EVOLUTION & DEVELOPMENT 7:3, 259–271 (2005)

Quantitative trait loci mapping of floral and leaf morphology traits in *Arabidopsis thaliana*: evidence for modular genetic architecture

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SUMMARY Morphological variation within organisms is integrated and often modular in nature. That is to say, the size and shape of traits tend to vary in a coordinated and structured manner across sets of organs or parts of an organism. The genetic basis of this morphological integration is largely unknown. Here, we report on quantitative trait loci (QTL) analysis of leaf and floral organ size in *Arabidopsis thaliana*. We evaluate patterns of genetic correlations among traits and perform whole-genome scans using QTL mapping methods. We detected significant genetic variation for the size and shape of each floral and leaf trait in our study. Moreover, we found large positive genetic correlations among sets of either flower or leaf traits, but low and generally nonsignificant genetic correlations between flower and leaf traits. These results support the hypothesis of independent floral and vegetative modules. We consider co-localization of QTL for different traits as support for a pleiotropic basis of morphological integration and modularity. A total of eight QTL affecting flower and three QTL affecting leaf traits were identified. Most QTL affected either floral or leaf traits, providing a general explanation for high correlations within and low correlations between modules. Only two genomic locations affected both flower and leaf growth. These results are discussed in the context of the evolution of modules, pleiotropy, and the putative homologous relationship between leaves and flowers.

INTRODUCTION

Morphological variation within organisms is usually integrated and often modular in nature. That is to say, the size and shape of particular traits tend to vary in a coordinated and structured manner across larger sets of traits in an organism (Olsen and Miller 1958; Raff 1996; Klingenberg 2004). This modular organization could arise from a variety of mechanisms. First, natural selection on functionally related traits could favor and maintain patterns of genetic variation that lead to either integrated or independent character complexes or "modules." In this case, integration allows the proper functioning of interrelated traits, whereas modularity allows adaptive flexibility or "evolvability" of different parts of an organism. For example, Berg (1959, 1960) hypothesized that natural selection to maintain proper fit between flowers and their pollinators would lead to increased correlations between floral organs and decreased correlations between flowers and the rest of the plant. Here, genetic modularity would allow flowers and leaves to follow independent adaptive paths over evolutionary time (Berg 1959, 1960; Conner and Via 1993; Raff 1996; Wagner 1996; Wagner and Altenberg 1996; Armbruster et al. 2004).

Alternatively, modular organization and character integration could reflect homologous developmental pathways that underlie the generation of character complexes (Hall 1984; Atchley and Hall 1991; Cheverud 1993, 1996; Wagner 1996; Klingenberg 2002, 2004). For instance, similar developmental mechanisms may be important in the generation of flattened laminar surfaces like plant leaves and petals, thus leading to correlations between their size and shape and a coupling of vegetative and floral organs. Finally, strong correlations between characters may be owing to similar responses of traits to environmental variation (environmental correlation). Despite the central importance of integration and modularity in development and phenotypic evolution, surprisingly little is known about the specific evolutionary processes generating these patterns in nature (Pigliucci and Preston 2004).

One common approach for the study of morphological integration is the partitioning of phenotypic correlations

among traits into environmental and genetic components. In general, quantitative genetic parameters can be considered population genetic descriptors of character structure and modularity (Lande 1979; Cheverud 1984, 1996; Zeng 1988; Armbruster and Schwaegerle 1996; Lynch and Walsh 1998). From an evolutionary perspective, a high degree of genetic correlation indicates integration of particular traits into a "module," whereas low or absent genetic correlations indicates trait independence. Traits within a module are expected to evolve as a coordinated unit and share similar evolutionary paths (Lande 1979; Lande and Arnold 1983; Zeng 1988; Riska 1989; Falconer and Mackay 1996; Lynch and Walsh 1998). At a proximate level, genetic modularity may be the product of pleiotropic effects of single genes on suites of related characters or due to linkage disequilibrium between separate genes with effects on different characters. Patterns of genetic correlation, particularly when driven by pleiotropy, play a critical role in understanding evolution because they can strongly influence the optima and trajectory of response to natural selection (Falconer and Mackay 1996). A major question then is which factors influence the evolution of genetic correlations.

A number of conceptual theories have been developed to explain the evolution of modular genetic architecture. Modular architecture may derive from a number of mechanisms including, for example, parcellation, integration, balanced pleiotropy, differential epistasis, or some combination of the above (Wagner 1996; Cheverud 2001). Parcellation consists of a differential suppression of pleiotropic effects between groups of characters through evolutionary time. Integration leads to modularity through the selective acquisition of pleiotropy among characters from the same functional group. Balanced pleiotropy results from the fact that a population genetic correlation is a weighted average of both positive and negative pleiotropy (Lande 1980; Gromko 1995; Cheverud 2001). Under a balanced pleiotropy model, trait or module independence may occur even in the presence of universal pleiotropy, through the balancing of positive and negative pleiotropic relationships. The role of parcellation and integration can be distinguished from balanced pleiotropy, as the former predicts that different genes will affect traits in different modules, whereas balanced pleiotropy allows for genetic overlap between modules. Finally, differential epistasis can lead to modularity through the differential suppression of pleiotropy in subsets of traits through the action of a modifier locus (Cheverud 2001).

Importantly, natural selection can play a critical role in the evolution of genetic correlations and their underlying genetic architecture. Responses to correlational selection can directly alter genetic correlations between sets of traits (Lande and Arnold 1983; Endler 1986; Brodie 1989, 1992; Conner 2002; Sinervo and Svensson 2002). Correlational selection occurs when several phenotypic characters jointly determine fitness, with particular trait combinations having higher or lower fitness. For example, it is easy to imagine how the pollination success of a plant may depend on the relative position or close matching of stigma, style, or corolla length. Here, reproductive success may be causally related to the covariances between floral organ sizes. This form of selection can lead to the evolution of strong genetic correlations by favoring (or disfavoring) new pleiotropic mutations (e.g., integration or parcellation) or by generating linkage disequilibrium between alleles at separate loci affecting the functionally related traits. The importance of linkage disequilibrium in the long-term of evolution of phenotypes is unknown, but theoretical models suggest that strong and persistent correlational selection can maintain genetic correlations at a balance of recombination, segregation, and selection (Hartl and Clark 1997; Lynch and Walsh 1998). Factors reducing recombination between loci affecting functionally related traits can increase the probability of maintaining modules or trait combinations through linkage disequilibrium (Hurst 1999; Kelly 2000). Therefore, the physical position of genes affecting characters within and among modules may be important in the evolution of the modules themselves.

Here, we use quantitative trait loci mapping to explore the pattern of character integration within and between flower organs and leaves in *Arabidopsis thaliana*. *Arabidopsis* flowers exhibit a concentric whorled pattern of sepals, petals, stamens, and carpels. The analysis of floral homeotic mutants in *Arabidopsis* and *Anthirrinum* has led to the proposal of the ABC model of flower pattern formation, in which the combinatorial activity of three homeotic functions specifies organ identities in the four floral whorls (reviewed in Weigel and Meyerowitz 1994). In *Arabidopsis*, class A genes *APETALA1* (*AP1*) and *AP2* specify sepals in the first whorl and the class C gene *AGAMOUS* (*AG*) specifies carpels in the fourth. The B function, encoded by the products of *AP3* and *PISTILLATA* (*PI*), is required for the identity of the second and third whorl tissues. Petal identity is determined by the combination of A and B functions in the second whorl and the identity of stamens by B and C function combination in the third whorl. *AP1*, *AP3*, *PI*, and *AG* genes encode members of the MADS-box family of transcription factors (Riechmann and Meyerowitz 1997). Functional studies of B and C homeotic genes in maize (Ambrose et al. 2000), rice (Kyozuka et al. 2000), and conifers (Mouradov et al. 1999) provide evidence for the evolutionary conservation of B and C function in angiosperms and gymnosperms, suggesting their ancient evolutionary origin.

Leaves are laterally determined organs produced reiteratively from a group of founder cells in the flanks of the shoot apical meristem (SAM). Indeterminate meristematic growth is maintained by the expression of *knotted-like homeobox* (*KNOX*) genes in the SAM (reviewed in Barton 2001). The products of the *ROUGH SHEATH2* (*RS2*; Schneeberger

et al. 1998; Tsiantis et al. 1999) gene of maize and their Orthologs *PHANTASTICA* (*PHAN*; Waites et al. 1998; Timmermans et al. 1999) of *Antirrhinum majus* and *ASYMMETRIC LEAVES1* (*AS1*; Byrne et al. 2000) of *Arabidopsis thaliana* are plant-specific transcription factors of the MYB family that repress *KNOX* genes in leaf primordia. In *Arabidopsis thaliana*, the expression of *SHOOTMERISTEMLESS* (*STM*) in the SAM represses *AS1*, which allows the *KNOTTED-like Arabidopsis thaliana1* (*KNAT1*) and *KNAT2* genes to be expressed (reviewed in Barton 2001). In leaf primordia, where *STM* is not expressed, *AS1* represses *KNAT1* and *KNAT2*, ensuring the proper developmental program of the leaf (Byrne et al. 2002).

Arabidopsis thaliana mutants defective in all the A, B, and C function genes display a conversion of floral organs to leaf-like structures (Bowman et al. 1991). These results support Goethe's famous conjecture that flowers are modified leaves (von Goethe 1790). However, ectopic expression of the ABC genes in leaves is not sufficient to transform them into floral organs (Krizek and Meyerowitz 1996; Mizukami and Ma 1997). Recently, the E function, encoded by the *SEPALLATA1* (*SEP1*), *SEP2*, and *SEP3* genes of the MADS-box family, has been described (Pelaz et al. 2000; Honma and Goto 2001). The ectopic expression of A and B genes together with *SEP* genes is sufficient to convert leaves into petals (Pelaz et al. 2001). These results support a homologous relationship between the leaf and the floral organs (Goto et al. 2001).

Although considerable insight into the genetic basis of pattern formation in plants has been gained (reviewed in Pruitt et al. 2003), many questions remain unanswered concerning the genetics of morphological variation and the growth of biological shapes (Day and Lawrence 2000; Mizukami 2001; Maloof 2003; Nath et al. 2003). Our experiments are motivated by an interest in the modular architecture of plant vegetative and reproductive organs. Here, we examine the pattern of genetic correlation between the size and shape of floral organs and leaf characteristics in *Arabidopsis thaliana* and the role of pleiotropy and genetic linkage underlying patterns of integration.

MATERIALS AND METHODS

Recombinant inbred lines

We used 162 recombinant inbred lines (RIL) generated from a reciprocal cross between Landsberg *erecta* (L*er*) and Cape Verde Islands (Cvi) accessions (Alonso-Blanco et al. 1998) to map QTL. These lines are available from the *Arabidopsis* stock center as accession number cs22000. We constructed a linkage map using 111 genetic markers (26, 19, 24, 15, and 27 markers, respectively, for chromosomes I, II, III, IV, and V). The RIL genotype at each marker locus was obtained from the published data available from the *Arabidopsis* stock center (http://arabidopsis.org). The linkage map was constructed using markers genotyped in at least 80% of the sampled lines. The map position of each marker (*d* cM) was estimated from the observed recombination frequencies (*r*) using the Kosambi mapping function (Kosambi 1944) as implemented by the software MapMaker 3.0 (Lander and Botstein 1989). These analyses provided a unique position for each marker that did not differ in order from the published *Arabidopsis* linkage maps.

Plant materials and growth conditions

Floral morphological data

Replicate plants were grown under standard growth chamber conditions using Promix BT™ potting soil (Premier Horticulture, Rivière-du-Loup, Quebec, Canada) and 36 cell flats in two independent Percival chambers. Plants experienced long-day photoperiod conditions (16 h light/8 h dark) provided by fluorescent supplemental lighting. Light conditions during the day period were maintained at 200 μmol/m/s Photosynthetic Photon Flux Density (PPFD). Chamber temperature was maintained at 20°C and 18°C during the day and night cycles, respectively. A randomized block design was used, incorporating 162 RIL and the 2 parentals derived from the L*er* × Cvi mapping population. Blocks corresponded to the two growth chambers—within each chamber, two replicates of each RIL and the parental accessions were randomly planted across flats. Several seeds were initially planted in each cell, flats were cold/wet stratified for 7 days at 4°C, and cells were subsequently thinned to a single replicate individual at the first true leaf stage.

Six floral characters were measured from each of the two flowers from each of the four replicates for all genetic lines in the experiment. The floral characters measured were petal length (Petal L) and width (Petal W), sepal length (SL) and width (SW), long stamen length (LSL), and pistil length (Pistil). The two flowers used were among the first six flowers to develop on each plant and were collected at anthesis (stage 13, Smyth et al. 1990). 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. Stamens were measured from the base of the filament to the tip of the anthers. Because pistils are still rapidly elongating at state 13, pistil length measurements may include considerable environmental variation related to differences in the timing of flower collection.

Leaf morphological data

Plants for leaf character studies were grown in growth chambers under continuous daylength at 100 μmol/m/s PPFD, 20°C, and 60–70% relative humidity as described in Ponce et al. (1998). Plants were grown in sterile conditions on agar in 150 cm Petri dishes. A total of 40 plants, 20 each from two randomly chosen RIL, were grown per Petri dish. Two sowings were made per RIL. Leaves from the third node from 15 plants of each RIL, chosen at random from within the Petri dishes, were excised with forceps 25 days after sowing, immediately placed on the surface of agar medium to prevent dehydration, and covered by a transparent film. Photographs were taken with a Sony Cybershot FV-505 digital camera using a resolution of 2240 × 1680 pixels. Images were digitally processed with the Adobe Photoshop 6.0 program (Adobe Systems Incorpo-

rated, San José, CA, USA) and analyzed using Scion Image (Scion Corporation, MD, USA) to obtain length, perimeter, and area values. Measurements were taken for lamina area (LA), lamina perimeter (LP), lamina length (LL), lamina width (LW), petiole length (PL), and petiole width (PW).

Statistical analyses were performed as previously described (Juenger et al. 2000; Pérez-Pérez et al. 2002) using appropriate mixed models with PROC MIXED in SAS (SAS Institute 1997). Growth chamber and cytoplasmic effects were incorporated in our statistical models as fixed effects. Variance components were estimated using REML and tests of significance were determined through log-likelihood ratio tests. Broad-sense heritability (H^2) was calculated as the ratio of among-RIL variance component (V_G) divided by the total phenotypic variance (V_G+V_E). In addition, we calculated the coefficient of genetic variation (CV_G) as $(100\sqrt{V_G})/X$, where V_G is the among-RIL variance component and X is the mean of the trait. Genetic correlations among floral and leaf traits were estimated as

$$\mathrm{cov}(i,j)/\sigma_i\sigma_j,$$

where cov(i,j) is the covariance among recombinant inbred line means for traits i and j, and σ_I and σ_j are the square roots of the respective among-RIL variance components (V_G) for each trait (Robertson 1959). The significance of each correlation was determined using a t-test after a z-transformation of the correlation coefficient as described by Sokal and Rohlf (1981). As we studied a large number of traits, we applied a strict Bonferroni correction for multiple tests within each set of correlation analyses performed (floral vs. floral traits, leaf vs. leaf traits, and floral vs. leaf traits) (Rice 1989). Given that the leaf and floral traits in this study were measured in independent experiments, and thus under differing environmental conditions, our estimation of the genetic correlations between these traits depend on an assumption of little QTL × environment interaction. In the presence of strong QTL × environment interaction, genetic correlations between floral and leaf traits will be underestimated. However, it is unlikely that all QTL affecting these traits would exhibit such interactions or that the QTL detected would be completely environmentally dependent. One benefit of this design, in contrast, is that it will reduce the impact of environmental correlations in biasing estimates of the genetic correlation between floral and leaf traits because phenotypic measurements were collected from independent replicates of RIL genotypes.

We also conducted a principle component analysis (PCA) on the line means for the flower and leaf traits to evaluate the overall morphological integration and structure of the data using the FACTOR procedure in SYSTAT 7.0 (SPSS 1997).

QTL mapping

QTL affecting floral and leaf morphology were mapped using multiple-QTL (MQM) methods and the software MAPQTL (version 4.0) (Van Ooijen and Maliepaard 1996). MQM tests the hypothesis that an interval flanked by two adjacent markers contains a QTL, and statistically accounts for the effects of additional segregating QTL using marker regression outside the test interval. The QTL analyses were performed using the RI line means. The number of cofactors for each MQM model was determined through an iterative process involving an initial interval mapping scan (IM) followed by an automated backward elimination of cofactors identified through IM. These preliminary analyses were followed by a "restricted" multiple-QTL scan including all significant cofactors—these analyses were "restricted" in that cofactors occurring on the same linkage group as a test position were excluded from the model. This is a conservative approach as it only controls for the segregation of unlinked QTL when performing a test at a particular genomic position. The control of additional variation owing to linked QTL can be problematic and in a number of situations will lead to false-positives or inaccurate estimates of QTL locations. A genome-wide critical threshold value for the experiment-wise 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). We present estimates of the additive genotypic effect (a) and the percent of the total genetic variation explained (PVE) as calculated under the restricted MQM model. Positive additive effects indicate that the L*er* parental genotype has the higher mean. Two Logarithm of odds (LOD) support intervals were established as a 95% confidence level for the location of QTL (Van Ooijen 1992).

RESULTS

The L*er* × Cvi RIL population displayed considerable transgressive segregation for all of the measured flower and leaf traits (Table 1 and Fig. 1). On average, floral and leaf traits measured in the RIL exhibited a 22.8- and 19.5-fold increase in phenotypic range compared with the phenotypic differences between parental lines, respectively. In the extreme case, sepal width exhibited a 96-fold increase in the phenotypic range in the RI population (Table 1). Significant genetic variation was detected for all measured floral and leaf traits. In general, broad sense heritabilities (V_G/V_p) were moderate to high and averaged 0.62 and 0.64 for flowers and leaf traits (Table 1), respectively. A marginally significant cytoplasmic effect was detected for petal length ($P<0.0110$).

We found a number of significant genetic correlations (r_G) among the measured flower and leaf traits (Table 2A–C and Fig. 2). Genetic correlations among floral organs were generally positive and of moderate to high strength. Sets of floral organ lengths [Petal L, SL, LSL, Pistil] or organ widths [Petal W, SW] were highly correlated (lengths, average $r_G = 0.85$; width, $r_G = 0.63$), whereas genetic correlations between length and width measures were generally not significant (average $r_G = 0.17$) (Table 2A). All of the leaf traits were positively genetically correlated (average $r_G = 1.0$) (Table 2B). A number of the leaf trait correlations extend beyond the range of the genetic correlation owing to sampling variance. In contrast, we detected only three significant genetic correlations between flower and leaf characters (Table 2C). Petiole length (PL) was significantly genetically correlated with sepal length (SL), long stamen length (LSL), and pistil length (Pistil).

Table 1. Phenotypic values (mean ± 1 standard deviation) and quantitative genetic parameters of floral and leaf traits

Trait	L*er*	Cvi	Mean of the RIL	Highest value (RIL)	Lowest value (RIL)	V_G	V_E	H^2	CV_G
Petal length (Petal L)	4.03 ± 0.38	4.43 ± 0.89	4.14 ± 0.25	6.00	2.80	0.91	0.44	0.67	9.2
Petal width (Petal W)	1.27 ± 0.38	1.53 ± 0.38	1.44 ± 0.13	2.12	0.96	0.18	0.06	0.75	11.8
Sepal length (SL)	2.44 ± 0.25	2.55 ± 1.02	2.45 ± 0.13	3.68	1.72	0.32	0.12	0.75	9.24
Sepal width (SW)	0.99 ± 0.25	1.00 ± 0.38	1.01 ± 0.00	1.60	0.64	0.04	0.05	0.43	7.9
Long stamen length (LSL)	3.18 ± 0.64	3.62 ± 1.02	3.27 ± 0.25	4.68	2.00	1.01	0.28	0.78	12.3
Pistil length (Pistil)	3.18 ± 1.52	3.95 ± 1.78	3.51 ± 0.25	5.44	1.80	0.66	1.28	0.34	9.25
Leaf area (LA)	63.85 ± 9.04	56.83 ± 7.46	51.24 ± 15.75	89.67	13.93	152.63	74.91	0.67	24
Leaf perimeter (LP)	30.60 ± 2.58	29.02 ± 2.30	26.98 ± 4.65	37.93	14.67	13.30	6.54	0.67	13.5
Leaf length (LL)	9.86 ± 0.98	9.97 ± 0.87	9.01 ± 1.58	13.28	5.41	1.72	0.90	0.66	14.5
Leaf width (LW)	8.38 ± 0.63	7.18 ± 0.61	7.04 ± 1.22	9.56	3.25	0.89	0.49	0.64	13.4
Petiole length (PL)	4.96 ± 0.67	6.00 ± 0.98	4.87 ± 1.32	8.26	2.10	1.47	0.74	0.66	24.9
Petiole width (PW)	1.05 ± 0.15	0.89 ± 0.13	0.91 ± 0.15	1.41	0.53	0.02	0.017	0.55	15.5

The among-RIL variance (V_G) and the residual variance (V_E) were used to calculate broad-sense heritabilities (H^2) and coefficients of genetic variation (CV_G) as described in the Materials and Methods section.
RIL, recombinant inbred lines; L*er*, Landsberg *erecta*; Cvi, Cape Verde Islands.

The results of the genetic PCA of the 12 traits are presented in Table 3. The first three principle components explain 39.4, 28.4, and 14.9 percent of the variation for the RI population, respectively. Principle component 1 reveals a consistent positive loading for all leaf traits and a consistent negative loading for all flower traits. Principle component 2 reveals a consistent negative loading for lamina, petiole, and sepal width measures and a consistent positive loading for the remaining traits. Finally, principle component 3 reveals a negative loading for petiole, sepal, long stamen, and pistil lengths along with large positive loadings for sepal and petal width, and petal length (Fig. 3).

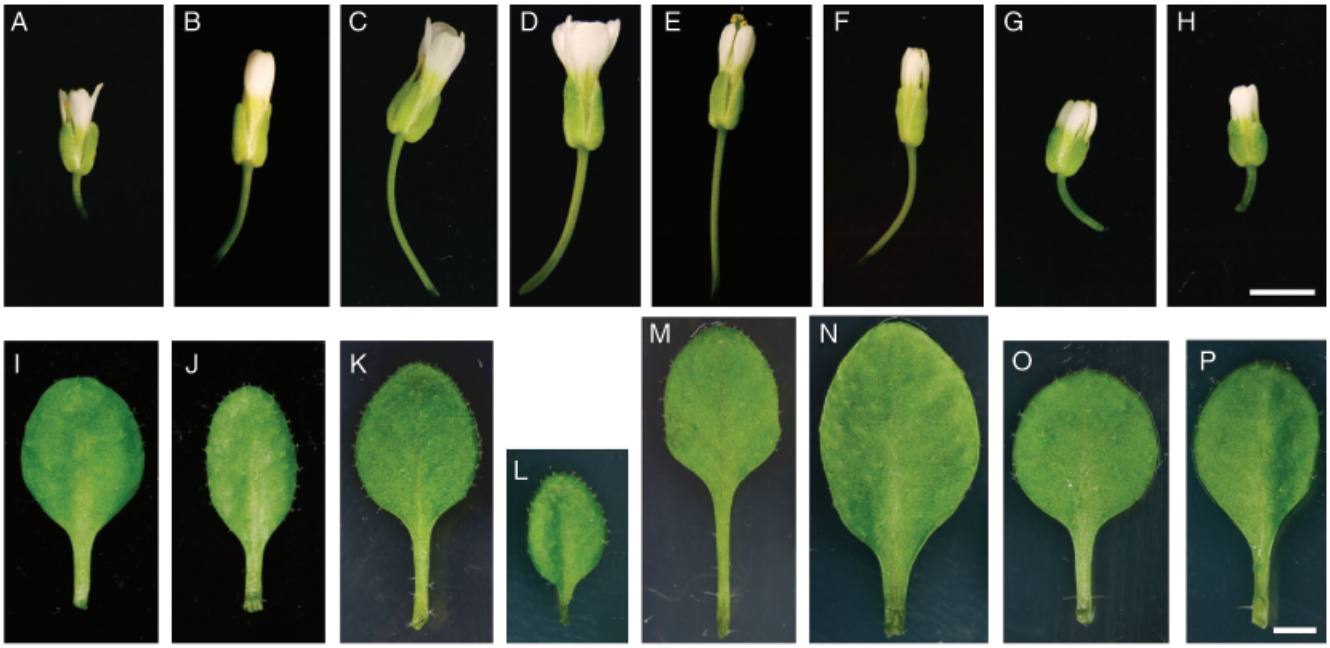

Fig. 1. Photographs of flowers (A–H) and third node leaves (I–P) of parental accessions Landsberg *erecta* (L*er*) (A, I) and Cape Verde Islands (Cvi) (B, J) and selected recombinant inbred lines (RILs) displaying extreme phenotypes: N22075 (C, K), N22014 (D, L) and N22143 (E, M) with large flowers (C, D, and E, respectively), N22143 (F, N) with medium-sized flowers (F); and N22022 (G, O) and N22041 (H, P) with small flowers (G and H, respectively). Leaf phenotypes show no consistent correlation with flower size. Pictures of flowers and leaves were taken 25 and 45 days after sowing, respectively. Scale bars indicate 2 mm.

Table 2. Genetic correlations among floral (A), leaf (B), and floral and leaf traits (C)

(A)	Petal L	SL	LSL	Pistil	Petal W	SW
Petal L						
SL	**0.80**					
LSL	**0.73**	**0.87**				
Pistil	**0.86**	**0.90**	**0.92**			
Petal W	**0.55**	0.06	− 0.09	0.18		
SW	0.02	− 0.04	− 0.26	− 0.18	**0.70**	

(B)	LA	LP	LL	PL	LW	PW
LA						
LP	**1.62**					
LL	**1.48**	**1.50**				
PL	**0.72**	**0.77**	**0.76**			
LW	**1.64**	**1.57**	**1.36**	**0.65**		
PW	**0.89**	**0.77**	**0.84**	**0.29**	**0.77**	

(C)	LA	LP	LL	PL	LW	PW
Petal L	− 0.02	0.00	0.02	0.18	− 0.07	− 0.16
SL	0.00	0.02	0.06	**0.38**	− 0.06	− 0.24
LSL	− 0.16	− 0.14	− 0.09	**0.45**	− 0.23	− 0.31
Pistil	− 0.05	− 0.03	0.03	**0.44**	− 0.14	− 0.13
Petal W	− 0.06	− 0.08	− 0.07	− 0.30	− 0.09	0.00
SW	− 0.05	− 0.05	− 0.05	− 0.17	− 0.04	− 0.07

Significant correlations are indicated in bold. For abbreviations, see Table 1.

Table 4A–C lists the QTL detected in this experiment that significantly affect some aspect of *Arabidopsis* floral morphology (A), leaf morphology (B), or PCA scores (C). Each QTL is designated by the identifier FQTL (floral QTL), LQTL (leaf QTL), or PCAQTL followed by a unique number. QTL presented in Table 4 were significant at the empirically determined threshold value corresponding to $P = 0.05$ based on permutation. For each QTL, we indicate the chromosome on which it resides, the estimated cM position of the QTL with 2-LOD confidence intervals, the genetic marker associated with QTL, the additive genotypic effect (a), and the proportion of the total genetic variance it explained in the full QTL model (PVE).

We detected eight and three QTL with strong effects on the floral and leaf traits, respectively. Several QTL were detected on each chromosome II (3QTL), IV (3QTL), and V (3QTL). A single QTL was detected on each of the chromosomes I and chromosome III. The number of QTL affecting floral and leaf traits ranged from a maximum of 5 (Petal L) to a single QTL (Petal W, PL). The proportion of total variation explained by each QTL (PVE) ranged from 4.1% to 61.0% (average 20.8%) and 8.3% to 15.5% (average 11.5%) for floral and leaf traits, respectively.

Five genomic regions affected only a single trait (*FQTL-1*, *FQTL-5*, *FQTL-6*, *FQTL-8*, *LQTL-2*). Six genomic regions affected two or more traits (*FQTL-2*, *FQTL-3*, *FQTL-4*, *FQTL-7*, *LQTL-1*, *LQTL-3*). A QTL on chromosome II (*PCAQTL-1, LQTL-1, FQTL-2*) affected the size of several floral and leaf traits (Petal L, Sepal L, LSL, Pistil, PL, SW, PW, LW), including both length and width measurements. Interestingly, this QTL has a negative additive effect for length measurements and a positive additive effect for width measures. The remaining QTL had consistent positive or negative effects on all of the traits they affected.

We detected six QTL with effects on principle component scores derived from PCA of 12 morphological traits. A single QTL (*PCAQTL-6*) was mapped for the first principle component. This QTL co-localizes with *LQTL3*/*FQTL-8*, a QTL region with effects restricted primarily to the leaf module. Two QTL were detected for the second principle component (*PCAQTL-1* and *PCAQTL-2*). The position of *PCAQTL-1* overlaps with the confidence intervals of both *FQTL*-2 and *LQTL-1*. *FQTL-2* and *LQTL-1* have large negative effects on leaf and floral organ lengths and positive effects on leaf and floral organ widths. *PCAQTL-2* co-localizes with *FQTL-3*, a QTL with effects restricted to floral organ lengths. Finally, five QTL were detected for principle component 3, including *PCAQTL-1*, *PCAQTL-3*, *PCAQTL-4, PCAQTL-5*, and *PCAQTL*-6. *PCAQTL*-5 is the only QTL localized to a genomic region not detected in single trait mapping analyses.

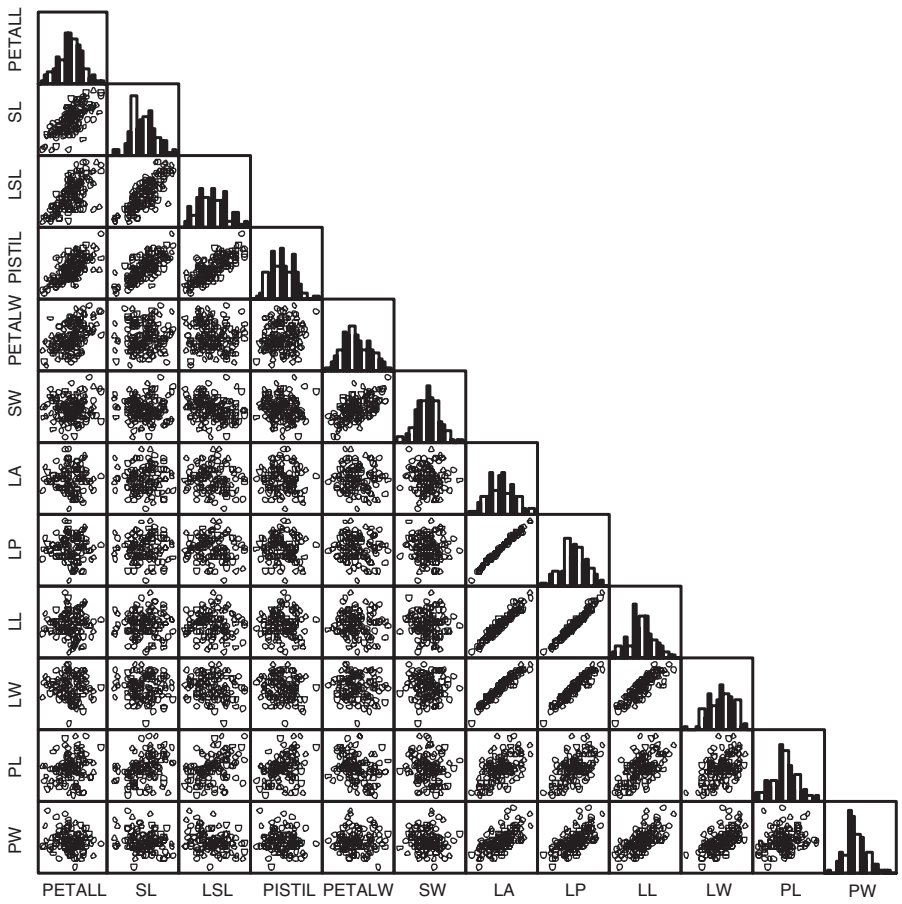

Fig. 2. Genetic scatterplot matrix of floral and leaf traits generated from the recombinant inbred line means for the studied traits. The histograms along the diagonal provide a visual representation of the genetic variance for each of the traits. The off-diagonal scatterplots provide a visual representation of the genetic correlation among pairs of traits.

Table 3. Principle component analysis of flower and leaf traits in the RIL population

Trait	Component 1	Component 2	Component 3
Lamina area	0.984	0.040	0.080
Lamina perimeter	0.987	0.060	0.065
Lamina length	0.969	0.097	0.061
Petiole length	0.583	0.456	− 0.272
Lamina width	0.942	− 0.018	0.071
Petiole width	0.721	− 0.177	0.088
Petal length	− 0.083	0.837	0.376
Sepal length	− 0.51	0.899	− 0.003
Long stamen length	− 0.152	0.905	− 0.237
Pistil length	− 0.058	0.891	− 0.007
Sepal width	− 0.078	− 0.110	0.805
Petal width	− 0.127	0.136	0.916
Percent variance explained	39.1	28.4	14.9

RIL, recombinant inbred lines.

Typically, QTL underlying principle component 3 exhibited a complex mixture of positive and negative effects on organ lengths and widths.

DISCUSSION

Over the past decade, remarkable progress has been made in understanding plant development, in particular, with respect to the molecular genetic basis of pattern formation in flowers (Weigel and Meyerowitz 1994), the establishment of polarity of lateral organs (Bowman et al. 2002), and the processes governing flowering time (Koornneef et al. 1998). These efforts have relied largely on loss- or gain-of-function mutant screening and transgenic experiments. Surprisingly few genetic studies have explored the molecular and developmental basis of size and shape variation in plant organs or have utilized natural genetic variation for understanding developmental processes (Mizukami 2001; Maloof 2003). Here, we

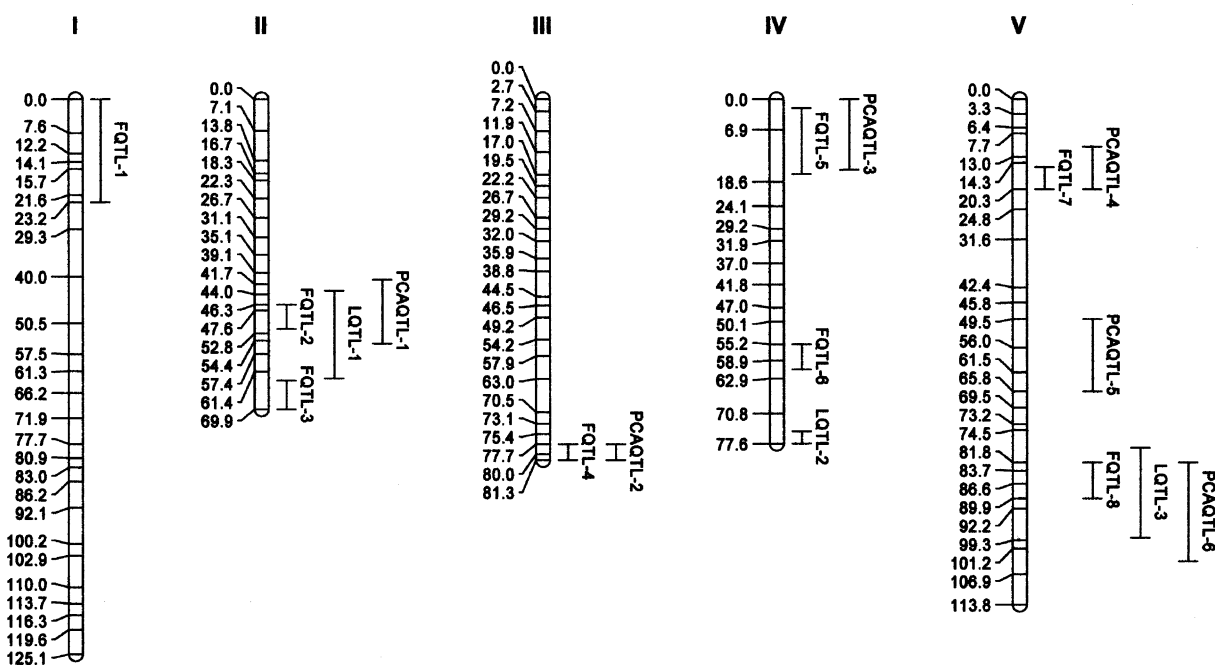

Fig. 3. Genomic positions of floral and leaf quantitative trait loci (QTL) detected in the Cape Verde Islands (L*er*) × Cape Verde Islands (Cvi) mapping population. Chromosome number is indicated above each linkage group and centimorgan (cM) position is indicated to the left. Each QTL is indicated by bars corresponding to 95% confidence intervals (corresponding to the average 2-LOD score drop for trait QTL mapped to a particular overlapping genomic region).

investigate the genetic architecture underlying size and shape variation in both flowers and leaves derived from a cross of natural accessions of *Arabidopsis thaliana*.

We discovered a number of QTL with small to moderate effects on organ size in the L*er* × Cvi mapping population. This finding supports the standard quantitative genetics assumption that continuous variation results from both the segregation of multiple genes with relatively small effects and environmental variation (Falconer and Mackay 1996; Lynch and Walsh 1998). Typically, we found that the L*er* and Cvi parents contained alleles that both increased and decreased floral and leaf measurements. This pattern of allelic distribution resulted in quite large transgressive segregation within the recombinant inbred population for most of the studied traits.

We found a rather striking pattern of genetic morphological integration. We detected strong positive genetic correlations among floral characters (particularly among organ lengths) and among leaf characters, but typically weak or absent genetic correlations between flower and leaf structures. These results support the notion of independent floral and vegetative modules. Furthermore, our findings reveal strong genetic integration within each module. The "within" flower or leaf module genetic correlations were generally greater than 0.70 and a genetic PCA clearly separates flower and leaf traits into distinct clusters. These results are consistent with a large body of literature reporting standing genetic variation in floral and leaf traits in natural populations as well as the differentiation of floral and vegetative modules (Berg 1960; Conner and Via 1993; Campbell 1996; Mitchell et al. 1998; Armbruster et al. 1999, 2004; Juenger et al. 2000; Conner 2002; Frary et al. 2004). Furthermore, it extends and complements previous quantitative genetic results in other *Arabidopsis thaliana* mapping populations (Juenger et al. 2000; Pérez-Pérez et al. 2002; Juenger, Pérez-Pérez, and Micol, unpublished results) and a close relative, *Raphinus sativus* (Conner and Via 1993; Conner 2002).

We used QTL mapping to localize genomic regions controlling variation in organ size and shape. It is important to emphasize that QTL mapping cannot directly identify the causal gene(s) underlying genetic variation in a trait. Typically, 95% confidence intervals surrounding detected QTL span approximately 5–50 cM, corresponding to 1.2–12 Mb of DNA (Koornneef et al. 2004). Nonetheless, it is a useful technique that can provide initial insight into the genetic basis of phenotypic variation, including identifying the minimum number of genes controlling trait variation, allow inference of modes of gene action (e.g., dominance, epistasis, GxE), and generate hypotheses concerning pleiotropy or tight linkage. Here, we consider overlapping QTL with effects on different traits as support for a pleiotropic basis of trait correlation and morphological integration (Cheverud et al. 1997; Juenger et al. 2000; Mezey et al. 2000; Cai and Morishima 2002).

Most of the QTL detected in our genome-wide screens have module-specific effects, being restricted to either floral or

Table 4. Results of QTL analyses of floral organs (A), leaf traits (B), and PCA scores (C) in *Arabidopsis* using multiple QTL mapping of RIL means

QTL	Traits	Chromosome	Position (cM)	Marker	LOD	PVE	*a*
(A)							
FQTL-1	Petal L	1	7.6 (0–23.2)	AXR-1	5.06	9	0.30
FQTL-2	Petal L	2	49.6 (46.3–56.4)	*erecta*	8.97	17.7	− 0.43
	SL	2	49.6 (45.6–51.6)	*erecta*	16.97	35.3	− 0.37
	SW	2	46.3 (44–49.6)	GD.460L-Col	5.99	14.7	0.09
	LSL	2	47.6 (46.3–51.6)	*erecta*	29.45	61	− 0.82
	Pistil	2	49.6 (47.6–51.6)	*erecta*	15.15	39.5	− 0.60
FQTL-3	SL	2	67.4 (61.4–69.9)	EC.235L-Col/247C	12.19	28.2	− 0.34
	Pistil	2	69.9 (65.4–69.9)	EC.235L-Col/247C	7.66	21.7	− 0.45
FQTL-4	Petal L	3	81.3 (77.7–81.3)	HH.90L	2.59	4.10	0.21
	SL	3	81.3 (77.7–81.3)	HH.90L	9.74	17.7	0.26
	LSL	3	81.3 (77.7–81.3)	HH.90L	5.87	6.7	0.28
FQTL-5	Petal W	4	8.9 (2.0–16.9)	GH.250C	5.34	9.9	− 0.15
FQTL-6	Petal L	4	58.9 (55.2–60.9)	HH.159-Col	2.81	4.8	0.23
FQTL-7	Petal L	5	18.3 (16.3–20.3)	BH.107L-Col	6.21	11.8	− 0.38
	Petal W	5	18.3 (14.3–20.3)	BH.107L-Col	14.52	34.2	− 0.28
FQTL-8	SW	5	85.7 (81.8–99.3)	HH.445L-Col	6.73	16.9	0.10
(B)							
LQTL-1	PW	2	49.6 (35.1–61.4)	*erecta*	3.11	9.4	0.05
	PL	2	52.8 (41.7–57.4)	GD.298C	3.69	11.1	− 0.44
	LW	2	59.4 (52.8–69.9)	BH.120L-Col	3.30	10.4	0.41
LQTL-2	PW	4	77.6 (74.8–77.8)	BH342/347L-Col	2.92	8.3	0.05
LQTL-3	LW	5	85.7 (73.2–96.2)	HH.445L-Col	4.88	15.5	− 0.53
	LA	5	85.7 (81.8–97.2)	HH.445L-Col	3.90	13.4	− 6.34
	LP	5	85.7 (81.8–97.2)	HH.445L-Col	3.53	12.2	− 1.75
	LL	5	85.7 (83.7–101.2)	HH.445L-Col	3.29	11.7	− 0.57
(C)							
PCAQTL1	PCA Factor 2	2	47.6 (46.3–57.4)	*erecta*	17.86	49.8	− 0.71
	PCA Factor 3	2	41.7 (35.1–52.8)	GB.150L-Col	4.74	8.6	0.30
PCAQTL2	PCA Factor 2	3	81.3 (77.7–81.3)	HH.90L	5.65	12	0.35
PCAQTL3	PCA Factor 3	4	6.0 (0–15.9)	GH.250C	3.56	6.5	− 0.27
PCAQTL4	PCA Factor 3	5	17.3 (10.7–20.3)	BH.180C	6.84	20.9	− 0.49
PCAQTL5	PCA Factor 3	5	61.5 (49.5–65.8)	CH.60C	3.65	11.5	− 0.35
PCAQTL6	PCA Factor 1	5	86.1 (81.8–101.2)	HH.445L-Col	4.54	17.3	− 0.45
	PCA Factor 3	5	95.2 (81.8–106.9)	GB.102C-Col	3.37	11.9	0.38

For abbreviations, see Table 1.
QTL, quantitative trait loci; RIL, recombinant inbred lines.

leaf complexes. Morphological integration within modules is owing to overlapping QTL effects, and thus likely the result of either pleiotropic loci or extremely tight linkage. Module independence is the result of unique QTL influencing traits within each module. Only two genomic regions contained QTL that affected both floral and leaf traits (*FQTL-2*/*LQTL-1* and *FQTL-8*/*LQTL-3*), and these QTL were primarily limited to petiole traits within the leaf module. One of these loci (*FQTL-2*/*LQTL-1*) is likely caused by the well-known *erecta* mutation (the confidence interval surrounding these QTL surround the *ERECTA* locus), which has been shown to influence organ elongation of *Arabidopsis thaliana* (Torii et al. 1996). Unfortunately, the confidence intervals surrounding the majority of the remaining QTL are large and it is virtually impossible to propose additional candidate genes with any certainty. Additional QTL mapping experiments with different parental accessions, fine-mapping with additional crosses, and the development of near-isogenic lines (NIL) will be needed to evaluate the generality of these QTL and to more fully explore the role of particular candidate genes. Moreover, we reiterate that the occurrence of QTL-by-environmental interactions may have resulted in an underestimation of the degree of genetic correlation between leaf and flower traits, given our study design (see Materials and Methods). Additional studies exploring the possibility of QTL × environmental interactions for these traits, as well as studies mapping

QTL for organs over the course of development (e.g., heteroblastic leaf development), may reveal additional insights into the genetic structure of leaves and flowers.

A classic hypothesis in plant developmental biology is that flower structures are homologs of ancestral leaf structures, which have been modified through evolutionary time (Goethe 1790). Recent molecular evidence supports this conjecture with the striking experimental transformation of leaves to petal-like and petals to leaf-like structures through the manipulation of important regulatory genes in *Arabidopsis thaliana* (Pelaz et al. 2000, 2001; Honma and Goto 2001; Goto et al. 2001). Interestingly, our study reveals only modest overlap in the genes controlling morphogenesis and the final size of floral and leaf organs. A recent F_2 study of tomato (Frary et al. 2004) found similar QTL results with little overlap in the position of QTL for leaf and floral characters. Taken together, these results suggest that the developmental genetic processes controlling organ growth in flowers and leaves have been decoupled over evolutionary time.

A number of hypotheses have been generated to explain the evolution of separate trait clusters or modules from a "universal" pleiotropic state in an ancestor. In general, decoupling of trait sets is thought to arise through the suppression of old or the acquisition of new pleiotropic effects as a result of selection for modularization (Wagner 1996). This process will lead to restricted sets of pleiotropic loci generating the modules of an organism. An alternative hypothesis is that genetic integration and modularization is the result of the balancing of positive and negative pleiotropy within and among modules as a product of selection. Under the balanced pleiotropy model, the same genetic loci are important across modules, but the pattern of their effects varies with module. These are clearly nonmutually exclusive hypotheses and it is likely that any particular set of modules will result from some contribution of each mechanism. Nonetheless, our QTL results suggest that the modularization of flowers and leaves in *Arabidopsis* is primarily the product of the selective acquisition or suppression of pleiotropy, rather than balanced pleiotropy across modules. The fact that two of the three leaf QTL co-localized with floral QTL suggests that selective acquisition of new pleiotropic loci may be particularly important for differentiating floral structures from leaves.

One possible mechanism leading to the genetic decoupling of flowers and leaves may be the loss or gain of pleiotropic effects following gene duplication. In particular, the *Arabidopsis thaliana* genome is thought to have undergone several ancient rounds of polyploidy and has therefore experienced widespread duplication of the genome. Recent studies have explored the functional divergence of duplicated genes formed by ancient polyploidy, have estimated the date of polyploidy events, and have mapped and characterized the resulting duplicated chromosomal sets in the sequenced *Arabidopsis* genome (Blanc et al. 2002; Simillion et al. 2002; Vision et al. 2002). Because duplicated genes have redundant functions immediately following polyploidy, one of the copies can accumulate new mutations with either deleterious or beneficial effects. Subsequent functional divergence can occur by neofunctionalization (a copy acquiring a new function) or by subfunctionalization (the copies retain different subsets of the functionality of the ancestral gene) (Force et al. 1999; Lynch and Conery 2000). One scenario for the development of an independent floral module from an ancestral leaf module would be sub-functionalization, perhaps through tissue-specific expression of genes with effects on organ morphogenesis. This hypothesis could be tested by comparing the genomic locations of QTL affecting flower or leaf morphology and evaluating the frequency of overlapping QTL (suggesting pleiotropy), QTL effects that are restricted to particular modules but derived from duplicated regions (suggesting sub-functionalization), or strictly novel QTL. Alternatively, sequence comparisons made between cloned floral and leaf QTL could directly test for the occurrence of duplicated genes with restricted modules of influence.

One of the main limitations of our study is that it is restricted to detecting genetic variation that segregates between two parental strains. It is possible that a large number of loci control both flower and leaf development, but these loci do not vary in natural populations. This might be expected for genes involved in developmental pathways with central or vital roles in growth and development. A more thorough characterization of the pleiotropic effects of known loss-of-function mutants may shed light on this hypothesis. As an example, Kim et al. (1999) found changes in the shapes of both leaves and flowers upon overexpression of a cytochrome *P*450 (*ROT3*) in *Arabidopsis*. In this case, changes in organ length were associated with changes in cell shape. Similarly, Mizukami and Fischer (2000) found that loss of *AINTEGUMENTA* (*ANT*) function reduces the size of petals and leaves by decreasing cell number. Conversely, gain of *ANT* function, via ectopic expression of *35S::ANT* transgenes, enlarges all shoot organs by increasing cell number. Finally, *rotunda2* mutants display wide rosette leaf laminae due to an increase in cell expansion (Cnops et al. 2004). The *RON2* gene was found to be identical to *LEUNIG* (*LUG*), previously identified in a genetic screen to isolate enhancer mutations of the floral homeotic mutant *apetala2-1* (Liu and Meyerowitz 1995). In flowers, *LUG* acts together with *SEUSS* (*SEU*) and *APETALA2* (*AP2*) to repress *AGAMOUS* (*AG*) in the sepals and petals (Conner and Liu 2000; Franks et al. 2002). In later stages of leaf development, *RON2* (*LUG*) is involved in the control of leaf size and shape through nonpolar cell expansion (Cnops et al. 2004). It is not known whether natural polymorphisms at *ROT3*, *ANT*, or *LUG* underlie size and shape variation of organs among ecotypes of *Arabidopsis*. We have isolated a large number of mutants with altered leaf size and shape, most of which carry loss-of-function mutations (Berná

et al. 1999; Robles and Micol 2001; Serrano-Cartagena et al. 2002; Cnops et al. 2004). A more complete analysis of these mutants may shed further light on the extent of common versus unique genetic control of flower and vegetative morphogenesis.

In conclusion, we have detected significant genetic variation for size and shape of both floral organs and rosette leaves and mapped a number of QTL underlying these traits in the L*er* × Cvi mapping population. We found large positive genetic correlations *among* sets of either flower or leaf traits, but low and generally nonsignificant genetic correlations *between* floral and leaf traits. Furthermore, we found that QTL effects were generally restricted to either flower or leaf structures. Only two QTL had overlapping effects in both regions of the plant. These results support the hypothesis of independent floral and vegetative modules and suggest that pleiotropic effects are typically restricted to functionally related character complexes.

Acknowledgments

We are grateful to the NASC and ABRC for providing seeds and to J. M. Serrano for expert technical assistance. We are especially indebted to M. Purugganan, T. F. C. Mackay, and C. Alonso-Blanco for continuous support and advice. We thank two anonymous reviewers for improving earlier drafts of this manuscript. This research was supported by a Miller postdoctoral fellowship to T. J. and BMC2002-02840 grant from the Ministerio de Educación y Cultura of Spain to J. L. M.

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