Quantitative Trait Loci for Floral Morphology in *Arabidopsis thaliana*

Thomas Juenger,* Michael Purugganan† and Trudy F. C. Mackay†

**Department of Integrative Biology, University of California, Berkeley, California 94720-3140 and †Department of Genetics, North Carolina State University, Raleigh, North Carolina 27695-7614*

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ABSTRACT

A central question in biology is how genes control the expression of quantitative variation. We used statistical methods to estimate genetic variation in eight *Arabidopsis thaliana* floral characters (fresh flower mass, petal length, petal width, sepal length, sepal width, long stamen length, short stamen length, and pistil length) in a cosmopolitan sample of 15 ecotypes. In addition, we used genome-wide quantitative trait locus (QTL) mapping to evaluate the genetic basis of variation in these same traits in the Landsberg *erecta* × Columbia recombinant inbred line population. There was significant genetic variation for all traits in both the sample of naturally occurring ecotypes and in the L*er* × Col recombinant inbred line population. In addition, broad-sense genetic correlations among the traits were positive and high. A composite interval mapping (CIM) analysis detected 18 significant QTL affecting at least one floral character. Eleven QTL were associated with several floral traits, supporting either pleiotropy or tight linkage as major determinants of flower morphological integration. We propose several candidate genes that may underlie these QTL on the basis of positional information and functional arguments. Genome-wide QTL mapping is a promising tool for the discovery of candidate genes controlling morphological development, the detection of novel phenotypic effects for known genes, and in generating a more complete understanding of the genetic basis of floral development.

GENES that control various aspects of floral development have been discovered in the model plant *Arabidopsis thaliana*. These genes have been identified largely on the basis of their mutant phenotypes, which range from transformations in floral organ identity to aberrant morphologies of floral and inflorescence structures. Molecular analyses indicate that many of the identified loci that control floral development encode either DNA-binding transcriptional activators, RNA binding proteins, or members of signal transduction pathways (reviewed in Coen 1991; Weigel 1995; Howell 1998).

Several genes [*e.g., APETALA1-3 (AP1-3), AGAMOUS (AG), PISTILLATA (PI), SEPALLATA1-3(SEP1-3)*] that influence the identity and position of floral organs have been cloned in *A. thaliana* (Coen 1991; Pelaz *et al.* 2000). These studies have culminated in a simple conceptual model, the "ABCD" model, describing the pattern formation of flowers. The ABCD model posits that four classes of homeotic regulatory genes, A, B, C, and D, influence the identity and position of floral organs. In each floral whorl, several homeotic genes are expressed, and it is the particular combination of gene expression that determines the identity of floral structures in each whorl (Bowman *et al.* 1991; Pelaz *et al.* 2000). Several genes have also been discovered with effects on other qualitative aspects of flower development, including genes that activate the expression of floral organ identity genes [*CAULIFLOWER (CAL)* and *LEAFY (LFY)*], influence floral meristem size [*CLAVATA (CLV)*], mediate the interactions between floral meristem and organ identity genes [*LEUNIG (LUG)* and *UNUSUAL FLORAL ORGANS (UFO)*], influence the setting of boundaries between whorls [*SUPERMAN (SUP)* and *UFO*], and alter the number of organs in each whorl [*CLV, PERIANTHA (PAN)*, and *SUP*].

In contrast, we know very little about the genes that control quantitative aspects of floral morphology. These quantitative characters, such as flower size and shape, tend to vary continuously among individuals within populations and can arise from both environmental and genetic factors. Since plant cells do not migrate during development, variation in floral organ size is probably the result of variation in patterns of cell division and cell elongation among genotypes (Meyerowitz 1997). Several molecular genetic studies have reported relatively large qualitative effects of mutations on the size or shape of floral organs. In addition, recent studies have shown that mutations and/or constitutive expression of several floral genes qualitatively affect floral organ size [*ALTERED AUXIN RESPONSE* (*AXR1*), Estelle and Somerville 1987; *REVOLUTA* (*REV*), Talbert *et al.* 1995; *AINTEGUMENTA* (*ANT*), Elliott *et al.* 1996; Krizek 1999; Mizukami and Fischer 2000; *NAC-like ACTIVATED BY AP3/PI* (*NAP*), Sablowski and Meyer-

Corresponding author: Thomas Juenger, Department of Integrative Biology, 3060 Valley Life Sciences Bldg. #3140, University of California, Berkeley, CA 94720-3140. E-mail: tj@socrates.berkeley.edu

owitz 1998; *ROTUNDIFOLIA3* (*ROT3*), Kim *et al.* 1999; *ANT*; *Arabidopsis SKP1-LIKE-1* (*ASK1*), Zhao *et al.* 1999; *ANT*]. These genes are all excellent candidates as factors segregating isoalleles underlying naturally occurring quantitative variation in floral organs.

Understanding quantitative variation in floral morphology is crucial to attempts to dissect the genetic basis of phenotypic integration in flowers. Angiosperm floral organs tend to show strong positive phenotypic correlation (Berg 1960). This pattern of correlation may be due to similar responses of traits to environmental variation (environmental correlation) or common inheritance (genetic correlation). Genetic correlation may be the product of pleiotropic effects of single genes on suites of developmentally related characters, or due to linkage disequilibrium between separate genes with effects on different characters. Patterns of genetic correlation play a critical role in understanding evolution because they can influence the trajectory of evolutionary responses to natural or artificial selection on complex multivariate phenotypes. Although many quantitative genetic studies have estimated genetic correlations using statistical genetic models in both natural and experimental populations, few studies have actually determined their underlying genetic basis. From an evolutionary perspective, evaluating the genetic architecture of integrated characters is central to understanding how developmental and genetic processes constrain or facilitate adaptive evolution.

We currently lack information on the polygenic basis of covarying traits because few organisms have the experimental versatility to allow an accurate mapping of quantitative trait loci (QTL), the cloning of potential candidate genes, and a sufficient quantity of DNA sequence data to allow tests of association between molecular variants and phenotypes (Mackay 1995; Falconer and Mackay 1996; Lynch and Walsh 1998). Moreover, there are few study systems with robust models of how genes choreograph morphological development. Until recently, such information was limited to studies of the fruit fly (*Drosophila melanogaster*), where technical advances have allowed incredible progress in elucidating the genetic basis of developmental programs (*e.g.*, early body plan formation, eye development, bristle formation; Lawrence 1992; Mackay 1995). This information is critical in making the step from statistical genetic models to mapped quantitative trait loci and, ultimately, the identification of genes that affect quantitative variation in a trait.

In the present work, we investigated quantitative genetic variation in the floral morphology of *A. thaliana* using two approaches. First, we partitioned genetic and environmental components of variation and covariation in eight floral traits (fresh flower mass, petal length, petal width, sepal length, sepal width, short stamen length, long stamen length, and pistil length) among a cosmopolitan sample of *A. thaliana* ecotypes. Second, we mapped QTL affecting these same eight traits (and principal components of the traits) using a recombinant inbred line (RIL) population derived from a cross between Landsberg *erecta* (L*er*) and Columbia (Col) ecotypes (Lister and Dean 1993). Finally, we used positional information from this QTL analysis in conjunction with data on the physical location of known genes to generate hypotheses concerning candidate genes with quantitative affects on floral morphology.

MATERIALS AND METHODS

Ecotypes: We quantified phenotypic variation in the floral morphology of a cosmopolitan sample of 15 *A. thaliana* ecotypes (Arabidopsis Biological Resource Center accessions: Col, L*er*, Cvi, CS1332, CS903, CS6042, CS6048, CS6092, CS6803, CS970, CS996, CS1092, N1006, N1074, and N1316). We measured eight floral characters (Table 1) from three flowers of each of 12 replicate plants for each ecotype. The three flowers used were among the first six flowers to develop on each plant and were collected at anthesis (stage 13; Smyth *et al.* 1990). Fresh flower mass was obtained using a microbalance (Mettler UM3). All morphological measurements were made on dissected fresh flowers using a stereomicroscope equipped with an ocular micrometer. Measurements were conducted on a single randomly chosen organ from each sampled flower. Length measurements were collected only on medial sepals. Short and long stamens were measured from the base of the filament to the tip of the anthers. Because pistils are still rapidly elongating at stage 13, pistil length and fresh flower mass measurements may include considerable environmental variation related to slight differences in the timing of flower collection. Plants were grown in randomized flats under long-day light conditions in a temperature-controlled greenhouse at the University of Chicago.

Quantitative genetic analysis of floral traits: Variation for each of the floral traits was partitioned into sources attributable to ecotype (E), replicate plant (R) within ecotype, and error with a random effects nested analysis of variance (ANOVA) using the model $y = \mu + E + R(E) + \text{error}$. The among-ecotypic component of variance estimated from a nested ANOVA represents the total genetic variation among the population of inbred ecotypes. Since many of the measured floral traits were positively correlated (see below), a multivariate analysis of variance (MANOVA) was performed to test for significant ecotypic variation over all traits simultaneously. Tests of the significance of *F*-ratios were obtained using SAS procedure GLM. All traits were normally distributed. The variance components for the random effects were obtained using restricted maximum likelihood estimation (REML) with SAS procedure VARCOMP. Standard errors for the variance components were obtained by taking the square root of the variance for each variance component. We computed the ratio V_G/V_P for each trait, where V_G equals the among-ecotype variance component for each trait and V_P equals the total phenotypic variance. In addition, we calculated the coefficient of genetic variation (CV_G) as $(100\sqrt{V_G})/\bar{X}$ for each trait, where V_G is the among-ecotype variance component and $\bar{X}$ is the mean of the trait.

Genetic correlations (r_G) among floral traits were estimated as

$$\frac{\text{cov}(i, j)}{\sigma_i \sigma_j},$$

where $\text{cov}(i, j)$ is the covariance among ecotype means for trait i and j, and σ_i and σ_j are the square roots of the respective among-ecotype variance component for each trait (Robert-

TABLE 1

Candidate floral morphology loci

Candidate loci[a]	Chromosome	Location[c] (∼cM)	References[d]
CARPEL FACTORY (CAF)[b]	I	2	Jacobsen *et al.* (1999)
ALTERED AUXIN RESPONSE (AXR-1)[b]	I	7	Estelle and Somerville (1987)
CYP78A5[b]	I	17	Zondlo and Irish (1999)
UNUSUAL FLORAL ORGANS (UFO)	I	46	
CAULIFLOWER (CAL1)	I	52	
CLAVATA2 (CLV2)[b]	I	88	Kayes and Clark (1998)
NAC-LIKE, ACTIVATED BY AP3/PI (NAP)[b]	I	103	Sablowski and Meyerowitz (1998)
APETALA1 (AP1)	I	106	
CRABS CLAW (CRC)[b]	I	107	Bowman and Smyth (1999)
PERIANTHIA (PAN)	I	108	
ADHESION OF CALYX EDGES (ACE)	I	111	
ARABIDOPSIS SKP1-LIKE-1 (ASK1)[b]	I	115	Zhao *et al.* (1999)
CLAVATA1 (CLV1)	I	115	
CURLY LEAF (CFL)[b]	II	27	Goodrich *et al.* (1997)
ERECTA (ER)[b]	II	36	Torii *et al.* (1996); Yokoyama *et al.* (1998)
CLAVATA3 (CLV3)	II	36	
ETTIN (ETT)[b]	II	47	Session *et al.* (1997)
FILIMENTOUS FLOWER (FIL)[b]	II	62	Chen *et al.* (1999); Sawa *et al.* (1999)
TSO1[b]	III	29	Liu *et al.* (1997); Hauser *et al.* (1998); Hauser *et al.* (1998, 2000); Song *et al.* (2000)
SUPERMAN (SUP)	III	35	
APETALA3 (AP3)	III	71	
AGAMOUS (AG)	IV	49	
LEUNIG (LUG)[b]	IV	58	Liu and Meyerowitz (1995)
ROTUNDIFOLIA3 (ROT3)[b]	IV	73	Kim *et al.* (1999)
AINTEGUMENTA (ANT)[b]	IV	80	Elliott *et al.* (1996); Krizek (1999); Mizukami and Fischer (2000)
APETALA2 (AP2)	IV	80	
PISTILLATA (PI)	V	27	
TOUSLED (TSL)[b]	V	28	Roe *et al.* (1993)
WIGGUM (WIG)[b]	V	58	Running *et al.* (1998)
TINY (TNY)[b]	V	59	Wilson *et al.* (1996)
PINHEAD/ZWILLE (PNH)[b]	V	85	Lynn *et al.* (1999)
REVOLUTA (REV)[b]	V	122	Talbert *et al.* (1995); Ratcliffe *et al.* (2000)
LEAFY3 (LFY3)	V	126	
SNOWBALL (SNO)[b]	Unknown		Running *et al.* (1998)

[a] Inclusion of loci as candidate genes was determined from published molecular genetic studies of Arabidopsis floral development.

[b] Genes with strong evidence for quantitative effects on some of aspect of floral morphology.

[c] Where possible, BLAST searches were used to place cloned sequences for candidate genes on particular BAC clones comprising the Arabidopsis physical map. The linkage map position for each gene was then estimated by locating the RI marker nearest the clones containing candidates. The positions of *LEUNIG* and *WIGGUM* were estimated from published linkage data because they have yet to be cloned. These positions are approximate as placement accuracy depended on the quality of linkage data and the proximity of genes to markers on the study map.

[d] References providing evidence for the effect of particular genes on some quantitative aspect of floral morphology.

son 1959). The significance of each genetic correlation was determined using a *t*-test after a *Z* transformation of the correlation coefficient as described by Sokal and Rohlf (1981) and a sequential Bonferroni correction for multiple tests (Rice 1989).

We also conducted a principal components analysis (PCA) on the line means for the eight traits to evaluate overall morphological integration using the FACTOR procedure in SYSTAT 7.0.

Recombinant inbred lines: We used 74 RI lines generated from a cross between Col and L*er* ecotypes to map QTL for floral traits (Lister and Dean 1993; Alonso-Blanco and Koornneef 2000). F_1 progeny from the initial cross were taken through eight generations of selfing via single seed descent to produce nearly homozygous lines (residual heterozygosity is 0.42%). We constructed a linkage map using a subset of 169 markers (chromosome I, 43 markers; chromosome II, 24 markers; chromosome III, 26 markers; chromosome IV, 33 markers; and chromosome V, 43 markers), all of which have been genotyped in at least 80% of our sample lines. The map position of each marker (*d* cM) was estimated from the observed recombination frequencies (*r*) using the Kosambi

TABLE 2

Descriptive statistics and quantitative genetic partitioning of floral morphology in the Ecotype (A) and RIL (B) populations

Trait	$\bar{x} \pm$ (SD)	$V_G \pm$ (SE)	$V_R \pm$ (SE)	V_E	V_G/V_P	CV_G
A. Ecotype population						
Fresh flower mass (FM)	1.59 (0.44)	0.194 (0.07)	0.025 (0.00)	0.024	0.80	27.70
Petal length (PL)	3.83 (0.51)	1.008 (0.39)	0.120 (0.02)	0.199	0.76	13.09
Petal width (PW)	1.23 (0.17)	0.119 (0.05)	0.014 (0.05)	0.035	0.71	14.02
Sepal length (SL)	2.40 (0.30)	0.350 (0.13)	0.071 (0.01)	0.066	0.72	12.53
Sepal width (SW)	0.96 (0.09)	0.035 (0.01)	0.012 (0.00)	0.040	0.40	9.74
Short stamen length (SSL)	2.58 (0.41)	0.654 (0.25)	0.088 (0.02)	0.223	0.68	15.76
Long stamen length (LSL)	3.01 (0.43)	0.709 (0.27)	0.095 (0.02)	0.183	0.72	13.96
Pistil length (PIL)	3.19 (0.48)	0.920 (0.36)	0.074 (0.04)	0.508	0.61	15.03
B. RIL population						
Fresh flower mass (FM)	0.97 (0.23)	0.044 (0.00)	0.030 (0.00)	0.007	0.54	21.62
Petal length (PL)	3.04 (0.48)	0.780 (0.16)	0.432 (0.05)	0.135	0.58	14.50
Petal width (PW)	1.00 (0.13)	0.056 (0.01)	0.038 (0.00)	0.017	0.51	11.80
Sepal length (SL)	1.99 (0.21)	0.150 (0.03)	0.113 (0.01)	0.024	0.52	9.71
Sepal width (SW)	0.88 (0.08)	0.021 (0.00)	0.019 (0.00)	0.017	0.36	8.19
Short stamen length (SSL)	1.90 (0.48)	0.434 (0.09)	0.195 (0.03)	0.122	0.58	17.34
Long stamen length (LSL)	2.95 (0.36)	0.537 (0.10)	0.182 (0.02)	0.058	0.69	12.42
Pistil length (PIL)	2.48 (0.39)	0.426 (0.09)	0.123 (0.04)	0.463	0.42	13.13

Means and standard deviations for all linear measurements are presented in millimeters while fresh flower mass is presented in milligrams. Quantitative genetic analyses were conducted on the ocular measurement scale. V_G, the among Ecotype or RIL variance component; V_R, the variance component associated among replicate plants nested in either Ecotype or RIL; V_E, the residual within-plant variance component; V_P, total phenotypic variance component.

mapping function as implemented by the software MapMaker 3.0 (Lander *et al.* 1987). This analysis provided unique positions for each marker and a map spanning 482 cM of the Arabidopsis genome. This map did not differ in marker order from the published Arabidopsis RI linkage map.

We measured floral phenotypes on two flowers from each of two to six replicate plants for each of the 74 RI lines. Floral measurements were collected as described for the ecotype experiment (see above). Plants were grown in randomized flats under controlled conditions in a single Percival growth chamber [long-day light regime (16 hr light:8 hr dark); temperature control (20° light:18° dark)]. Quantitative genetic analyses were conducted on these data as described for the ecotypes.

QTL Analyses: QTL affecting floral morphology were mapped using the composite interval mapping (CIM) procedure described by Zeng (1993, 1994) with the software QTL Cartographer (Basten *et al.* 1994, 1997). This procedure tests the hypothesis that an interval flanked by two adjacent markers contains a QTL affecting the trait, while statistically accounting for the effects of additional segregating QTL using multiple regression on markers outside the tested interval. The likelihood-ratio (LR) test statistic is $-2 \ln(L_0/L_1)$, where L_0/L_1 is the ratio of the likelihood under the null hypothesis (there is no QTL in the interval) to the alternative hypothesis (there is a QTL in the interval). The QTL analysis was performed on the least-squares RI line means and principal component scores. The number of marker cofactors for each CIM model was set by forward-backward stepwise regression with the critical P value set at 0.05 (model 6). We used a window size of 10 cM and tests were performed at intervals of 2 cM. A genome-wide critical threshold value for the experimentwise type I error rate $\alpha = 0.05$ was set for each trait independently by randomly permuting the line means among genotypes 1000 times and using the empirical permutation false positive rate (Churchill and Doerge 1994; Doerge and Churchill 1996).

In addition, we fit the markers closest to each significant QTL likelihood peak in a multiple regression model to provide estimates for each trait of the standardized additive genotypic effects ($2a/\sigma_P$) and standard errors of each QTL, using the GLM procedure of SAS. The additive genotypic effect corresponds to the standardized difference in the means of the two homozygous genotypes at a particular QTL. Positive additive effects indicate that the Col parental genotype has the higher mean. The percentage of the total genetic variation explained by each significant QTL was determined by dividing the sums of squares associated with each marker by the total model sums of squares from GLM models.

We searched for pairwise epistasis between the significant QTL (detected with composite interval mapping) by incorporating marker interactions in linear models using the GLM procedure of SAS. For each trait, we fit a series of GLM models including the main effect of all significant markers detected in the CIM analysis and one additional marker × marker interaction. The significance of interaction terms was determined using a sequential Bonferroni procedure, correcting for the number of linear models investigating epistasis for each marker (Rice 1989). We calculated the epistatic effect of each significant interaction ($4i$) as $(A + D - B - C)/\mathrm{SD}$, where A and D represent the means of the homotypic classes (AA, BB), and B and C represent the means of the heterotypic classes (AB, BA; Mather and Jinks 1977).

Candidate genes: We identified potential candidate genes by relating QTL positions to the Arabidopsis physical genome map and known locations of genes affecting floral development. We placed conservative confidence intervals (CI) around each QTL as the distance in centimorgans on either

TABLE 3

Genetic correlations (r_G) among floral traits in the Ecotype (below diagonal) and RIL (above diagonal) populations

	FM	PL	PW	SL	SW	SSL	LSL	PIL
FM		1.08	1.00	0.89	0.83	0.87	0.85	0.94
				(0.83, 0.93)	(0.74, 0.89)	(0.80, 0.91)	(0.77, 0.90)	(0.92, 0.96)
PL	0.85		0.90	1.10	0.30	1.11	1.11	1.15
	(0.59, 0.95)		(0.84, 0.93)		(0.08, 0.49)			
PW	0.92	0.78		0.72	0.82	0.79	0.74	0.80
	(0.77, 0.97)	(0.45, 0.92)		(0.59, 0.81)	(0.73, 0.88)	(0.69, 0.86)	(0.62, 0.83)	(0.70, 0.87)
SL	0.86	0.82	0.79		0.30	1.08	1.07	1.13
	(0.62, 0.95)	(0.53, 0.94)	(0.46, 0.93)		(0.08, 0.49)			
SW	0.90	*0.25*	*0.29*	*0.34*		0.29	0.24	0.33
	(0.72, 0.97)	(−0.30, 0.67)	(−0.26, 0.70)	(−0.21, 0.72)		(0.07, 0.48)	(0.01, 0.44)	(0.11, 0.52)
SSL	0.79	0.81	0.70	*0.62*	*0.23*		1.10	1.09
	(0.46, 0.93)	(0.51, 0.93)	(0.20, 0.89)	(0.16, 0.86)	(−0.32, 0.66)			
LSL	0.81	0.84	0.71	*0.62*	*0.23*	1.02		1.11
	(0.51, 0.93)	(0.57, 0.94)	(0.31, 0.89)	(0.16, 0.86)	(−0.32, 0.66)			
PIL	0.87	0.98	0.72	0.74	*0.23*	0.73	0.97	
	(0.64, 0.96)	(0.94, 0.99)	(0.33, 0.90)	(0.36, 0.91)	(−0.32, 0.66)	(0.34, 0.90)	(0.91, 0.99)	

Genetic correlations were estimated as the covariance among ecotype and RIL means for two traits divided by the product of the standard genetic deviations for those traits. Significance was tested using a *Z* transformation and a sequential Bonferroni correction for multiple tests—each correlation is followed (in parentheses) by a 95% confidence interval based on the *Z* transformation. Several correlations extend beyond the range of the genetic correlation due to sampling variance—confidence intervals for these correlations are undefined. Correlations not significant at the Bonferroni-corrected α are indicated in italics.

side of the QTL locations where there was a drop in the LR statistic of ~9.22 (corresponding to a 2-LOD score drop; van Ooijen 1992). The RI markers closest to the confidence interval cutoffs were considered 2-LOD CI flanking markers. We searched the Arabidopsis physical map to identify the genetic clones contained within these flanking markers for each putative QTL. Once a marker was located on a specific clone, we inspected the annotated sequence database in the intervening regions for likely candidates based on functional studies. Because the Arabidopsis genome has not been completely sequenced (chromosomes I and III) or annotated (chromosome V), we also compared QTL locations to the positions of 33 candidate genes (Table 1). Inclusion of loci as candidates was determined from a review of published molecular genetic studies of Arabidopsis floral development. This list includes genes with both quantitative and qualitative (often homeotic) effects on flower development. Where possible, BLAST searches were used to locate the position of these candidate genes on particular bacterial artificial chromosome (BAC) clones comprising the Arabidopsis physical map.

Matching QTL and candidate positions is only a first step in understanding the genetic architecture of quantitative traits. To establish some statistically based criterion for the matching of QTL and candidate genes, Nuzhdin *et al.* (1998) have proposed a simple randomization test that asks whether genome locations with high QTL mapping statistics are associated with candidate gene locations more frequently than by chance alone. Nuzhdin *et al.* (1998) generated a hypothetical list of candidate genes affecting Drosophila bristle number based on *a priori* information of gene function and evaluated the sum of log-likelihood-ratio statistics at these locations for their mapping data on bristle number QTL. A test can then be implemented by randomly sampling 10,000 data sets for the same number of positions as candidate genes and calculating the sum of the likelihoods for each new set of positions. Support for the role of the specified candidates (as a set) underlying QTL is found if the sum of the likelihoods of the candidate positions is in the top 5% of the randomization distribution. We performed the Nuzhdin *et al.* (1998) randomization test for the eight floral traits using 33 candidate genes (Table 1) and sampling 10,000 data sets. In addition, we conducted the same test using a subset of 18 genes (asterisks in Table 1) with known quantitative effects on floral development. The gene *SNOWBALL* was excluded from this analysis because it has not been cloned and we could not locate linkage data for placement on the genome.

RESULTS

Quantitative genetic analysis: Means and standard deviations for each of the eight floral traits in the ecotype and RI populations are given in Table 2, A and B, respectively. In general, the means and levels of variability are slightly less in the RI mapping population compared to the ecotype population, as expected.

Results of the MANOVA indicate a highly significant overall effect of ecotype and RI line on floral morphology (ecotype, Wilks' $\lambda = 0.0007$, $F = 14.57_{112,902}$, $P < 0.0001$; RI line, Wilks' $\lambda = 0.0008$, $F = 2.93_{584,1196}$, $P < 0.0001$). Similarly, univariate ANOVAs indicate significant ecotype and RI line effects for each trait (in all cases, $P < 0.001$).

We estimated the ratio V_G/V_P for each trait as the among-ecotype or -RI line variance component divided

TABLE 4

Principal component analysis of eight floral traits in both the Ecotype and RIL populations

	Ecotype population		RIL population	
	Component 1	Component 2	Component 1	Component 2
FM	0.95	0.18	0.91	0.36
PL	0.87	−0.04	0.96	−0.24
PW	0.88	0.34	0.82	0.85
SL	0.93	0.31	0.92	−0.18
SW	0.84	0.33	0.44	0.44
SSL	0.90	−0.40	0.93	−0.23
LSL	0.89	−0.40	0.94	−0.28
PIL	0.94	−0.28	0.94	−0.21
Variance explained (%)	81	10	76	16

by the total phenotypic variation in the trait. These values were generally high and ranged from 0.40 to 0.80 (average 0.67) and 0.36 to 0.69 (average 0.52) for the ecotype and RI populations, respectively.

Broad-sense genetic correlations between the floral characters were all positive and significantly different from zero in both the ecotype and RI populations for all but one trait (Table 3). The exception is for sepal width, which was significantly correlated only to fresh flower mass in the ecotype population. In several instances, the genetic correlations were not significantly different from 1.0. The genetic correlations range in magnitude from 0.23 to 1.0 (average 0.66) in the ecotype population, and 0.24 to 1.0 (average 0.81) for the RI population. These genetic correlations may be due to pleiotropic effects of single QTL on floral traits or linkage between QTL affecting different traits.

Results of the principal components analysis of the eight traits are presented in Table 4. Principal components 1 and 2 (PC1 and PC2) account for 81 and 10% of the variation for the ecotype population and 76 and 16% of the variation for the RI population, respectively. The first component reveals a consistent positive loading for each trait in both populations and can be considered a general size vector. Ecotypes or RILs with extreme scores for PC1 had flowers that were either larger or smaller overall than the average flower from that population. The second component contrasts the highly variable length measurements [petal length (PL), sepal length (SL), pistil length (PIL), long stamen length (LSL), and short stamen length (SSL)] with the less variable width characters [sepal width (SW) and petal width (PW)] and fresh flower mass (FM).

QTL Mapping: Table 5 provides a listing of the QTL that significantly affected some aspect of Arabidopsis floral morphology. Each QTL is designated as QTLF (F, floral) followed by a two-number extension corresponding to chromosome and an ordering number from the left telomere. QTL presented in Table 5 were significant at the empirically determined threshold value corresponding to $P = 0.05$ (average LR threshold = 14.70) based on permutation testing. For each trait and QTL combination, we present the RI marker nearest to the LR statistic peak, the estimated centimorgan position and 2-LOD confidence interval surrounding the QTL, the standardized additive genotypic effect ($2a/\sigma_P$), the proportion of the total phenotypic variance explained by the QTL, and the flanking RI markers associated with a 2-LOD confidence interval. Likelihood-ratio statistic profile plots for each trait are presented in Figures 1 and 2.

Altogether, 18 significant QTL were detected. Several QTL were found on each chromosome (chromosome I, 5 QTL; chromosome II, 3 QTL; chromosome III, 2 QTL; chromosome IV, 4 QTL; chromosome V, 4 QTL). Two-LOD confidence intervals ranged from 4 to 22 cM, averaging 10.7 cM. Generally, these confidence intervals encompass only one or two RI markers directly flanking the marker closest to the maximum LR peak. The number of QTL affecting any particular trait ranged from 2 (flower mass and petal width) to 13 (petal length), averaging 5. The proportion of the total phenotypic variation explained for each trait by these QTL ranged from 35% (petal width) to 97% (sepal length), averaging 71% (estimated from GLM models composed of all significant QTL affecting a trait).

Seven QTL significantly affected single traits (QTLF-I-1, QTLF-I-4, QTLF-I-5, QTLF-II-3, QTLF-III-1, QTLF-III-2, and QTLF-IV-2), while 11 QTL were associated with two or more traits (QTLF-I-2, QTLF-I-3, QTLF-II-1, QTLF-II-2, QTLF-IV-1, QTLF-IV-4, QTLF-IV-3, QTLF-V-1, QTLF-V-2, QTLF-V-3, and QTLF-V-4). QTL had consistent positive or negative additive effects on all of the traits they affected. The standardized additive genotypic effect of these QTL (averaged across floral traits, but excluding PC1 and PC2) ranged from 0.24 to 1.30, with an average of 0.54. The proportion of the total phenotypic variation explained by individual QTL ranged from 2 to 77%, with an average of 15.6%.

We detected two significant additive × additive epi-

TABLE 5

Results of QTL analyses of floral traits in Arabidopsis using composite interval mapping of RI line means and principal component scores

QTL[a]	Marker	Position (cM)	Trait	Additive effect ($2a/\sigma_P$) ± 1 SE[b]	% variance explained	2-LOD CI flanking markers	Candidate loci[c]
				Chromosome I			
QTLF-I-1	mi113	20 (19–24)	SW	−0.93 ± 0.18	32	(mi348, ARR7)	
	mi113	22 (18–24)	PC2	0.92 ± 0.16	25	(EG17G9, ARR7)	
QTLF-I-2	mi265	32 (30–39)	PL	−0.45 ± 0.14	4	(m235, CDs12)	
	mi265	32 (28–38)	SL	−0.49 ± 0.14	7	(g3829, mi62)	
	mi163	33 (19–36)	FM	−0.74 ± 0.20	21	(mi348, mi62)	
	mi62	36 (30–40)	LSL	−0.46 ± 0.13	6	(m235, CDs12)	
QTLF-I-3	UFO	51 (39–54)	SSL	−0.46 ± 0.13	5	(CDs12, mi423a)	*UFO, CAL*
	m253	51 (38–56)	PC1	−0.54 ± 0.17	9	(mi62, mi423a)	
	m253	51 (39–56)	PIL	−0.42 ± 0.14	6	(CDs12, mi423a)	
QTLF-I-4	mi72	65 (62–71)	PC1	0.16 ± 0.18	0	(mi133, mi291a)	
	mi72	67 (60–71)	PL	0.39 ± 0.14	4	(mi133, mi291a)	
QTLF-I-5	mi425	115 (110–120)	SW	0.53 ± 0.17	9	(mi103, agp64)	*ASK1, CLV1, ACE*
				Chromosome II			
QTLF-II-1	mi421	8 (4–12)	PC1	0.75 ± 0.16	22	(g4553, g4133)	
	mi421	8 (4–12)	SSL	0.48 ± 0.16	6	(g4553, g4133)	
	mi421	10 (6–23)	FM	0.70 ± 0.22	18	(g4553, mi139)	
	mi421	10 (4–12)	PL	0.60 ± 0.14	10	(g4553, mi421)	
	mi421	10 (4–16)	LSL	0.55 ± 0.15	10	(g4553, PR1)	
	PR1	15 (12–19)	PIL	0.32 ± 0.15	3	(g4133, mi398)	
QTLF-II-2	g6842	36 (34–40)	PC1	0.81 ± 0.16	28	(g6842, ER)	*ER, CLV3*
	ER	36 (34–42)	SSL	1.03 ± 0.16	33	(g6842, m220)	
	ER	36 (34–40)	LSL	1.33 ± 0.15	59	(g6842, ER)	
	ER	36 (34–42)	PC2	1.15 ± 0.17	40	(g6842, m220)	
	ER	36 (34–40)	PIL	1.35 ± 0.15	66	(g6842, ER)	
	ER	38 (34–42)	PL	1.25 ± 0.17	30	(g6842, m220)	
	ER	38 (34–42)	SL	1.51 ± 0.14	77	(g6842, m220)	
QTLF-II-3	ve018	52 (47–59)	PL	−0.19 ± 0.15	0	(ve015, CK_97)	*ETT*
				Chromosome III			
QTLF-III-1	mi79b	45 (35–57)	PC2	−0.45 ± 0.16	6	(mi225, g4564b)	*SUP, TSO1*
	mi79b	45 (35–57)	SW	0.43 ± 0.17	6	(mi225, g4564b)	
QTLF-III-2	m457	65 (62–75)	SL	0.37 ± 0.14	3	(g4014, g2778)	*AP3*
				Chromosome IV			
QTLF-IV-1	mi51	2 (telomere-5)	PL	0.52 ± 0.15	5	(T, g3843)	
	mi204	3 (telomere-5)	PC1	0.58 ± 0.20	5	(T, g3843)	
	mi204	3 (telomere-5)	SSL	0.41 ± 0.13	4	(T, g3843)	
	mi204	3 (telomere-5)	PIL	0.35 ± 0.14	3	(T, g3843)	
QTLF-IV-2	Gsl_ohp	17 (16–20)	PL	−0.50 ± 0.20	0	(app, m448A)	
QTLF-IV-3	mi167	22 (16–32)	PC1	−0.38 ± 0.20	4	(app, Td23)	
	mi167	24 (21–29)	PL	0.40 ± 0.21	0	(mi87, RPS18C)	
QTLF-IV-4	mi465	36 (32–42)	PL	−0.44 ± 0.14	2	(Td23, m326)	*AG*
	mi326	42 (32–49)	PW	−0.62 ± 0.18	19	(Td23, AG)	
				Chromosome V			
QTLF-V-1	mi322	25 (21–33)	LSL	−0.38 ± 0.14	4	(mi174, mi90)	*TSL, PI*
	cor6.6	23 (18–32)	PL	−0.18 ± 0.14	0	(CDs5, mi138)	
QTLF-V-2	mi219	56 (49–58)	SL	−0.46 ± 0.14	6	(g4715b, mi125)	*TINY, WIG*
	mi291b	62 (50–73)	SSL	−0.40 ± 0.15	4	(mi219, mi323)	
	mi137	69 (64–73)	PL	−0.40 ± 0.14	4	(m291b, mi323)	

(*continued*)

TABLE 5

(Continued)

QTL[a]	Marker	Position (cM)	Trait	Additive effect ($2a/\sigma_P$) ± 1 SE[b]	% variance explained	2-LOD CI flanking markers	Candidate loci[c]
QTLF-V-3	mi83	88 (81–92)	SW	−0.70 ± 0.17	16	(mCH1, mi61)	
	ve027	95 (90–100)	PC1	−0.47 ± 0.17	4	(mi81, PAP3)	
	mi271	100 (97–104)	PW	−0.57 ± 0.18	16	(mi271, m435)	
	PAP3	100 (97–104)	PL	−0.93 ± 0.17	16	(mi271, m435)	
	PAP3	100 (97–104)	SSL	−0.96 ± 0.20	23	(mi271, m435)	
	m435	104 (93–107)	SL	−0.38 ± 0.13	4	(ve027, h2a1)	
QTLF-V-4	g2368	136 (129-T)	PC1	0.34 ± 0.17	2	(ve032, T)	
	g2368	136 (129-T)	PL	0.91 ± 0.18	14	(ve032, T)	
	g2368	136 (129-T)	SSL	0.72 ± 0.20	13	(ve032, T)	
	g2368	138 (132-T)	PIL	0.10 ± 0.13	0	(ve032, T)	

[a] QTL were identified using evidence from 2-LOD confidence intervals surrounding each LR peak, inspection of LR statistic profile plots, and the observance of abrupt changes in the sign of the estimated additive genotypic effects across intervals.

[b] For each trait affected by a QTL, we present an estimate of the standardized additive genotypic effect ($2a/\sigma_P$) and an associated standard error estimated from a GLM model including all significant QTL-associated markers. Markers that were significant in GLM models are indicated by underlining.

[c] Hypotheses concerning candidate genes underlying QTL were generated by inspecting the Arabidopsis physical genome map for cloned genes with known functional effects or expression in floral traits within the reported 2-LOD flanking markers.

static interactions involving three individual QTL (QTLF-II-2, QTLF-III-1, and QTLF-V-4) and two floral traits (PC2 and PL; Table 6). In both cases, interacting QTL were located on separate chromosomes, limiting the possibility of bias in estimating epistasis through simple linkage effects. The epistatic effect ($4i$) for these interactions was relatively weak when compared to the simple additive effects of these loci.

Candidate genes: Confidence intervals based on a 2-LOD score drop for the observed QTL corresponded to an average genetic distance of 10.7 cM surrounding any QTL peak. With respect to the *A. thaliana* physical map, this distance is generally equivalent to several thousand kilobases (~2500 kb, assuming 233 kb cM^{-1}) and therefore several hundred genes (~568 genes, assuming a gene density of 4.4 kb per gene). Despite this uncertainty, our QTL mapping study can help direct us to the genetic loci underlying quantitative genetic variation in these floral characters in at least two ways.

One approach is to ask whether candidate genes with known effects on floral traits (Table 1) also contribute to quantitative genetic variation in floral size and shape. For example, QTLF-II-2 affected all of the measured floral length characters (PC1, PL, SL, LSL, SSL, and PIL) and was located at 36 cM on chromosome II, near the *ERECTA* (*ER*) locus. The *ER* mutant was characterized as conferring a compact inflorescence through the reduction of internode and floral pedicel lengths, but it has also been shown to influence final silique length (Torii *et al.* 1996). *ER* encodes a putative receptor protein kinase (Torii *et al.* 1996) with strong expression in floral organ primordia and immature organs (Yokoyama *et al.* 1998). Furthermore, expression is greatest in cells predicted to divide and elongate in both the pedicel and floral organs. Therefore, we hypothesize that variation at *ER* plays an important role in determining the degree of cell elongation and division along both the pedicel and floral organ length axis in this mapping population. In contrast, QTLF-I-5 was located at 115 cM on the tip of chromosome I (near the mi425 marker) and had specific effects on SW. Several candidates occur near this QTL, including Arabidopsis *ASK1*, *ADHESION OF CALYX EDGES* (*ACE*), and *CLV1*. A recent study (Zhao *et al.* 1999) has shown that the *ask1-1* mutant has a general function in regulating development of vegetative and floral organs and specifically influences petal and stamen lengths. Using scanning electron microscopy, Zhao *et al.* (1999) show that size variation caused by the *ask1-1* mutant is due to changes in cell division and not from alterations of cell shape or elongation. Like *ER*, *CLV1* encodes a receptor kinase and controls shoot and floral meristem size (Clark *et al.* 1997). *ACE* mutants have fused sepal edges and altered sepal epidermis (T. Araki, personal communication). Additional hypotheses concerning the correspondence of QTL and candidate genes are presented in Table 5. We emphasize that these are only initial hypotheses. Clearly, additional studies are needed to confirm the genetic basis of these QTL.

Despite the co-occurrence of several candidate genes with QTL positions (Table 5), randomization tests provided little support for the role of the set of candidate genes proposed in Table 1 as factors underlying genomic regions with high LR statistics. For all traits, the sum of LR statistics for the candidate genes was less than the top 5% of the summed LR statistics for a random sample of genome positions (using both the complete list of candidate genes and a subset with putative

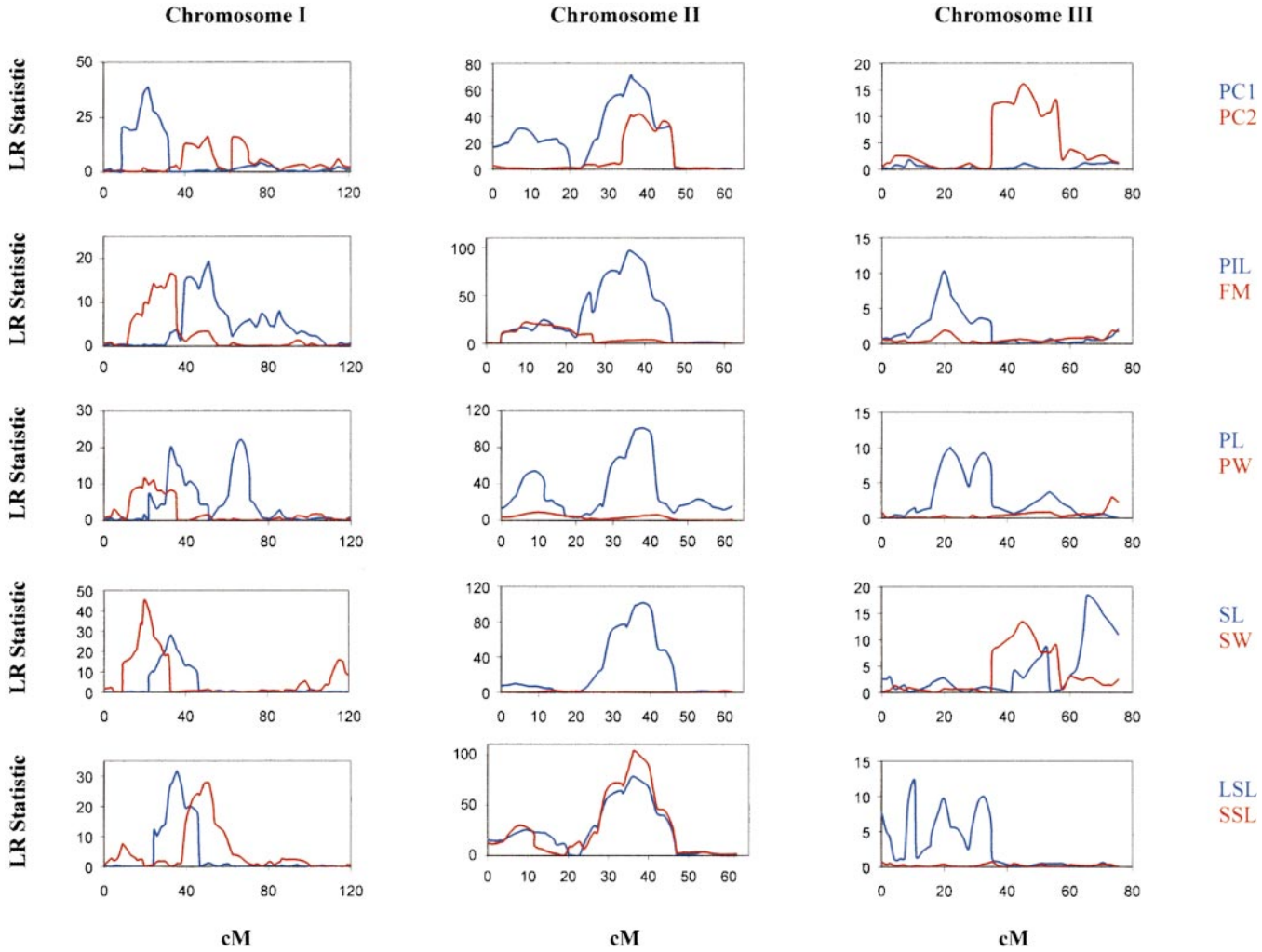

FIGURE 1.—LR statistic profile plots for chromosomes I–III generated from composite interval mapping analyses of floral traits. LR statistic thresholds corresponding to $\alpha = 0.05$ were set using 1000 random permutations of the data and averaged 14.70. Significance thresholds for each trait are as follows: PC1, 15.82; PC2, 14.14; PIL, 15.51; FM, 12.02; PL, 17.91; PW, 13.38; SL, 14.90; SW, 14.08; LSL, 13.78; SSL, 15.55. The number of background markers used in the CIM analysis was chosen using a forward-backward stepwise regression model: PC1, 15 markers; PC2, 9 markers; PIL, 12 markers; FM, 3 markers; PL, 20 markers; PW, 7 markers; SL, 6 markers; SW, 9 markers; LSL, 15 markers; SSL, 7 markers. Note variation in the scale of the *y*-axis among plots.

quantitative effects). We feel this test indicates our inability to make *a priori* predictions of candidate genes (even in the face of considerable molecular genetic data) and does not necessarily negate our hypotheses concerning specific candidate genes.

QTL mapping can also be used as a functional genomics tool to assign new pleiotropic effects (function) to known genes in regions not thought to affect floral development or to assign functions to new genes discovered by sequencing. For example, the entire sequence for *A. thaliana* chromosomes II and IV is currently available (LIN *et al.* 1999; MAYER *et al.* 1999), and the entire *A. thaliana* genome will be completely sequenced by the end of the year 2000. Given complete sequence information, all genes between the markers flanking QTL are potential candidate genes. In our study, we have identified 18 genomic regions that should contain genes with effects on floral organs. Seven of these regions occur on chromosomes II and IV. Four of these regions (intervals surrounding QTLF-II-1, QTLF-IV-1, QTLF-IV-2, and QTLF-IV-3) are of particular interest, as they do not contain obvious candidate genes on the basis of the current sequence annotation of chromosome II or IV. These QTL may therefore lead to the discovery of new genes influencing flower development.

DISCUSSION

Over the past decade, remarkable progress has been made in elucidating the molecular genetic basis of flower development (THEIBEN and SAEDLER 1999). Mutation screening and transgenic techniques have driven these advances by evaluating the roles of regulatory

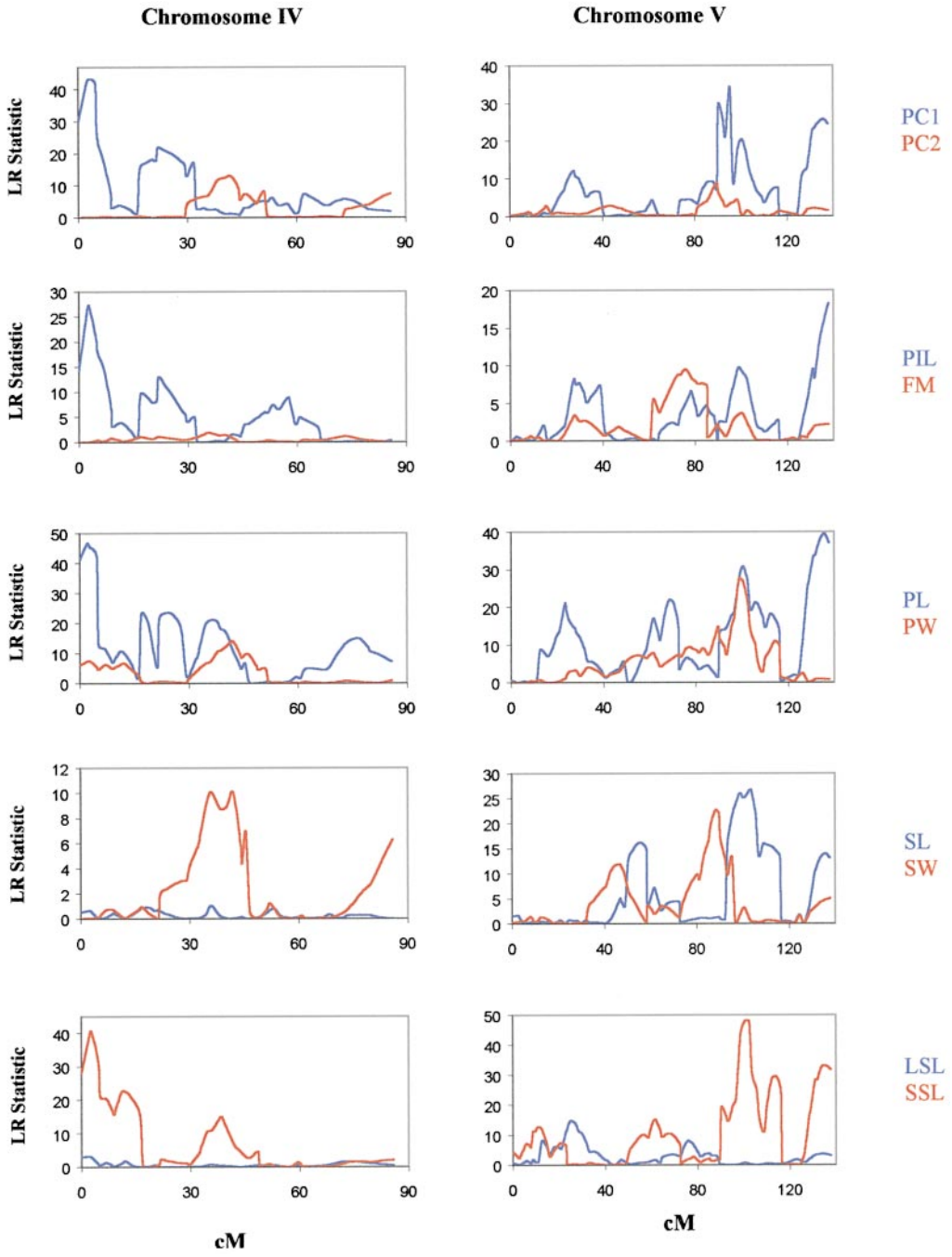

FIGURE 2.—LR statistic profile plots for chromosomes IV and V generated from composite interval mapping analyses of floral traits. Note variation in the scale of the *y*-axis among plots.

genes in the pattern formation of the basic flower. This approach has culminated in predictive models of how several classes of homeotic genes interact to set whorl and floral organ identity (COEN 1991; PELAZ *et al.* 2000). Despite these advances, surprisingly little is known about the underlying genetic basis of continuous variation in floral form, leaving a major gap in our understanding of flower development. This is by no means a simple task, as phenotypic variation in quantitative characters is influenced by the simultaneous segregation of alleles at many loci. Here, we report on the first experiments evaluating the quantitative genetic basis of floral form in *A. thaliana*.

We detected significant genetic variation for all of the measured floral characters within both the ecotype and L*er* × Col mapping population, providing evidence for considerable standing quantitative genetic variation in overall floral morphology in Arabidopsis. In addition, we detected significant positive genetic correlations between all of the measured floral traits, supporting strong genetic integration of *A. thaliana* flowers. These results are consistent with a large body of literature (across a broad sample of plant families) reporting standing genetic variation in floral traits and positive genetic correlation among flower parts (*e.g.*, CONNER and VIA 1993; FENSTER and RITLAND 1994; CAMPBELL 1996;

TABLE 6

Results of analysis of potential pairwise epistasis between QTL detected from composite interval mapping

Interaction	Traits	*P* value	Bonferroni-adjusted α	Marker 1	Marker 2	Mean ± 1 SD
QTLF-II-2 × QTLF-III-1	PC2	0.0180	0.05	er	mi79b	
$4i = -0.81$				A	A	0.17 ± 0.20
				A	B	1.15 ± 0.17
				B	A	−0.53 ± 0.14
				B	B	−0.36 ± 0.14
QTLF-II-2 × QTLF-V-4	PL	0.0045	0.0050	er	g2368	
$4i = -0.49$				A	A	7.10 ± 0.13
				A	B	6.66 ± 0.16
				B	A	6.22 ± 0.11
				B	B	5.13 ± 0.11

Only interactions significant at a Bonferroni-corrected α-level are presented.

Mitchell *et al.* 1998). Unfortunately, technical difficulties have limited the ability of most studies to evaluate the underlying genetic basis of floral integration (although see Bradshaw *et al.* 1998; Kim and Rieseberg 1999 for studies of interspecies crosses). This information is critical with respect to understanding how genetic architecture influences the evolution of integrated characters.

Composite interval mapping located many QTL with effects on multiple floral organs, supporting the role of pleiotropy (or very tight linkage) as the primary mechanism of floral integration in *A. thaliana*. Together, we detected 18 significant QTL—11 of these were associated with variation in several traits. Generally, QTL affecting multiple traits had consistent positive or negative effects on all length measures (SL, PL, SSL, LSL, and PIL) across all four floral whorls. Loci underlying these QTL may therefore function through generalized effects on cell division or elongation along the longitudinal floral axis. This hypothesis could be tested by performing similar QTL analyses on simple cell size or patterns of cell division in floral organs from this sample of RI lines.

Individual QTL effects varied in magnitude and ranged from additive genotypic effects ($2a/\sigma_P$) of 0.32 to 1.51 SD. Individual QTL explained between 2 and 77% of the total phenotypic variation in the traits (when controlling for other segregating QTL). We detected only two cases of QTL × QTL interaction, both involving QTLF-II-2 (possibly the *ER* locus). It is likely that our analyses have seriously underestimated the possibility of epistatic effects because we limited our screen to pairwise combinations of QTL with previously identified additive effects on at least one of the floral traits. Moreover, our design involving only 74 recombinant inbred lines had low power in detecting higher order marker × marker interactions.

There are only a handful of *A. thaliana* QTL studies (reviewed in Alonso-Blanco and Koornneef 2000). Many of these have investigated life history traits such as seed dormancy (van Schaar *et al.* 1997), flowering time (Kowalski *et al.* 1994; Clarke *et al.* 1995; Alonso-Blanco *et al.* 1998; Stratton 1998), growth (Mitchell-Olds 1995, 1996; Alonso-Blanco *et al.* 1999), seed size (Alonso-Blanco *et al.* 1999), and circadian rhythm (Swarup *et al.* 1999). One study focused on the genetic basis of physiology by quantifying the production of 10 enzymes involved in primary and secondary metabolism (Mitchell-Olds and Pedersen 1998). These studies generally report multiple QTL for traits and effects of similar magnitude to those reported here. Several studies have detected QTL with apparently pleiotropic effects (Mitchell-Olds 1995; Alonso-Blanco *et al.* 1999), pairwise epistasis (Clarke *et al.* 1995; Alonso-Blanco *et al.* 1998, 1999), and QTL × environment interactions (Clarke *et al.* 1995; van Schaar *et al.* 1997; Alonso-Blanco *et al.* 1998; Stratton 1998). Together, these studies suggest that quantitative traits in Arabidopsis mapping populations can be influenced by a modest number of QTL, that QTL effects can be relatively varied, and that epistatic and QTL × environment interactions are common. Additional information concerning the spectrum of allelic frequencies of QTL in natural populations is still needed, however, to make rigorous evolutionary inferences concerning the genetic diversity of quantitative traits.

There are several caveats with respect to our study. First, we evaluated floral phenotypic variation in a very restricted window of time. It is clear that competition among developing fruits and seeds along with architectural effects can have large impacts on overall patterns of phenotypic variation in Arabidopsis (Diggle 1997). We expect that resource limitation and architectural trade-offs will inflate the environmental variation (reducing heritability) associated with floral traits through ontogeny. QTL positions and effects are notoriously difficult to detect and quantify. Some "significant" QTL are likely to be false positives despite our relatively rigorous significance threshold—QTL should therefore be considered hypotheses until further evaluation.

We were able to propose several candidate genes with effects on continuous variation in floral morphology despite uncertainty in the exact position of QTL. These candidates include *UFO*, *CAL1*, *ASK1*, *CLV1*, *ACE*, *ER*, *CLV3*, *ETT*, *TSO1*, *SUP*, *AP3*, *AG*, *TSL*, *TINY*, and *WIG*. Seven of these candidate genes have previously been reported to influence quantitative variation in some aspect of *A. thaliana* floral morphology (Table 1). Several of these genes are thought to influence patterns of cell elongation and growth and cell proliferation in floral primordia, or may act as more general regulators of floral development. Despite the co-location of several QTL with candidate genes, we found no support for a matching of *a priori* candidate genes and QTL positions on the basis of a simple randomization test. We feel this result should not be interpreted as evidence against the role of the proposed candidate genes or the usefulness of a candidate gene approach, but instead that the test stresses the difficulty of predicting the genes underlying quantitative traits. It cannot be emphasized enough that QTL mapping methods are necessarily restricted to detecting genetic variation that segregates between two parental strains. Although many of the candidate genes proposed here do not appear to influence quantitative variation in flowers from this study, it is possible that they would in other mapping populations derived from different parental strains. Additional QTL mapping experiments are needed to evaluate the generality of QTL effects and to more fully explore sets of putative candidate genes.

Future experiments will help to confirm these loci as genes underlying the observed QTL and/or will generate new hypotheses concerning additional floral QTL or candidate genes. For example, fine-scale mapping with additional genetic markers or RI lines may improve our estimates of QTL positions and will increase our confidence surrounding those positions. Further fine-scale mapping is possible in this population because 200 additional RI lines and >1000 additional genetic markers are currently available. Moreover, multiple trait methods may increase the accuracy of mapping these genetically correlated floral characters by incorporating information from correlated error structures and will provide more direct tests of pleiotropy *vs.* tight linkage for individual QTL (Jiang and Zeng 1995). Besides QTL mapping, candidate gene hypotheses can also be directly tested using quantitative complementation approaches and near isogenic lines (NILs; Long *et al.* 1996; Mackay and Fry 1996; Lynch and Walsh 1998; Alonso-Blanco and Koornneef 2000) or through transgenic methods. Genome-wide QTL mapping, fine-scale mapping in locations of interest, complementation testing with NILs, and association studies in natural populations will be needed to unravel the complexity of most polygenic traits, particularly in organisms with high gene density genomes like Arabidopsis.

In conclusion, we have found a relatively large number of QTL with effects on some aspect of flower development. In many cases, individual QTL had pleiotropic effects that generate overall floral morphological interaction. Several of these QTL map to genomic regions overlapping with several candidate genes on the basis of strong functional arguments. QTL mapping is a promising tool for the discovery of candidate genes, the detection of novel phenotypic effects for known genes, and in generating a more complete understanding of the genetic basis of continuous variation.

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