parent. In backcrosses to both species, F1 plants were used as the paternal parent. Because individual plants did not produce enough flowers to generate the sample sizes necessary for QTL analysis, F2 crosses and backcrosses were carried out on three and two pairs of plants, respectively (Fig. 1), and genetic and phenotypic data for each cross type were pooled for analysis.

For genotypic and phenotypic analysis, 100 plants from each backcross, 100 plants from each of two F2 populations, and 257 plants from a third F2 population were grown during the winter of 1998. In addition, 50–70 plants for each F1 population and parental species were grown simultaneously for phenotypic analysis. Plants of all parental and cross populations were grown in standard potting mix in subirrigated Conetainers (Stuewe and Sons, Corvallis, OR) at the University of British Columbia greenhouses.

**Phenotypic Measurements**

Three flowers of each plant were scored for corolla tube length, corolla lobe length, corolla lobe width, stigma-anther separation, stigma lobe length, and anther length. All floral measurements were made using digital calipers except for stigma lobe and anther length, which were measured using a dissecting microscope with an ocular micrometer. Corollas of both species elongate somewhat during the three days that flowers are open. Therefore, to minimize error, all measurements except anther length were made between 1200 and 1500 h on the day that flowers first opened. Because anthers dehisce when flowers first open, anther length was measured in buds on the evening prior to anthesis. Phenotypic correlations between traits were calculated for each of the F2, BCb, and BCj datasets. Experimentwise significance of correlations was determined using a sequential Bonferroni method (Rice 1989).

**Amplified Fragment Length Polymorphism Markers**

DNA was extracted from plant tissue of F2 and backcross populations using a CTAB method (Doyle and Doyle 1990). For analysis of AFLP markers with a LiCor (Lincoln, NE) automated sequencer, we modified protocols developed by Remington et al. (1999). Genomic DNA was digested with EcoRI and MseI, adaptor sequences were ligated to the cut sites, and ligated fragments were amplified with primers having a single selective nucleotide: MseI+C and EcoRI+A. The final selective amplification was modified from Vos et al. (1995) to include tailed primers. The tailed primer method was originally designed to create a universal primer for microsatellite amplification based upon M13 phage sequence (Steffens et al. 1993) and was adapted in this study for AFLP analysis. In this protocol, we used three primers in the final amplification: (1) a standard selective primer for the MseI site adaptor sequence (GAT GAG TCC TGA GTA A[+3 selective nucleotides]); (2) a selective primer for the EcoRI site adaptor sequence with an added 5’ tail that is complementary to M13 sequence (CAC GAC GAT TGA AAA CGA CGA CTG CGT ACC AAT TC[+3 selective nucleotides]); and (3) a standard M13 forward primer with an infrared label. The advantage of the tailed primer method is that labeled M13 primers can be purchased in bulk, and custom-labeled primers are not required. The EcoRI adaptor sequence was modified slightly from Vos et al. (1995) for the tailed protocol (S‘-AAC GAC TGC TGA CC/S‘-AAT TGG TAC GCA GTC GTC; boldface denotes nucleotides that were changed). Amplifications were carried out in 10-μl reactions. Amplified fragments from eight selective primer pairs...
(EcoRI + ACC/MseI + CGG; EcoRI + ACC/MseI + CGG; EcoRI + AGC/MseI + CGG; EcoRI + AGA/MseI + CGG; EcoRI + AAG/MseI + CGC; EcoRI + AAC/MseI + CGC; EcoRI + AGG/MseI + CGC; EcoRI + ATG/MseI + CGC) were separated on a LiCor automated sequencer. Genotypes were scored for the parental plants and the F2 and backcross progeny arrays. For genetic analysis, we sought markers that were fixed present in one species and absent in the other. Because AFLPs are dominant markers, it was necessary to infer parental genotypes from segregation in the progeny. We selected bands that were present in the parental plants of one species (plants used to generate F1 or backcrosses), invariably present in the progeny of backcrosses to that species, absent in plants used as parents of the second species, and variable in both F2 progeny and backcrosses to the second species. Several bands were present in parental plants of one species, absent in the other, but segregated in backcrosses to both species, indicating that the parents with bands present were heterozygous. These loci were not scored. Loci selected for analysis were scored by eye as present or absent in all progeny sets. Genotyping was replicated for a subset of approximately 5% of the progeny to test repeatability. For each dataset, loci were tested for departure from Mendelian segregation ratios using chi-square tests.

**Genetic Mapping**

For each of the F2, BCb, and BCj progenies, genetic linkage maps were estimated using Mapmaker/EXP (Whitehead Institute for Biomedical Research, Cambridge, MA). Linkage thresholds for both backcross populations were set at LOD > 5.0 and a maximum distance of 30 cM. For the F2 data, LOD threshold for linkage was increased to 7.0. Marker order was estimated for linkage groups in each data set by comparing likelihoods of map orders (‘compare’ function in Mapmaker/EXP). In linkage groups with many markers, pairwise distances were used to first select subsets of five markers distributed throughout the linkage group. After marker order was established with strong support (LOD > 3.0) for a subset of the markers, remaining markers were added to the map using the ‘try’ command. Additional markers were added when LOD > 2.0. We estimated genome length by adding two times the average intermarker distance to the length of each linkage group and then summing across linkage groups. We estimated genome coverage from marker number and genome length using a function proposed by Lange and Boehnke (1982), which has been shown to yield coverage estimates that are consistent with those of other methods (Remington et al. 1999).

**Quantitative Trait Loci Analysis**

We used QTL Cartographer (Basten et al. 1994, 2002) to locate QTL for the five floral traits with each dataset. Composite interval mapping (Zmapqtl, model 6) was used to control for the effect of genetic background in the analysis (Zeng 1993, 1994). In this method, several selected markers control for the genetic background while testing for the presence of QTL, and background markers are blocked out within a window of specified size around the point of analysis. For each trait and dataset, the number of background markers included in the composite mapping model was determined using the forward stepwise regression function in QTL Cartographer (Smmapqtl). In preliminary analyses, other regression functions (e.g., backward and forward) gave essentially equivalent results. Background marker number ranged from four to a set maximum of six. Window size was set at 20 cM in the F2 analysis and 15 cM for both BCj and BCb. Relatively large window sizes were chosen because of marker order uncertainties and sparse coverage in the genomic maps. Small adjustments in window size did not affect the qualitative results. Significant LOD scores (experimentwise \( \alpha = 0.05 \)) for QTL detection were determined using permutation tests for each trait and dataset (\( n = 1000 \)), a function provided in QTL Cartographer (Doerge and Churchill 1996).

The additive effect of each significant QTL was obtained from QTL Cartographer. Additive effects (the effect of substituting two alleles from L. jepsonii) were expressed as percent of the mean difference between parental species. For comparison with other QTL studies, the magnitude of QTL effects was also calculated as the percent of phenotypic variance explained by the QTL. Significant QTL in different datasets were hypothesized to correspond to the same genetic locus if they fell within 15 cM of a genetic marker shared by the two datasets.

**Dominance of Quantitative Trait Loci**

The ratio of QTL effects in reciprocal backcrosses can be used to estimate dominance. The genotypic values at a locus with two alleles (A1 and A2) are expressed as \( g(A_1A_1) = 0 \), \( g(A_1A_2) = (1 + k)a \), and \( g(A_2A_2) = 2a \), where \( a \) is the additive effect of the A2 allele and \( k \) is the dominance deviation (Lynch and Walsh 1998). From these values, \( k \) can be estimated as \( k = [2g(A_1A_2) - g(A_1A_1)]/g(A_1A_1) \). We used this relationship and the estimates of additive effect in both backcrosses to calculate dominance effects of QTL. We compared these to dominance estimates provided by QTL Cartographer analyses of F2 progeny.

**RESULTS**

**Phenotypic Variation**

For corolla tube length, corolla lobe length, corolla lobe width, anther length and stigma lobe length, the mean for L. jepsonii was 67–85% higher than that of L. bicolor, representing a difference of 7–17 standard deviations. For stigma–anther separation, the difference between parental species was only 2.3 standard deviations in magnitude. Repeated measurements of stigma-anther separation on individual plants were highly variable, indicating that the trait was sensitive to environmental or temporal factors. In the F2 progeny, for example, the mean coefficient of variation among replicate flowers was 0.375, as compared to a mean coefficient of variation of 0.028 for corolla lobe length and comparable values for the other four traits. As a result, stigma-anther separation was not included in further analyses.

For all traits, variability of F2 progeny was greater than that of F1 plants, and trait values within the range of the parental species were recovered at the ends of the F2 distribution (Fig. 2). This suggests that a limited number of genetic
Fig. 2. Phenotypic distributions of floral traits in parental and progeny populations. BCb, backcross to Leptosiphon bicolor; BCj, backcross to L. jepsonii. Arrows for F1, F2, and backcross progenies indicate mean values. Arrows labeled M indicate the midpoint between means for L. bicolor and L. jepsonii.
TABLE 1. Pearson product correlation coefficients, P-values, and sample size (in parentheses) for five phenotypic traits in the F2 progeny for a cross between Leptosiphon bicolor and L. jepsonii. Correlation coefficients and P-values that are significant after adjustment for multiple tests are shown in bold type.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Corolla tube length</th>
<th>Corolla lobe length</th>
<th>Corolla lobe width</th>
<th>Anther length</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corolla lobe length</td>
<td>0.598</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(441)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corolla lobe width</td>
<td>0.477</td>
<td>0.810</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>(441)</td>
<td></td>
<td>(441)</td>
<td>(441)</td>
</tr>
<tr>
<td>Anther length</td>
<td>0.469</td>
<td>0.536</td>
<td>0.422</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>(302)</td>
<td>(302)</td>
<td>(302)</td>
<td>(302)</td>
</tr>
<tr>
<td>Stigma lobe length</td>
<td>0.075</td>
<td>0.256</td>
<td>0.140</td>
<td>0.285</td>
</tr>
<tr>
<td></td>
<td>(308)</td>
<td>(308)</td>
<td>(308)</td>
<td>(308)</td>
</tr>
</tbody>
</table>

Factors contribute to each trait. The phenotypic data show overall dominance in all five floral traits toward the large-flowered parent, L. jepsonii, as indicated by a difference between the midparent value and the means for the F1 and F2 progeny (Fig. 2). Most pairs of traits showed positive phenotypic correlations, with statistically significant correlation coefficients in the F2 progeny ranging from 0.140 to 0.810 (Table 1). Stigma lobe length was the least strongly correlated to the other floral traits, with generally lower and nonsignificant correlation coefficients. Correlation matrices for the backcross data were highly congruent with those for F2 progeny (data not shown).

Of the 457 F2 plants, 21 individuals (4.8%) were found to be male sterile, with aborted anthers that completely lacked pollen. Male sterility was not observed in any other progeny set.

### Linkage Mapping

The eight primer combinations yielded bands ranging in size from 54 bp to 425 bp. The number of bands provided by each primer combination that met our criteria for use in the analysis (i.e., segregating in the F2 and one backcross progeny and fixed present in the other backcross progeny) ranged from 16 to 28. However, a number of these were not in linkage groups in the F2 population that corresponded to significant QTL in at least one other dataset, even though their LOD scores were slightly below the threshold.

Quantitative Trait Loci Detection

QTL analysis indicated that all of the floral traits measured are affected by multiple loci (Table 2). LOD significance thresholds ranged between 9.1 and 10.8 in the backcross datasets and between 13.9 and 18.1 in the F2 dataset. For each of the five traits analyzed, between two and seven QTL were detected in each dataset (Table 2). Additive effects of individual QTL explained 2% to 28% of the phenotypic variance and accounted for 6.4% to 54.2% of the difference in trait value between parental species (Table 2). With all traits included, 25, 25, and 23 QTL were detected at LOD levels above the significance threshold in the F2, BCb, and BCj datasets, respectively. We inferred four additional QTL in the F2 population that corresponded to significant QTL in at least one other dataset, even though their LOD scores were slightly below the threshold.

Although the BCb, BCj, and F2 maps have substantial incongruencies, in many cases a given QTL was detected in two or all three of these maps. For example, a QTL of large effect for corolla tube length was detected on linkage group 2 for all datasets, in each case, falling within 10 cM of a shared marker locus (Fig. 3). Of the 29 QTL detected in the F2 dataset, 11 had corresponding QTL in both backcrosses that met our criterion for identity across datasets. For five
we found QTL in corresponding linkage groups for both backcrosses, but only one mapped within 15 cM of a shared marker (Table 2).

**Dominance of Quantitative Trait Loci**

We calculated dominance of QTL from the relative effect size in backcross populations for 16 QTL for which these data were available. Of these, seven were essentially codominant \((-0.05 < k < 0.05)\), eight showed dominance of the *L. bicolor* allele \((-0.114 < k < -0.379)\), and one showed dominance of the *L. jeppsonii* allele \((k = 0.112)\). Four QTL were mapped in the F2 and BCb datasets, but were not detected in the backcross to *L. jeppsonii*, suggesting strong dominance of the *L. jeppsonii* allele. The presence of three QTL for anther length in the F2 and BCb datasets, but not the BCb dataset, suggest dominance of *L. bicolor* alleles. Dominance estimates from the QTL Cartographer analysis of F2 progeny indicate a higher proportion of essentially additive loci \((22 \text{ of } 29)\), four with dominant *L. bicolor* alleles and three with dominant *L. jeppsonii* alleles.

**DISCUSSION**

**Polygenic Control of Floral Divergence**

Our results indicate that several loci contribute to each of the floral size traits that have diverged between *L. jeppsonii* and *L. bicolor* (Table 2). Compiling information from the two backcross and F2 populations, we detected seven QTL for corolla tube length, seven for corolla lobe length, six for corolla lobe width, five for anther length, and three for stigma length. Characterization of genetic architecture is likely to be influenced by a number of methodological factors, such as the approach used (i.e., biometric vs. QTL mapping), population sample size, and marker density, which limits the extent to which meaningful comparisons can be made among different experiments. However, the number of QTL detected in our study falls within the range of gene or QTL numbers reported for floral and mating system traits in other taxa, and some consensus is emerging from the accumulating literature.

In the most comprehensive study of this kind to date, Fishman et al. (2002) identified 11 to 15 QTL affecting several floral traits that differentiate *Mimulus guttatus*, an outcrossing species, and *M. nudatus*, a selfing species. Four to fourteen QTL were found to have influenced the divergence of floral traits associated with the mating system in a wild and a cultivated rice (Uga et al. 2003). Lin and Ritland (1997) detected fewer QTL for floral traits distinguishing *M. guttatus* and a different selfing species, *M. platycalyx*, but a large amount of phenotypic variation was unexplained, suggesting the action of a number of undetected QTL. Similarly, in a QTL mapping experiment with limited marker coverage, Hodges et al. (2002) found one to three QTL contributing to the floral traits that distinguish two species of *Aquilegia*. Four to six QTL were detected for floral size and shape traits that differ in a bee-pollinated and hummingbird-pollinated species of *Mimulus* (Bradshaw et al. 1998). Biometrical studies of mating system traits in *Mimulus* species have also yielded comparable estimates. Using a modification of Wright’s (1968) method for inferring the number of genes contributing to quantitative traits, Fenster and Ritland (1994) found that minimum genetic factors for several floral traits ranged from five to 12, and Macnair and Cumbe’s (1989) study of *M. guttatus* and a derived selfing species, *M. cuprichilus*, found between three and seven genetic factors underlying floral size characters.

Populations of both *L. jeppsonii* and *L. bicolor* undergo substantial selfing, so the divergence of the two species represents only a part of the full transition from cross- to self-fertilization. Although corolla size in *L. jeppsonii* is similar to that of some obligately outcrossing congeners (e.g., *L. parviflorus*), the most recent SI ancestor of the *L. jeppsonii/L. bicolor* lineage (L. *androsaceus*) has corolla lobes that are approximately 75% longer than those of *L. jeppsonii* (Schemske and Goodwillie 1996). As Fenster and Ritland (1994) noted in their study of selfing and partially outcrossing species of *Mimulus*, the shift from one mating system extreme to the other is likely to have involved a larger number of genes than one involving a species in an intermediate stage, such as *L. jeppsonii*.

**Potential Biases in Quantitative Trait Loci Estimation**

The actual numbers of QTL underlying each trait are likely to be somewhat higher than our estimates. Beavis (1994) showed that any finite sample size will bias estimates of QTL number downward and QTL effect upward. While our F2 population \((N = 457)\) falls near or exceeds the minimum sample size recommended for estimation of QTL of minor or moderate effect (Beavis 1994; Doerge et al. 1997), each backcross population consisted of only 200 individuals and therefore must be viewed with caution. This raises specific concerns in interpreting the results for stigma lobe length and anther length, for which the sample sizes for phenotypic measurements were considerably smaller than for the other three floral traits. Across all three datasets, the number of QTL was lower and mean effect size higher for these traits, and the estimated additive effects of QTL in anther length sum to substantially more than 100% percent of the difference between parental species in the F2 and BCb datasets.

Assessing biases associated with limited sample size is complicated in this study because the expectations differ for F2 and backcross populations. While the sample size required to detect strictly additive QTL of a given effect is expected to be approximately double for backcross relative to F2 populations, that relationship for nonadditive QTL depends on the direction and strength of dominance (Lynch and Walsh 1998). Additionally, phenotypic variation due to minor background loci that limits QTL detection power is expected to be lower in backcross populations, which improves detection relative to F2 analyses (Lynch and Walsh 1998). Thus, backcross analyses may have had higher or lower power than the F2 analysis for detecting individual QTL.

We might have detected more QTL had we used inbred lines chosen to represent extremes for floral traits in each species. These traits are generally nonoverlapping between the two species (Schemske and Goodwillie 1996), which indicates the presence of largely fixed genetic differences. However, phenotypic variation within populations is substantial, especially in *L. jeppsonii* (Fig. 2, also see below),
Fig. 3. Linkage maps and quantitative trait loci (QTL) locations from three mapping populations for a cross between *Leptosiphon bicolor* and *L. jepsonii*. Locations of QTL are designated with boxes, with box width indicating the magnitude of additive effect (expressed as percent of difference between parental species). Asterisks above QTL indicate that the allele from *L. jepsonii* decreased the trait value. Dotted lines are drawn between corresponding loci on F2 and backcross datasets.
FIG. 3. Continued.
suggesting allelic variation for some loci. The parental plants in this experiment, chosen at random, may not have contained some of the alleles for larger flower size in *L. jepsonii*. Moreover, the power to detect those QTL for which parental plants were heterozygous is expected to be low. Limitations of the data are evident in the discrepancies in marker order and placement between F2 and backcross datasets. Several factors may account for these differences. Although AFLP markers allow rapid generation of genetic data that span the genome, they have low information content in F2 designs (Remington and O’Malley 2000). Moreover, errors in estimation of recombination frequency and marker order often occur with repulsion F2 matings (Knapp et al. 1995). Consistent with this explanation is the observation that map distances between markers were often considerably shorter in the backcross maps relative to the F2 map (Fig. 3). Genotyping error may also account for some of the discrepancies. Although replicate genotyping for a subset of individuals and markers indicated high repeatability, consistent genotyping error could have occurred due to amplification artifacts, and their effects might vary in the different genetic backgrounds of the crossing populations. Low resolution in the genetic maps limited our ability to distinguish multiple QTL on a linkage group. Finally, the considerable segregation distortion seen in our data (see below) might have affected our estimates of QTL number and effect size by limiting the effective sample size of our progeny sets.

Although the potential biases in our analysis discussed above likely limited our estimate of QTL number, phenotypic variability and the recovery of parental phenotypes among the F2 progeny suggest that the numbers of genetic factors are not dramatically larger than our estimates. Additional factors, such as epistasis and transgressive segregation, could contribute to phenotypic variation in the F2 progeny and complicate the inference of gene number from phenotypic distributions. However, we would expect the magnitude of these effects to be low unless gene number is in fact somewhat limited.

### Segregation Distortion

Substantial segregation distortion at marker loci is common in interspecific crosses (Zamir and Tadmor 1986) and often is reported in mapping experiments (Bradshaw et al. 1998; Virk et al. 1998; Fishman et al. 2001). Causes of segregation distortion may include inbreeding depression and selection occurring at gamete formation, fertilization, or during seed development (Zamir and Tadmor 1986; Fishman et al. 2001). In backcross populations of interspecific crosses, distorted ratios most often favor alleles from the recurrent parent, that is, the species to which the F1 is crossed (Rieseberg and Carney 1998). In contrast, we found that the majority of loci in both backcrosses showed overrepresentation of alleles from the large-flowered *L. jepsonii*. Although several explanations are possible, our results are consistent with the hypothesis that segregation ratios are caused, at least in part, by alleles for pollen tube growth rate. If pollen tube growth rate is higher in the long-styled parent, gametes bearing alleles for faster tube growth would have greater fertilization success. This could result in an overrepresentation of markers from the large-flowered parent that are linked to genes affecting pollen tube growth. Because F1 plants served as the male parents for both backcrosses, the distorted ratios we observed in backcross progeny are expected under this scenario. Pollen performance loci have been invoked as a possible explanation for directional segregation distortion in crosses of small- and large-flowered species of *Mimulus* (Fishman et al. 2001) and *Eucalyptus* (Myburg et al. 2004).

### Male Sterility

While all F1 and backcross progeny appeared to produce normal pollen, male sterility was observed in 4.8% of the F2 progeny. The presence of sterility in the F2 but not F1 progeny is indicative of epistasis, and the frequency at which it occurs is consistent with a classic model suggested by Dobzhansky.

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**TABLE 2.** Significant quantitative trait loci (QTL) detected for each trait for a cross between *Leptosiphon bicolor* and *L. jepsonii*. Additive effects for each QTL (the effect of substituting two alleles from *L. jepsonii*) are expressed as percent of the mean difference between parental species. A negative value for percent of mean difference between parents indicates that the effect of alleles from *L. jepsonii* (the large-flowered species) decreased the phenotypic value. An asterisk denotes a QTL for which LOD scores were slightly below the significance threshold, but that correspond to significant QTL in other datasets; “no marker” indicates that the linkage group or region of the linkage group had no amplified fragment length polymorphism marker coverage in the map for that dataset. QTL shown in bold fell within 15 cM of a marker shared on corresponding linkage groups, meeting our criterion for identity across datasets.

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(1936) and Muller (1942) for the evolution of reproductive isolation in diverging lineages. The Dobzhansky-Muller model proposes that hybrid sterility results from epistatic interactions among two or more loci for which substitutions have occurred in diverging populations. This genetic pathway to reproductive isolation does not require transient fitness reductions in either population, as would be the case when hybrid inferiority is caused by heterozygosity. Fitness is reduced only when alleles in diverging populations interact epistatically.

The observed frequency in the F2 progeny is not significantly different \( (P = 0.144) \) from the Mendelian expectation of 1:16, or 6.25% male sterility for a two-locus model in which the sterile genotypes are homozygous for alleles from \( L. \) bicolor at one locus and homozygous for alleles from \( L. \) jepsonii at another. In a study of genetic factors contributing to reproductive isolation in two species of \( M. \) jepsonii, Fishman and Willis (2001) obtained similar results, reporting a substantial number of completely male sterile individuals in F2 but not in F1 hybrids. In detailed analyses of components of male and female fertility in parentals and F1 and F2 hybrids, they concluded that epistatic Dobzhansky-Muller incompatibilities have played a major role in speciation. Our results provide preliminary evidence for that process in \( L. \) leptosiphon.

**Trait Correlations and Quantitative Trait Loci Effects**

QTL for corolla tube length, lobe length and lobe width often mapped to the same or adjacent positions on linkage groups (Fig. 3). These results are consistent with our observation of strong positive phenotypic correlations among these floral traits (Table 1). Stigma lobe length was less strongly correlated with other traits in the phenotypic analysis and, correspondingly, QTL for stigma lobe length appear not to be closely linked to those for the other traits. For anther length, however, strong positive phenotypic correlations with other traits were observed, yet QTL for anther length often mapped to independent locations. Environmental effects may contribute to the observed phenotypic correlations with another length, as is also true for correlations between the other traits. The phenotypic results for \( L. \) leptosiphon are congruent with the finding of positive correlations among floral size traits in \( M. \) jepsonii (Macnair and Cumbes 1989; Fenster and Ritland 1994), and colocalization of QTL for correlated floral traits has been found in other taxa (Lin and Ritland 1997; Fishman et al. 2002). In a study of mating system divergence in \( M. \) guttatus and \( M. \) nasutus, 22 of 24 QTL identified were found to affect at least two floral traits associated with the mating system (Fishman et al. 2002).

The colocalization of QTL for different traits could indicate genetic loci with overall effects on floral size. Pleiotropy seems particularly likely for a QTL on linkage group 6: loci for corolla tube length and corolla lobe length map to adjacent locations in both backcross datasets and, for both traits, the allele from the large-flowered species reduces the corolla size. As is generally true of interval mapping studies, however, our results are only suggestive of pleiotropy (Erickson et al. 2004) and do not allow us to reject an alternative explanation that loci for different traits are closely linked. In studies of crop species, fine mapping of some putatively pleiotropic QTL has revealed distinct, tightly linked genes for different traits (Paterson et al. 1990; Han et al. 1997; Monforte and Tanksley 2000).

Pleiotropy, if present, could play a role in facilitating adaptive evolution in flower size. In this scenario, selection for reduced allocation to the corolla would result in simultaneous decreases in several dimensions of flower size. This is consistent with the observation of convergent or parallel evolution of remarkably similar floral morphology in selving species of \( L. \) leptosiphon (Goodwillie 1999; Goodwillie and Stiller 2001). Conversely, in a study of correlated floral and mating system variation in \( L. \) jepsonii, we observed differences among populations in floral allometry (Goodwillie and Ness 2005). Although more selving populations had generally smaller flowers, the ratio of corolla lobe to tube length varied, indicating that different floral size dimensions are at least partially controlled by genes with independent effects.

**Dominance**

Estimates of QTL dominance drawn from two analytical methods yielded qualitatively different results. Because both methods have limitations, resolving the conflict is difficult. Dominant markers limit the accuracy of dominance estimation from F2 progeny. However, our method comparing effect sizes in backcross progeny may be influenced by errors in map construction and QTL detection in both datasets. Analysis of F2 progeny indicated a predominance of additive QTL, whereas the backcross comparison method suggested a substantial number of QTL with dominance of \( L. \) bicolor alleles. Assuming that selving is the derived condition, either result could be viewed as support for the theoretical prediction that adaptive evolution in outcrossing populations is likely to involve alleles with at least partial expression in heterozygotes (Haldane 1927; Charlesworth 1992). However, because the divergence of floral traits in \( L. \) jepsonii and \( L. \) bicolor likely occurred in a population already undergoing some self-fertilization, it could be argued that ‘Haldane’s sieve’ would be relatively weak.

Contrary to either estimate of QTL dominance, comparison of F1 and F2 means to midparent values indicated overall dominance toward \( L. \) jepsonii for all traits. Interestingly, Fishman et al. (2002) found a similar result in their study of mating system divergence in \( M. \) jepsonii; although phenotypic data showed overall dominance toward the large-flowered \( M. \) guttatus, dominance of QTL was more or less evenly distributed between the two parental species. A number of explanations demand consideration. The discrepancy is consistent with initial evolution of inbreeding by major genes of additive or dominant effect for selving, against a background of genes of minor effect, which are undetected as QTL, with alleles that exhibit dominance with respect to floral size traits. Segregation distortion favoring \( L. \) jepsonii could contribute to the apparent dominance in the F2 phenotypes, but cannot explain the comparable degree of dominance toward \( L. \) jepsonii observed in the F1 progeny. Apparent dominance of F1 and F2 progeny could result from maternal effects, since the maternal plant in initial parental crosses was \( L. \) jepsonii. Maternal effect QTL have been shown to account for a considerable proportion of phenotypic variation in other mapping
studies (Wolf et al. 2002). In our crossing design, however, we would expect maternal effects in both backcrosses to favor the recurrent parent species because these served as maternal plants. In fact, trait means for both backcrosses showed overall dominance toward L. jepsonii, as compared to the mean expected with strictly additive effects (calculations not shown). Finally, since all of the traits were size related, apparent dominance toward the larger species might reflect general hybrid vigor unrelated to genes with specific effects on floral traits. Under this scenario, however, we would predict stronger overall dominance in F₁ than in F₂ progeny, a pattern that is evident for only one of the five traits (corolla tube length, Fig. 2).

**The Genetic Architecture of Mating System Adaptation**

Theoretical work that models the temporal sequence of genetic changes in the process of adaptation predicts a skewed distribution of QTL effects with few major genes and many of small effect (Orr 1998). This is expected because mutations of large effect are more likely to be advantageous very early in the process of adaptation, whereas mutations of smaller effect are selected as populations approach their optima and adaptation is fine tuned. In their study of floral adaptation to pollinators in *Mimulus*, Bradshaw et al. (1998) found support for this model; for many of the traits studied, one or two major QTL and several smaller QTL were found.

In contrast, our study showed a fairly even distribution of QTL effects; most were found to be similarly moderate in size, and, as argued above, the few QTL with especially large estimated effects on stigma and anther size may have been inflated by small sample sizes. Our results are congruent with a study of mating system divergence in *Mimulus* (Fishman et al. 2002). In interpreting the lack of fit between their results to Orr’s model, Fishman et al. (2002) discussed several ways in which real populations may violate the model’s assumptions, some of which are relevant to our study of *Leptosiphon*. First, in the early stages of adaptation, selection on standing variation for genes of small effect might move a population swiftly to a position near the optimum phenotype, yielding a relatively even distribution of QTL underlying an adaptive trait. Considerable phenotypic variation for floral size traits is present in the *L. jepsonii* study population, at least some of which appears to be genetically based. For example, in a common garden study of this population, variation in corolla tube length among unrelated individuals was substantial (coefficient of variation = 12.6; unpubl. data), and the variance greatly exceeded that among *L. jepsonii* siblings measured in this study (variance ratio test, df = 30/33, *P* < 0.0001). Thus, standing variation might have contributed substantially to the adaptive shift in floral morphology.

Second, real populations may violate the assumption of Orr’s model that the phenotypic optimum is fixed (Fishman et al. 2002; Orr 2005b). Adaptive walks that track a moving optimum might yield an even distribution of QTL of small effect because the distance between the phenotype and the optimum is relatively small and constant throughout the process. A moving optimum seems particularly plausible in the evolution of *Leptosiphon* floral morphology. For instance, consider the following scenario: changes in the timing of self-compatibility from delayed to competing selfing cause an increase in the selfing rate and, thereby, promote selection to reduce allocation to corollas. The evolution of smaller flowers leads to a reduction in pollinator visitation and, as a result, a further increase in the selfing rate. As the selfing rate continues to increase, the optimal flower size is reduced even further. If such a sequence occurred in *Leptosiphon*, we might expect a relatively even distribution of QTL contributing to the present divergence in floral morphology.

In characterizing the architecture of mating system adaptation in *Leptosiphon*, we need to consider the genetic basis of pollen-pistil traits as well. The contemporary differences in floral traits between *L. jepsonii* and *L. bicolor* are likely to have been fixed after the divergence of transient SI and complete self-compatibility. Within populations of *L. jepsonii*, the flowers of most individuals exhibit transient SI, but a few individuals are fully self-compatible upon flower opening, as in *L. bicolor* (Goodwillie et al. 2004; Goodwillie and Ness 2005). Results of crosses in some populations indicate that the shift from transient SI to full self-compatibility may be controlled by only two genes (C. Goodwillie, unpubl. data). Thus, a substitution in a gene of large effect on pollen-pistil interactions, conferring changes in the timing of selfing, may have shifted the adaptive optimum for morphological traits, leading to the fixation of numerous QTL of small to moderate effect on floral size traits.

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**Literature Cited**


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