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ORIGINAL PAPER

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A comparative study of the genetic bases of natural variation in tomato leaf, sepal, and petal morphology

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Abstract In an effort to better understand the dramatic differences in vegetative and floral morphology that differentiate species within the genus *Lycopersicon*, quantitative trait loci (QTL) for leaflet and perianth size and shape characters were mapped in an interspecific F_2 population of tomato (*Lycopersicon esculentum* × *L. pennellii*). Thirty-six highly significant ($P \leq 0.001$) QTL were associated with 18 separate traits. QTL for correlated traits were generally not colocalized in the genome unless there was a clear codependence between the traits (e.g., organ length and area). Little or no overlap in QTL positioning between different organs was observed, suggesting that the genes determining the size and shape of leaflets, sepals, and petals are organ specific. Thus, while leaves are considered the developmental and evolutionary precursors to floral organs, genes acting late in development to determine certain aspects of morphology (namely shape and size) must have specialized to exert control over individual organs. Five of the leaflet-trait QTL map to analogous regions in the genome of eggplant, and therefore it appears there has been some conservation in the genes controlling leaf morphology within the Solanaceae.

Introduction

Tomato has been the focus of a large number of quantitative trait mapping studies in recent years. These studies have revealed the genetic basis of many traits of agronomic importance, including fruit size and shape, yield, soluble solids content, and pigmentation (Fulton et al. 1997, 2000; Grandillo et al. 1999; Bernacchi et al. 1998; Grandillo and Tanksley 1996; Tanksley et al. 1996; Alpert et al. 1995; Eshed and Zamir 1995). In several cases, the genes underlying fruit quantitative trait loci (QTL) have been cloned and characterized at the molecular level (Liu et al. 2002; Frary et al. 2000). Yet, despite the fact that great diversity in leaf and flower morphology exists among species within the tomato genus (*Lycopersicon*), the genetic basis of the natural variation found in these organs has been largely unexplored.

Virtually all of the research on the genetics of leaf and floral development has used mutagenesis to create loss-of-function mutations that dramatically alter organ size and shape. Using this approach, a number of major genes causing gross abnormalities in tomato leaf and flower development have been identified and characterized (Bharathan and Sinha 2001; Kessler et al. 2001; Hareven et al. 1996; Pnueli et al. 1991, 1994a, b; Dengler 1984). However, it is unclear whether these same genes shape the more subtle morphological variation observed in natural populations.

Our goal was to use QTL mapping to unravel the genetic basis of the differences in leaf and floral morphology that characterize two highly divergent tomato species: cultivated tomato, *L. esculentum*, and its wild relative, *L. pennellii*. These two species differ dramatically in ecological adaptation, morphology, and floral/pollination biology. (Rick and Tanksley 1981). *L. pennellii* is a xerophytic species occupying desert habitats along the western coast of Peru. Adapted to arid conditions, the leaves of *L. pennellii* are relatively small and consist of small, rounded, thick leaflets attached to the rachis on short petiolules. In contrast, the leaves of *L. esculentum* are larger and characterized by numerous elongated leaflets with narrowed tips on elongated petiolules. All accessions of *L. esculentum* are self-compatible and usually produce flowers with flush or recessed stigmas, conducive to self-pollination. However, most *L. pennellii* accessions are self-incompatible and produce flowers with

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exserted stigmas, suited for cross-pollination by insects (Fig. 1).

Our quantitative analysis examined the lateral leaflets of tomato's compound leaves and the most leaflike of the floral organs, the sepals and petals, which together comprise the perianth. In this way, we could best gauge whether the same QTL determine aspects of both vegetative and floral morphology. A comparative study of leaves and flowers is of particular interest, given that biologists have long subscribed to Goethe's hypothesis that leaves are the evolutionary precursors of floral organs (Arber 1950). The ABC model of floral-organ identity determination in *Arabidopsis* provides evidence that leaf production is indeed a default developmental pathway—flowers lacking all three classes of the homeotic floral-organ identity genes consist solely of leaflike organs (Coen and Meyerowitz 1991). Thus, because all flower parts are modified leaves or parts of leaves, one might speculate that the morphology of leaves and floral organs is regulated by many of the same genes. While the QTL underlying variation in floral and leaf morphology have been identified in several plant species—including *Arabidopsis*, *Gossypium*, *Mimulus*, and *Populus* (Juenger et al. 2000; Jiang et al. 2000; Bradshaw et al. 1998; Wu et al. 1997)—a comprehensive, combined analysis of the genes controlling natural variation in both these plant organs had not been undertaken.

In conducting such a combined analysis, we have asked three questions: (1) How many genes control the differences in leaf, sepal, and petal morphology between *L. esculentum* and *L. pennellii*? (2) What proportion of these loci correspond to known mutants? (3) To what degree is the morphology of different organs shaped by variation in shared genes? The answers to these questions should help lay a foundation for an evolutionary, developmental, and molecular understanding of plant species diversification.

Materials and methods

Plant material

The mapping population consisted of 83 F_2 individuals derived from the cross *L. esculentum* LA925 × *L. pennellii* LA716 (Fulton et al. 2002; http://www.sgn.cornell.edu). LA716 is a homozygous accession of *L. pennellii*. The F_2 population was generated from a single F_1 plant. The population was grown in a greenhouse at Cornell University in Ithaca, New York, USA. A duplicate population was generated by in vitro propagation and transplanted to a greenhouse at Mount Holyoke College in South Hadley, Massachusetts, USA.

Phenotypic analysis

Four leaves from each individual were scanned on a computer scanner to generate digital images—two leaves from the Ithaca population and two from the South Hadley population. To ensure comparison of leaves of similar developmental stages, only leaves five to nine nodes below the shoot apex were harvested. Nine leaflet traits were evaluated as illustrated in Fig. 2. The number of ateral leaflets (LFN) >0.5 cm in diameter was counted for each leaf, and the primary pairs of leaflets identified and numbered according to position. Length (LFL) and width (LFW) (at the widest point) measurements were taken from each of these labeled leaflets. Two shape indices were calculated. The LR index (LFLR), a measure of overall shape, was calculated as the ratio of width to length (deVicente and Tanksley 1993; Wu 2000). The DLR index (LFDLR) distinguishes between ovate and deltoid shapes by determining where along the length the widest part of an object is; this was calculated as the ratio of the distance from the leaflet base to the maximum width (D):length (L) (Wu 2000). The leaflet apex angle (LFAA) was evaluated as the angle between two lines drawn tangential to the tip of the leaflet. Leaflet surface area (LFSA) was quantified using the public-domain software program NIH Image (developed at the US National Institutes of Health and available on the internet at http://rsb.info.nih.gov/nih-image/). The petiolule length (PLL) of each leaflet and the leaf rachis length (RAL) were also measured. Values for each character were averaged in several ways to account for any differences due to position of the leaflet pairs along the rachis. Thus, measurements from the left and right leaflets of a pair at each position along the leaf were averaged, and

Fig. 1 Representative leaves and dissected perianths of *Lycopersicon esculentum* (*left*) and *L. pennellii* (*right*)

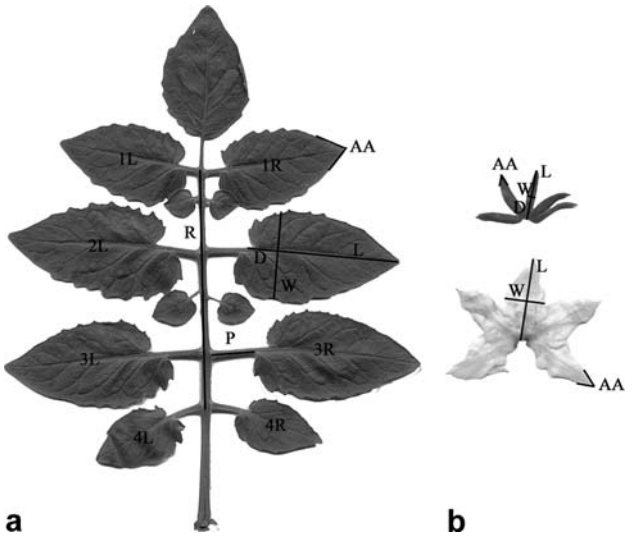


Fig. 2a, b Representative F_2 generation leaf (**a**) and perianth (**b**) illustrating how traits were measured. Major leaflets were identified and numbered according to relative position. Length (*L*), width (*W*), and apex angle (*AA*) were found for each primary lateral leaflet, sepal, and petal. Distance from base to widest points (*D*) was determined for leaflets and sepals. Petiolule lengths (*P*) and the portion of the rachis (*R*) to which all the lateral leaflets attach were also measured for each leaf. Ratios to describe shape were calculated from W:L (*LR*) and D:L (*DLR*)

the total average value for all the leaflets belonging to the same genetic individual was found. Pairwise and total averages were calculated for each location and across the two locations for the combined Mount Holyoke and Cornell populations.

Four to six flowers from each individual were dissected and scanned on a computer scanner and stored as digital images. A total of 13 perianth (sepal and petal) traits were evaluated from the images as shown in Fig. 2. The numbers of each floral part [sepal number (SEN), petal number (PEN)] were counted. Length measurements were taken from the sepals (SEL) and petals (PEL). The sepals width (SEW) at their widest point was determined. Petal width (PEW) was measured at the point of petal fusion. The LR index was calculated for the sepals (SELR) and petals (PELR) and the DLR index for the sepals (SEDLR). The apex angles for the sepals (SEAA) and petals (PEAA) were also found. The surface areas of the calyx and corolla of each flower were quantified using NIH Image and divided by the number of floral organs in each whorl to obtain an estimate of sepal (SESA) and petal surface area (PESA).

Genotypic and statistical analysis

Of the over 1,000 molecular markers genotyped in this population (Tanksley et al. 1996; Fulton et al. 2002), 391 markers that could be ordered at LOD≥3.0 (RIPPLE function of MAPMAKER) were selected for QTL analysis. This set of markers, a mixture of restriction fragment length polymorphism (RFLP), simple sequence repeat (SSR), and conserved ortholog set (COS) markers derived from ESTs, covers all 12 tomato chromosomes at an average spacing of 4 cM. Chromosome maps were constructed using MAPMAKER, version 2.0 (Lander et al. 1987). Correlation coefficients between traits were calculated by QGENE (Nelson 1997), version 3.06, and displayed graphically using the multidimensional scaling function of SAS (SAS Institute). Single-point regression and interval-mapping analyses were done using QGENE to identify putative QTL and estimate their effects. A LOD threshold of 2.8 was used as the criterion for QTL declaration. Based on marker density and genome size, this value is approximately equivalent to a locuswise significance level of P=0.001 (Lander and Botstein 1989). The

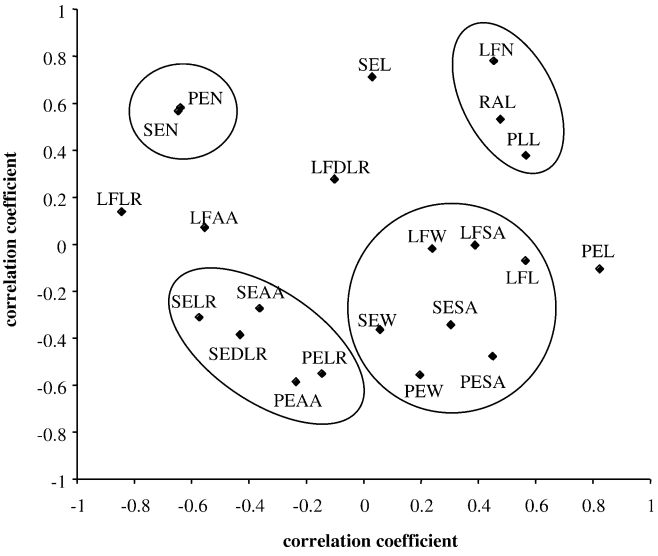


Fig. 3 Correlations between all leaflet and perianth characters (P<0.001) graphically displayed using multidimensional scaling analysis. The distance between traits is inversely proportional to the size of the correlation coefficient; thus, strongly related traits tend to cluster

percentage of phenotypic variation explained (r^2 from QGENE), trait means, and gene actions (*d/a*) were determined for the most significant marker for each QTL via single-point analysis. Multiple-regression analysis was performed with the program StatView (SAS Institute) to estimate the percentage of phenotypic variation accounted for by all significant QTLs for each trait. Interactions between QTL for each trait were assessed via two-way ANOVAs using StatView.

Results and discussion

A total of 22 traits encompassing differences in organ shape and size were analyzed in 83 F_2 individuals derived from the cross *L. esculentum* LA925 × *L. pennellii* LA716.

Correlations between traits

A number of significant (P≤0.001) correlations were observed among the various traits measured. Strong positive correlations were observed between interrelated size characters such as width, length, and surface area for each organ as revealed by the clustering of these traits in the multidimensional scaling analysis (Fig. 3). Thus, the highest correlations within the leaflet traits were those between surface area (LFSA) and leaflet width (LFW) (r=0.89) and length (LFL) (r=0.84). The size of sepals and petals (SESA and PESA) was most closely associated with the width (SEW and PEW) of those organs (r=0.84 and 0.81, respectively).

Two of the parameters describing organ shape, LR index and apex angle, were also positively correlated for each organ type, with r values ranging from 0.53 in sepals to 0.66 in petals, supporting the observation that rounded leaflets, sepals, and petals tend to have blunter tips.

A relationship was found between the length of the leaf axis and the number of organs along the axis. Thus, LFN was highly correlated with RAL, which in turn, was significantly correlated with PLL, suggesting some common control of these traits (Fig. 3).

A few salient correlations that may have implications with respect to shared genetic control of leaf and floral-organ development were also noted. One of the most striking observations was that comparable sepal and petal parameters were much more likely to be correlated to each other than to leaf parameters. For example, variation in SEN and PEN was highly correlated (r=0.98). Similarly, the shapes (SELR, SEDLR, PELR) of sepals and petals were associated as were their areas (SESA, PESA) as can be seen by the clustering of these traits in Fig. 3. In contrast, leaflet number and shape were not significantly correlated with their corresponding parameters in petals and sepals. Surface area and, to a lesser degree, organ width were the only traits for which correlations were observed amongst all three organs: leaves, petals and sepals. However, even in these cases, the correlations were stronger between petal and sepal traits. Finally, the leaflet shape parameters (LFLR, LFDLR) were not well correlated with each other, nor with shape parameters of sepals or petals.

QTL analysis

A total of 36 highly significant ($P \leq 0.001$) QTL were associated with 18 of the 22 leaf and flower characters. These QTL were mapped over 11 of the 12 tomato chromosomes; Fig. 4 shows their map locations. For comparative purposes, this figure also depicts the map positions of previously identified mutants known to affect one or more of the same traits. Overall, the greatest number of QTL was detected on chromosome 5 (seven loci); however, noteworthy numbers of QTL (between four and six loci) were also found on chromosomes 7, 11, and 12. No QTL were mapped to chromosome 6, and no significant loci were identified for leaf RAL and three of the flower traits: sepal length, petal width, and petal width: length ratio. The QTL for each trait are described below (see also Table 1).

Leaf traits

LFN Two QTL located on chromosomes 1 (*lfn1.1*) and 5 (*lfn5.1*) affected LFN. At both loci, the *L. esculentum* (LE) alleles acted to increase the number of leaflets, as plants homozygous for LE alleles averaged about 12 leaflets compared to nine leaflets in the plants homozygous for the *L. pennellii* (LP) alleles at these loci. The LE alleles at *lfn5.1* behaved in a dominant manner, while those at *lfn1.1* showed additive gene action. Simultaneous fit of the two QTL accounted for 22% of the variation in LFN.

LFW and LFL Two QTL were identified as controlling LFW in the Cornell population only: *lfw7.1* and *lfw12.1*. As predicted from the parental phenotypes, LP alleles at both loci served to increase LFW. *lfw7.1* on chromosome 7 was partially recessive (d/a=−0.3) in nature, whereas the locus on chromosome 12 (*lfw12.1*) showed a degree of dominance (d/a=0.75). Together the loci explained 28% of the phenotypic variation in LFW.

LFL mapped to three QTL on chromosomes 2 (*lfl2.1*) and 11 (*lfl11.1* and *lfl11.2*). These loci showed similar effects on phenotype with the LP alleles somewhat unexpectedly, contributing toward longer leaflets. Gene action of the LP alleles at these loci ranged from partially (*lf11.1* and *lfl11.2*) to fully dominant (*lf2.1*). In addition to the aforementioned loci, the Mount Holyoke population supported a fourth QTL, *lfl12.1*, at which the LE allele increased LFL and was dominant.

LFLR and LFDLR Leaflet shape—as assessed with the LFLR and LFDLR indices—was controlled by three loci: *lflr1.1*, *lflr4.1*, and *lfdlr5.1*. LP alleles at these loci had the expected effect of promoting round and ovate leaves (higher LFLR and LFDLR) and exhibited partial and full dominance at *lflr4.1* and *lfdlr5.1*, respectively. When fit simultaneously, the LFLR QTL explained 18% of the phenotypic variation.

LFAA QTL on three chromosomes were associated with the shape of the leaflet tip. *lfaa4.1*, *lfaa5.1*, and *lfaa5.2* were supported in both populations, while *lfaa7.1* was detected only in plants grown at Cornell. LP alleles at all four loci showed some degree of dominance (d/a values ranged from −0.2 to −0.76) and, as predicted from the parental phenotype, contributed toward blunter leaflets (i.e., increased apex angle). The loci also showed similar magnitudes of effect.

LFSA Overall leaflet size was affected by three loci that mapped to chromosomes 10 (*lfsa10.1*) and 11 (*lfsa11.1* and *lfsa11.2*). While all three QTL were of approximately equal significance and magnitude of effect, those on chromosome 11 showed an effect opposite to that expected. Thus, the leaflet area of plants homozygous for LP alleles at *lfsa11.1* and *lfsa11.2* averaged around 14 cm^2, while the leaflets of LE homozygotes were significantly smaller at about 9.5 cm^2. Gene action at *lfsa11.2* was additive, while the LP alleles at *lfsa11.1* were partially dominant. Dominant LE alleles augmented leaflet size at *lfsa10.1*.

PLL Evidence for loci controlling the length of the leaflet stalk was obtained only from plants grown at Cornell. Two QTL were identified, *pll7.1* and *pll12.1*, which accounted for approximately 16% of the variation in this trait. Surprisingly, the mean PLL was greater in the LP homozygotes at both loci. However, while the LP alleles were fully dominant at *pll7.1*, they showed partial recessivity at *pll12.1*.

Table 1 List of quantitative trait loci (QTL) detected for each trait

Trait	Chromosome	QTL designation	Interval[a]	*P*-value	Source[b]	%PVE (r^2)[c]	Trait means by genotype			*d*/*a*[d]
							AA	aa	Aa	
Sepal number	2	*sen2.1*	cLEZ17J15A-cLPT1A21	0.0008	LE	19.2	5.83	5.07	5.26	−0.50
	11	*sen11.1*	TG393-TG105A-T1648	0	LE	54.2	6.39	5.07	5.13	−0.91
Sepal width	3	*sew3.1*	T482-T781-T772	0	LE	31.6	0.19	0.15	0.17	0
Sepal width: length ratio	5	*selr5.1*	T1640-CD64-TG441	0.0001	LP	24.2	0.16	0.20	0.18	0
	8	*selr8.1*	CT92-TG176-CT156B	0.0001	LP	23.6	0.16	0.20	0.18	0
Sepal distance: length ratio	5	*sedlr5.1*	TG96-cLET7N9	0.001	LP	19.0	0.40	0.49	0.42	0.55
	8	*sedlr8.1*	T721	0.0005	LP	19.6	0.39	0.49	0.43	0.20
	11	*sedlr11.1*	TG651	0.0009	LP	18.5	0.40	0.47	0.40	1.00
Sepal apex angle	5	*seaa5.1*	CT93-T1335	0.0006	LP	19.7	39.1	47.3	33.5	2.36
	9	*seaa9.1*	T1267-T1212-TG223	0	LP	26.6	25.3	46.6	40.7	−0.45
Sepal surface area	3	*sesa3.1*	CT243-T781-T196	0	LE	31.2	0.16	0.11	0.14	−0.20
Petal number	11	*pen11.1*	TG393-TG105A-T1071	0	LE	51.0	6.32	5.06	5.15	−0.86
Petal length	7	*pel7.1*	T643-cLED22K8	0.0003	LE	21.7	1.57	1.36	1.34	−1.19
	12	*pel12.1*	T1483-P68-T1684	0	LE	26.8	1.35	1.26	1.50	4.33
Petal apex angle	11	*peaa11.1*	cLEX4G10-T675	0.0003	LP	21.6	33.4	59.4	47.8	−0.11
Petal surface area	12	*pesa12.1*	P68	0.0007	LE	18.0	0.40	0.40	0.51	NA[g]
Leaflet number	1	*lfn1.1*	T1284-T67	0.0005	LE	18.3	12.5	9.23	11.2	0.20
	5	*lfn5.1*	CD64-T1335-TG441	0.0009	LE	17.2	11.2	8.89	11.5	1.26
Leaflet width	7	*lfw7.1*[f]	T463A-T848-cLEX13I13	0.0001	LP	21.7	2.83	3.46	3.25	−0.33
	12	*lfw12.1*[f]	P68-T1684-cLET5 M3B	0.0007	LP	18.5	3.00	3.57	3.07	0.75
Leaflet length	2	*lfl2.1*	ORFX3-cLPT1A21-T347	0.0004	LP	18.5	4.09	4.84	4.86	−1.05
	11	*lfl11.1*	cLEX4G10-SSR46-TG523	0.0001	LP	24.0	3.98	4.02	4.61	−0.21
	11	*lfl11.2*	TG46-T1014-TG400	0.0002	LP	20.4	3.96	4.98	4.63	−0.31
	12	*lfl12.1*[f]	P64	0.0002	LE	21.0	5.03	4.17	5.27	1.56
Leaflet width: length ratio	1	*lflr1.1*	T67-T1957	0.0002	LP	19.7	NA[g]	0.74	0	0
	4	*lflr4.1*	TM19-T1232-T1068	0.0001	LP	22.5	0.62	0.73	0.71	−0.64
Leaflet distance: length ratio	5	*lfdlr5.1*	cLEC37C6A-T1640-CT167	0	LP	15.9	0.33	0.36	0.36	−1.00
Leaflet apex angle	4	*lfaa4.1*	CT173-cLED19B12-T974	0.001	LP	16.9	75.8	88.9	86.4	−0.62
	5	*lfaa5.1*	TG96-cLET7N9	0.0009	LP	17.3	78.5	89.4	88.1	−0.76
	5	*lfaa5.2*	T730-TG318-T328	0.0007	LP	17.7	77.3	90.7	85.9	−0.28
	7	*lfaa7.1*[f]	T1366-TG216	0.0005	LP	18.7	81.2	98.3	91.5	−0.20
Leaflet surface area	10	*lfsa10.1*	T246	0.0005	LE	18.1	13.5	10.0	13.1	0.77
	11	*lfsa11.1*	cLEX4G10-CT182-TG523	0.0004	LP	19.9	9.42	13.5	12.3	−0.41
	11	*lfsa11.2*	T1014-T1648	0.0006	LP	17.7	9.70	13.9	12.1	−0.14

Table 1 (continued)

Trait	Chromosome	QTL designation	Interval[a]	*P*-value	Source[b]	%PVE (r^2)[c]	Trait means by genotype			d/a[d]
							AA	aa	Aa	
Petiolule length	7	*pll7.1*[e]	TG499-T848	0.001	LP	17.8	0.80	1.15	1.20	−1.28
	12	*pll12.1*[e]	T801-P68-TG394	0.0005	LP	19.4	0.90	1.35	0.95	0.78

[a]For QTL that were significant for more than one marker, the flanking markers are given with the most significant marker *underlined*
[b]*LE L. esculentum, LP L. pennellii*
[c]%PVE (r^2) Percentage phenotypic variation explained
[d]Means are average phenotypic values for plants with the following genotypes: *AA* homozygous *L. esculentum*, *aa* homozygous *L. pennellii*, *Aa* heterozygous
[e]*d/a* gene action
[f]QTL denoted here were significant in only one location
[g]*NA* Not available

Flower traits

SEN and PEN Two QTL were detected as controlling the number of sepals: *sen2.1* and *sen11.1*. PEN was also found to be associated with both locations on chromosomes 2 and 11; however, the chromosome 2 locus, with a *P*-value of 0.002, just exceeded the significance threshold. At both loci, LE alleles increased the number of floral organs to six in the LE homozygotes and showed a degree of recessivity. The QTL on chromosome 11 accounted for the majority of the phenotypic variance, while that on chromosome 2 also exhibited a substantial influence. Together, *sen11.1* and *sen2.1* explained 39% of the variation in SEN.

SEW and PEL A single QTL was found for SEW (*sew3.1*). LE alleles at this locus, acting in an additive fashion (*d/a*=0), had the somewhat unexpected effect of increasing SEW.

PEL was affected by two loci on chromosomes 7 (*pel7.1*) and 12 (*pel12.1*). LE alleles served to increase petal length at both loci, acting in a recessive manner at *pel7.1* and displaying overdominance at *pel12.1* (*d/a*=4.33). As indicated earlier, no significant QTL were identified for SEL or PEW.

SELR and SEDLR QTL on three chromosomes (5, 8, and 11) played a role in determining sepal shape. Two QTL were linked with the SELR index and three with the SEDLR index. In all cases, the LP alleles enhanced the roundness of the sepals (higher SELR and SEDLR). The two loci for SELR and SEDLR on chromosome 5 (*selr5.1* and *seldlr5.1*) overlapped slightly, though *selr5.1* showed additive gene action, while *sedlr5.1* showed partial dominance of the LE alleles. The QTL had similar magnitudes of effect on their corresponding traits (SELR and SEDLR, respectively). The SELR and SEDLR QTL on chromosome 8 showed complete overlap, as *sedlr8.1* resides in the interval spanned by *selr8.1*. Alleles at both loci behaved in an additive fashion. Together, the two SELR QTL accounted for approximately 36% of the variance in the trait, while the three SEDLR QTL accounted for 37% of the phenotypic variation in that shape index.

SEAA and PEAA Three separate QTL were identified as influencing the angle of the sepal and petal tips. Two loci were specific to sepals (*seaa5.1* and *seaa9.1*), while one was associated with petals (*peaa11.1*). As expected, LP alleles at all loci increased apex angle, thereby producing a blunter tip on the floral organs. The LE alleles at *seaa5.1* showed overdominance (*d/a*=2.36), while those at *seaa9.1* were partially recessive. Simultaneous fit of the two QTL accounted for 21% of the variation in SEAA. Approximately 22% of the variation in PEAA was attributed to *peaa11.1*, which exhibited additive gene action.

SESA and PESA Single QTL were associated with SESA and PESA—one on chromosome 3 (*sesa3.1*) and one on chromosome 12 (*pesa12.1*). LE alleles at these two loci served to increase floral-organ size in an additive manner. The r^2 values indicated that a substantial proportion of the phenotypic variance is explained by these loci.

Allelic trends

The two parent species contributed toward organ morphology in different but generally predictable ways. LP alleles promoted ovate and round forms and blunt tips in sepals, petals, and leaflets, whereas LE alleles had the opposite effect on shape. Alleles of LE enhanced the size of the floral organs and increased the numbers of leaflets, sepals, and petals. Somewhat surprisingly, LP alleles lengthened leaflets and petiolules and increased leaflet surface area (at two of the three detected loci).

QTL with pleiotropic effects on more than one parameter

QTL controlling many of the correlated traits tended to be colocalized within the genome. While this could be due to close linkage of two or more genes, it most likely reflects the pleiotropic activity of a single gene as, in the majority of cases, these overlaps involve characters that are obviously codependent. Thus, not surprisingly, SEW and SESA mapped to the same region of chromosome 3; PEL and PESA overlapped on chromosome 12; LFL and LFSA were found in the same two regions of chromosome 11. Although they just missed the significance threshold, LFW QTL were detected on chromosomes 2 and 11, in positions corresponding to *lfl2.1*, *lfl11.1*, and *lfl11.2* (Fig. 4). Nevertheless, some regions of the genome did contain single, independent loci for the organ size traits (e.g., *pel7.1*, *lfw7.1*, *lfsa10.1*). The fact that linkage was not always observed among these characters suggests that measurements of width and length along a single axis are insufficient indicators of total organ surface area.

Interestingly, while the shape and apex angle of perianth organs were significantly correlated, the major QTL for these traits did not colocalize on the genetic map (with the exception of SELR and SEAA on chromosome 5), suggesting that they are independently inherited parameters. If there is any degree of shared genetic control of these traits, it must be attributed to QTL of lesser effects that were not detected in this analysis (Fig. 4).

The two major QTL controlling the number of parts within the perianth appear to be pleiotropic, exerting their effects in both floral whorls, as SEN and PEN mapped to the same intervals of chromosomes 11 and 2 (the PEN chromosome 2 QTL just missed the significance threshold, Fig. 4). However, colocalization of other QTL for leaflet and perianth traits would appear to be purely coincidental, as no strong correlations were observed between those organs. These results suggest that the genes determining the size and shape of leaflets, sepals, and petals are organ specific. However, one must bear in mind that, because tomato leaves are compound, leaflets are not precisely homologous to individual flower parts such as sepals or petals. Moreover, given the relatively small size of the mapping population, it is likely that QTL with smaller effects on the traits of interest were not detected in this study. Some of these minor QTL may have overlapping functions in both vegetative and floral organs.

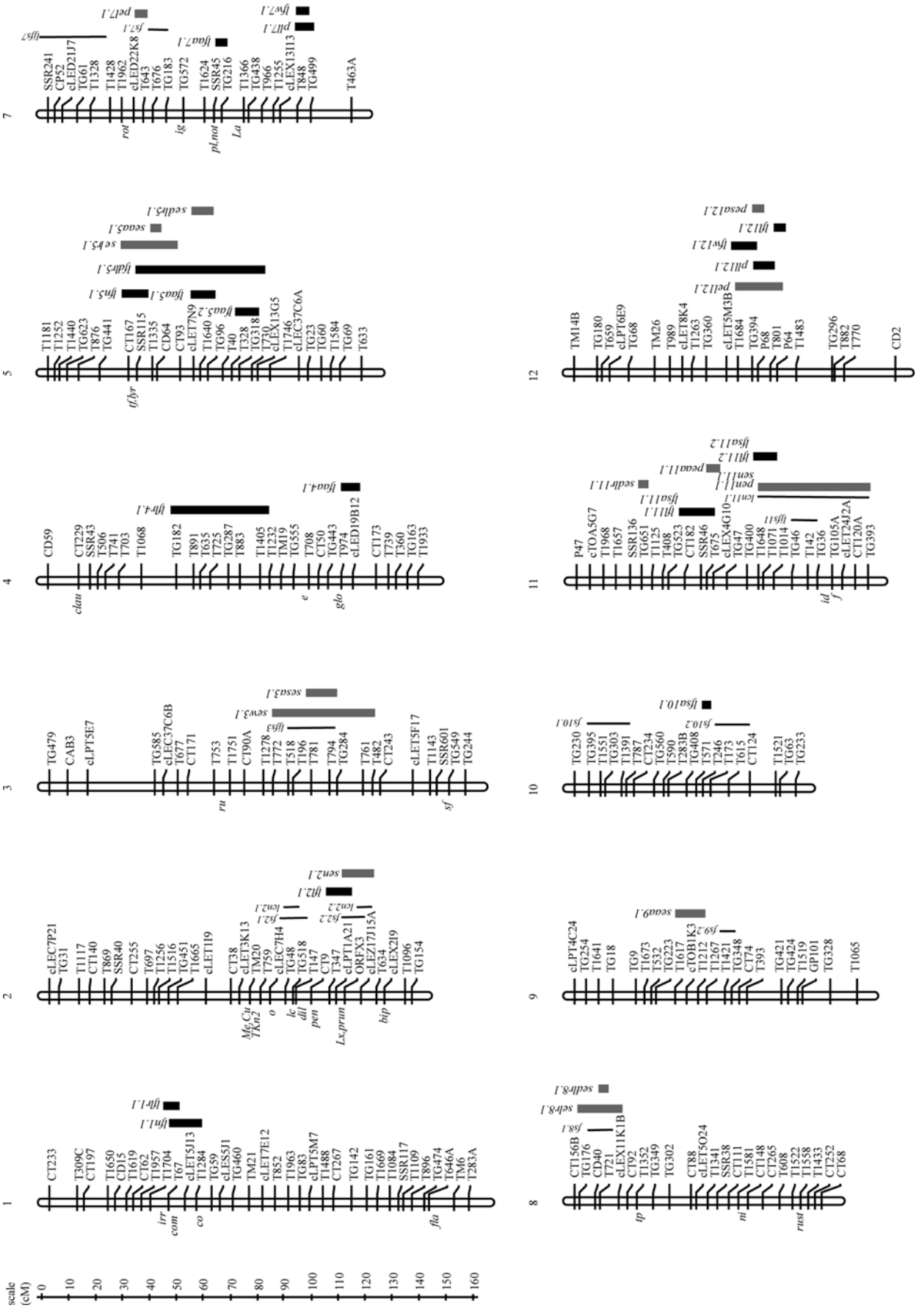
Epistasis between QTL

Two-way ANOVAs were performed between all significant markers within each trait to reveal any epistatic interactions between loci. A single significant ($P<0.005$) interaction was detected between *sen2.1* and *sen11.1* for the control of SEN. Figure 5 depicts the synergistic interaction between these loci: when both are homozygous for LE alleles, a disproportionate increase in SEN results.

Classical genetic studies have described two genes which affect carpel number in tomato: *fasciated* (*f*, MacArthur 1934) and *locule number* (*lc*, Yeager 1937). QTL analysis then identified these same two loci (designated *lcn11.1* and *lcn2.1*) as controlling locule number, mapped the loci to distal portions of chromosomes 11 and 2, and revealed that they interact epistatically (Lippman and Tanksley 2001). The SEN and PEN QTL detected in the current study occupy positions close to *lcn11.1* and *lcn2.1* and exhibit a similar epistatic interaction (Fig. 5). Given these similarities, and because fasciation is often manifested in other floral parts (including the corolla and calyx), it seems probable that *f* and *lc* are responsible for determining the number of organs in all whorls of the tomato flower. On the other hand, LFN is clearly under separate genetic control, mapping as it does to chromosomes 1 and 5.

Correspondence of QTL to known mutant loci in tomato

In addition to *lc* and *f*, a number of other single genes controlling leaf and flower characters have been described. Among the best studied of these are the MADS-box family of genes that control floral-organ identity (Pnueli et al. 1991, 1994a, b) and the *knotted1*-like homeobox (*KNOXI*) genes that regulate leaf morphogenesis (Avivi et al. 2000; Janssen et al. 1998; Chen et al. 1997; Parnis et al. 1997; Hareven et al. 1996). Moreover, a large number of loci affecting vegetative and floral morphology have been identified through mutant analysis (Stevens and Rick 1986). To explore the possibility that allelic variation at these known genes may be responsible for the subtle differences in leaflet and perianth morphology that were observed in our population, we compared the approximate map positions of relevant morphological markers with the QTL identified in this study (Fig. 4). Significant overlaps among loci controlling leaflet characters were found and,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CT197
T1650
CD15
T1619
CT62
T1957
T1704
T67
cLET5J13
T1284
TG59
cLES5J1
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TM21
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T852
T1963
TG83
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T1488
CT267
TG142
TG161
T1669
T1084
SSR117
T1109
T896
TG474
T646A
TM6
T283A
irr
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ljfn1.1
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2
cLEC7P21
TG31
T1117
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SSR40
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T1256
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lcn2.1
lcn2.2
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TG479
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TG585
cLEC37C6B
T677
CT171
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T518
T196
T781
T794
TG284
T761
T482
CT243
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TG549
TG244
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T739
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TG163
T1933
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ljfa4.1
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T1252
T1440
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T876
TG441
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TG318
T750
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T1746
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TG23
TG60
T1584
TG69
T633
tfdyr
ljfa5.1
ljfa5.2
ljfn5.1
ljfdh5.1
sefh5.1
seaa5.1
sedh5.1
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TG183
TG572
T1624
SSR45
TG216
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ljfw7.1
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CT92
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TG302
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SSR38
CT111
T1581
CT148
CT265
T608
T1522
T1558
T1433
CT252
CT68
tp
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rust
jsf8.1
sefh8.1
sedh8.1
9
cLPT4C24
TG254
T1641
TG18
TG9
T1673
T532
TG223
T1617
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T1212
T1267
T1421
TG348
CT74
T393
TG421
TG424
T1519
GP101
TG328
T1065
jsf9.2
seaa9.1
10
TG230
TG395
T1551
TG303
T1391
T787
CT234
TG560
T590
T283B
TG408
T571
T246
T173
T615
CT124
T1521
TG63
TG233
jsf10.1
jsf10.2
ljfa10.1
11
P47
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T1968
T1657
SSR136
TG651
T1125
T408
TG523
CT182
SSR46
T675
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TG47
TG400
T1648
T1071
T1014
TG46
T142
TG36
TG105A
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TG68
TM26
T989
cLET8K4
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TG360
cLET5M3B
T1684
TG394
P68
T801
P64
T1483
TG296
T882
T770
CD2
pel12.1
plf12.1
ljfw12.1
ljfl12.1
pesa12.1

◀ **Fig. 4** Linkage map derived from the F_2 population of the cross *L. esculentum* LA925 × *L. pennellii* LA716. Only those chromosomes with QTL are shown. *Solid black bars* indicate significant (LOD>2.8) QTL for leaflet traits and *gray bars* mark loci for perianth traits. *Lines* designate the position of previously mapped fruit-shape QTL. The approximate positions of relevant morphological mutants are indicated to the *left*

based on this positional and functional information, several candidate genes can be proposed as underlying the QTL.

Thus, the variation in leaflet number attributed to *lfn1.1* and *lfn5.1* may reflect the action of the genes *Cochlearis* (*Co*) on chromosome 1 and *Trifoliate* (*Tf*) and *Lyrata* (*Lyr*) on chromosome 5 as *co*, *lyr*, and *tf* mutants all display a drastic reduction in leaflet number. The concurrence of the leaflet length locus *lfl2.1* and the genes *Laxa* (*Lx*) and *Prunoidea* (*Prun*) is highly suggestive as, judging from their mutant phenotypes, both genes appear to have a role in controlling leaf elongation. The differences in leaflet shape observed in this study may also be attributable to two previously characterized loci. *Lanceolate* and *lfaa7.1* are located in the same region of chromosome 7, and both affect the shape of the leaflet apex (Dengler 1984). On chromosome 1, the proximity between *Irregularis* (*Irr*) and *lflr1.1* would appear to be more than coincidental, as *irr* mutants have shortened leaves which will clearly impact the leaflet shape index LR. The overlaps described above suggest that, while the mutant forms of single genes produce major aberrations in form, they may also play a role in normal development and contribute to quantitative variation in traits.

Conservation of size and shape QTL in tomato and related species

While no previous reports regarding the quantitative genetics of perianth morphology in the Solanaceae have appeared, a substantial number of QTL influencing ovary development have been identified. In several instances, fruit-shape loci map to the same positions as QTL governing aspects of leaflet and perianth size. Only one significant correspondence occurred between genetic factors governing organ shape: sepal shape QTL on chromosome 8 (*selr8.1* and *sedlr8.1*) colocalize with the major determinant of fruit shape in tomato and pepper, *fs8.1* (Grandillo et al. 1999; Ben Chaim et al. 2001; Fig. 4). The intersection of QTL controlling matters of organ size and shape is not particularly surprising and suggests that these loci may contain genetic factors affecting the extent or timing of cell division and/or cell expansion.

A few studies have investigated leaflet traits in tomato and related solanaceous species. Thus, it seems likely that *lflr1.1* corresponds to two shape loci, *lr1a* and *lr1b*, detected in an earlier analysis of an LE × LP F_2 population (deVicente and Tanksley 1993). In addition, *lflr4.1* may coincide with the locus *lr4* described in the same study. A recent study has identified leaf size and shape QTL in eggplant, another solanaceous crop (Frary et al. 2003). Although eggplant has simple leaves, five of the nine loci that were detected for leaflet width, length, and shape parameters in tomato overlap with similar QTL in eggplant. A locus orthologous with *lfw7.1* mapped to linkage group 7 of eggplant (*lw7.1*), and a counterpart of the shape QTL *lflr1.1* was found on linkage group 1 (*lsh1.1*). Loci for leaf length in eggplant (*ll11.1*) and leaflet shape in tomato (*lflr4.1*) are located in syntenic regions of the genome, and an eggplant QTL for leaf shape (*lsh5.1*) spans the region occupied by two tomato leaflet QTL: *lfw12.1* and *lfl12.1* (Frary et al. 2003). These overlaps indicate that there has been some conservation in the genes controlling leaf morphology within the Solanaceae. Moreover, given the different form of eggplant and tomato leaves (simple versus compound), these overlaps suggest some common genetic control over the determination of shape in both leaves and leaflets.

Evolutionary implications

Striking differences in vegetative and floral morphology differentiate species within the genus *Lycopersicon*

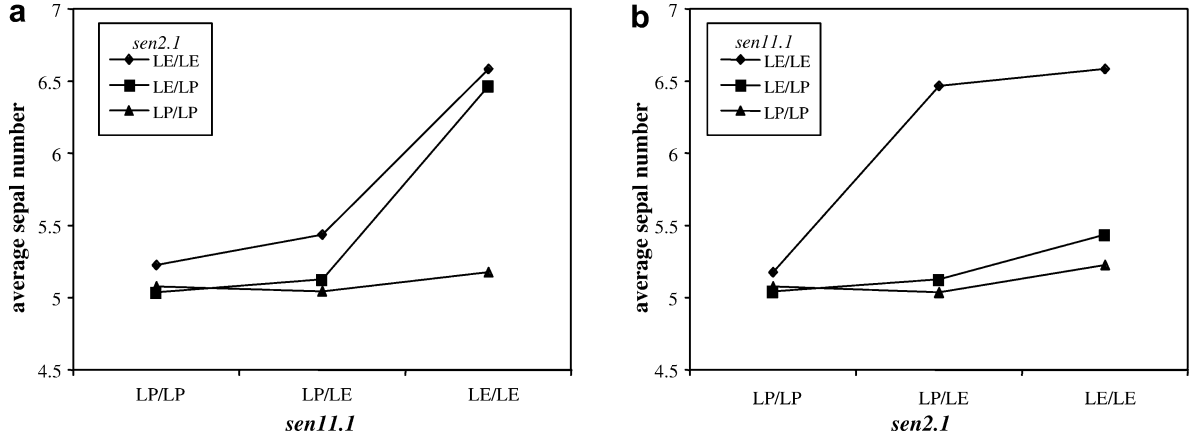

Fig. 5 **a** *sen2.1* × *sen11.1* sepal number interaction plot. **b** *sen11.1* × *sen2.1* sepal number interaction plot. *LP/LP* homozygous for *L. pennellii* alleles, *LE/LE* homozygous for *L. esculentum* alleles, *LP/LE* heterozygous

(Luckwill 1943). The current study has succeeded in mapping the major QTL accounting for some of the natural variation in leaf, sepal, and petal morphology that exists between two species in the genus. While major QTL were identified for each organ, little or no overlap in QTL positioning between organs was observed. This finding—that there is not a shared set of loci controlling traits such as organ width, length, and shape—has certain evolutionary implications. While leaves have long been considered the antecedents of the floral organs, genes determining certain aspects of their morphology (namely shape and size) must have specialized to exert control over individual organs. The obvious clustering of QTL mediating leaflet and floral morphology on chromosomes 5, 7, 11, and 12 could lead one to hypothesize that gene duplication and dispersal events may have generated an array of organ-specific size and shape genes. However, DNA sequence information would be needed to ascertain if there is any degree of conservation among these clustered loci.

While there is now little overlap in the genetic control of the shape of different organs within tomato, we do report common QTLs for control of leaflet shape between tomato and eggplant. Thus, in the 12 million years or so since the divergence of eggplant and tomato (Wikstrom et al. 2001), several of the genes underlying natural variation in leaf morphology have been conserved.

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