Quantitative Trait Locus Analysis of Leaf Dissection in Tomato Using *Lycopersicon pennellii* Segmental Introgression Lines

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ABSTRACT

Leaves are one of the most conspicuous and important organs of all seed plants. A fundamental source of morphological diversity in leaves is the degree to which the leaf is dissected by lobes and leaflets. We used publicly available segmental introgression lines to describe the quantitative trait loci (QTL) controlling the difference in leaf dissection seen between two tomato species, *Lycopersicon esculentum* and *L. pennellii*. We define eight morphological characteristics that comprise the mature tomato leaf and describe loci that affect each of these characters. We found 30 QTL that contribute one or more of these characters. Of these 30 QTL, 22 primarily affect leaf dissection and 8 primarily affect leaf size. On the basis of which characters are affected, four classes of loci emerge that affect leaf dissection. The majority of the QTL produce phenotypes intermediate to the two parent lines, while 5 QTL result in transgression with drastically increased dissection relative to both parent lines.

T HE leaf is the primary organ of all plants and is predominantly responsible for photosynthesis and gas exchange. The architecture of many leaves is complex with a correspondingly protracted developmental program. The most common and clear example of this is seen in plants with dissected leaves where the blade of the leaf is subdivided (Figure 1B). Despite the great diversity of leaf morphology, all leaves are defined by a flattened dorsiventral transactional symmetry, determinate development, and initiation from the flanks of the shoot apical meristem (Figure 1A).

A number of studies have recently described the genetic control of leaf dissection in several species. Generally, these studies involve mutant analysis, expression studies, and transgenic systems (GOLIBER *et al.* 1999; TSIANTIS and HAY 2003). The rich history of the study of leaf morphology in tomato is reflected by the availability of 307 different mutant lines affecting leaf form and size from the Tomato Genetics Resource Center. A broad study of many of these mutants has allowed researchers to group them on the basis of the nature of their leaf phenotypes (KESSLER *et al.* 2001). Mutant analysis has also shown that these mature leaf phenotypes can result from perturbations of early stages of leaf primordia development (DENGLER 1984; SEKHAR and SAWHNEY 1990). Similarities between phenotypes seen in mutant lines and phenotypes caused by manipulation of plant growth factors have implicated roles for gibberellic acid (SEKHAR and SAWHNEY 1991) and polar auxin transport (SEKHAR and SAWHNEY 1991; AVASAR-

ALA *et al.* 1996) in controlling leaf dissection. Several studies have implicated *knotted*-like homeobox (*KNOX*) genes in the control of leaf dissection in tomato and transgenic Arabidopsis plants (HAREVEN *et al.* 1996; CHEN *et al.* 1997; AVIVI *et al.* 2000; HAY *et al.* 2002). The role of *KNOX* genes in leaf morphology has also been inferred from an expanded domain of expression seen in species with dissected leaves (BHARATHAN *et al.* 2002). However, since *KNOX* genes are required for the development of the shoot system, it has not been possible to test whether these genes are required for leaf dissection through the study of mutant lines.

A systematic approach to the problem, however, has not taken place, leaving us with little information concerning the number of genetic loci involved in leaf dissection in tomato and their relative importance. Quantitative trait loci (QTL) analysis provides an effective way of evaluating complex traits by capitalizing on existing diversity and natural variation. Specifically, QTL analysis provides a measure of the phenotypic differences between two different lines or species and determines the genetic locations of all the loci contributing to these differences. Two recent studies have used QTL analysis to look at leaf shape in other dicots. One study used a set of recombinant inbred lines to discover 21 QTL affecting leaf morphology in *Arabidopsis thaliana* (PEREZ-PEREZ *et al.* 2002). This work highlights the complexity of leaf morphology even in plants with morphologically simple leaves. Another study described 62 QTL that affect leaf morphology in cotton (JIANG *et al.* 2000). Cotton leaves are dissected into a number of lobes, and this study analyzed the variability in lobe size and frequency in detail. Researchers have also used isozymes

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to map QTL affecting some aspects of leaf morphology segregating from an interspecific backcross between *Lycopersicon esculentum* and *L. pennellii* (Tanksley *et al.* 1982).

QTL analysis can describe only those loci that actually differ between the two parental lines, possibly ignoring genes with crucial roles in the process in question. However, this feature is a strength as well as a weakness because it focuses the analysis on loci that are more likely to be important in evolution. Because QTL contribute to phenotypic differences between naturally occurring populations or species, these loci can have particular significance for questions about the process of evolutionary divergence. For this reason QTL analysis has been used to describe the loci crucial to the evolution of modern maize from teosinte (Doebley and Stec 1993; Doebley *et al.* 1997; Westerbergh and Doebley 2002) and many characters of modern tomato (Paterson *et al.* 1990, 1991). Genes responsible for some of these QTL have subsequently been demonstrated to play important roles in the evolution of these species (deVicente and Tanksley 1993; Doebley *et al.* 1997; Frary *et al.* 2000).

This study utilized a previously existing tool for QTL analysis, a population of introgression lines (IL) between *L. esculentum* cultivar M82 and *L. pennellii* (Eshed and Zamir 1994). We capitalized on the great difference in leaf morphology between *L. esculentum* and *L. pennellii* to identify QTL for leaf dissection. The difference in leaf morphology was quantified as differences of eight characters: leaf length, leaf width, primary leaflet count, secondary leaflet count, tertiary leaflet count, intercalary leaflet count, lobing frequency, and lobing acuteness. We evaluated these characters across the IL and revealed a number of QTL affecting each of the characters we measured. Together these QTL are responsible for the divergence in leaf morphology between the parental lines.

MATERIALS AND METHODS

Plant materials: The 50 *L. pennellii* segmental introgression lines and their parental lines *L. pennellii* and *L. esculentum* cv. M82 were obtained from the Tomato Genetics Resource Center. These unique IL were generated and made available to the research community by Y. Eshed and D. Zamir at the Hebrew University of Jerusalem (Eshed and Zamir 1995). These 50 lines were more recently subdivided to include 26 new introgression lines (Qilin *et al.* 2000). These resources provide a total of 76 IL that define 104 bins through uniquely overlapping regions across the genome. Each of these IL is nearly isogenic and homozygous for a small contiguous segment of *L. pennellii* chromosome estimated to span an average of 12.3 cM.

In summary, these lines were generated by first crossing *L. pennellii* to M82 and generating a segregating F_2 generation. To fix the positions of the *L. pennellii* and *L. esculentum* genomic content, the plants were put through six generations of self-fertilization, each time selecting 100 individuals from a population of 1500 on the basis of their morphological similarity to

the M82 parental line. This process favored the selection of lines that have the minimal number of QTL affecting plant morphology per line. For our analysis of morphological traits, this selection maximizes the likelihood that the morphological change seen in any IL is caused by a single QTL. Finally, these individuals were crossed back to *L. esculentum* three times and monitored with 175 restriction fragment length polymorphism markers to limit the *L. pennellii* content in any one line to a single contiguous fraction of one chromosome. A collection of 50 IL was chosen that together cover the entire genome with overlapping introgressed regions of *L. pennellii* DNA. This set was characterized with 350 markers, including the most distal chromosomal markers available so as to represent the entire tomato genetic map at minimal intervals.

To localize the significant loci to smaller bins we analyzed 8 of the 26 recently developed subdivisions of the introgression lines (Koltai and Bird 2000). The subdivision lines were chosen on the basis of which IL appeared significant in the preliminary results from the first planting of the set of 50 ILs. The subdivision lines chosen for study were 1-1-2, 1-1-3, 2-1-1, 4-3-2, 8-2-1, 9-1-2, 9-1-3, and 9-2-5.

Growth conditions: All plants were grown in a greenhouse with 16 hr light and 8 hr dark with temperatures of 25° in the day and 18° at night. Plants were grown two plants per 12-inch pot, and pots were distributed randomly around the room to normalize environmental effects. Plants from each of the 50 IL and the parental lines were grown three times—in winter, spring, and summer. The subdivision lines were grown alongside the final planting of the 50 IL in the summer.

Phenotypic measurements: Leaf five and six were analyzed from each plant, making the number of leaves analyzed twice as high as the number of individuals involved. Leaves were measured after they reached full size and before they showed signs of senescence. Leaf length (LL) was measured from the tip of the terminal leaflet down to the base of the petiole at the site of attachment to the stem. Leaf width (LW) was determined as the sum of the lengths of the two largest leaflets on either side of the rachis. Leaflet types were differentiated on the basis of position within the leaf and were differentiated from lobes by the presence of a petiolule (Figure 1B). Primary leaflets (1°L) were differentiated from intercalary leaflets (InL) by size. Secondary leaflets (2°L) and tertiary leaflets (3°L) were defined by arising from the flanks of primary leaflets and secondary leaflets, respectively. The degree of lobing was determined by eye and assigned a score from 1 to 10 for acuteness (the average depth and sharpness of the lobes) and for frequency (the average spacing of lobes over the whole leaf). Because these two lobing scores were subjective, and sometimes difficult to separate from each other, they were averaged to give a single lobing score for each leaf.

Statistical analysis: All statistical analysis was performed in Microsoft Excel. Statistical significance of the values of each sample as compared to the M82 *L. esculentum* background was determined using the Wilcoxon rank sum test, which tests the hypothesis that two random samples were taken from populations with the same distribution and median. This comparative test was made between M82 as the control group and each of the introgression lines. Resulting *P*-values of <0.05 were considered significant. Student's *t*-test was also applied with similar results, but with more IL shown to be significant (supplemental S1, available at http://www.genetics.org/supplemental/). Because we do not know that the values have normal distribution, the Wilcoxon test is preferred over the Student's *t*-test.

Covariance is described using the Pearson calculation and is reported as the Pearson product (r^2). The Pearson's correlation coefficient (r) summarizes the linear relationship between two variables having ranked categories and has a value of 1.0 if there is perfect correlation between the two variables being analyzed.

Measurements of leaf five and six from all the plants of all three plantings were combined. All calculations, including standard deviation, arithmetic mean, and percentage of variation were based on the full data set.

RESULTS

Leaf development in tomato

The development of a tomato leaf from initiation to full size takes place over several weeks. This process can be broken into three phases: initiation, organogenesis, and histogenesis. The initial phase includes the initiation and establishment of the leaf primordium from the peripheral zone of the shoot apical meristem (Figure 1A). The organogenic phase occurs when the leaf primordium initiates all the leaflets, lobes, and serrations seen in the mature leaf. The histogenic phase encompasses the time during which the majority of growth and differentiation of tissues occur. By considering the estimated leaf initiation rate of one leaf every 2 days and counting the number of leaves that initiate between developmental phases, we can approximate the duration of these phases in *L. esculentum*. The first leaflets initiate on the third youngest leaf present on the meristem, plastochron three (P_3), indicating that the initial phase lasts ∼6 days. The initiation of new structures continues through P_8, indicating that the organogenic phase lasts ∼10 days. The characters we analyze in this study (excluding leaf size) initiate during the organogenic phase. The duration for these phases is not known for *L. pennellii*, but all the structures initiated late in the organogenic phase of *L. esculentum* (secondary, tertiary, and intercalary leaflets) are missing. Thus we expect that the organogenic phase of *L. pennellii* leaves is shortened relative to *L. esculentum*.

L. esculentum **leaf morphology:** *L. esculentum* leaves have two or three orders of dissection, depending on the genetic background. The *L. esculentum* background used in this study is cultivar M82, which displays three orders of dissection in some but not all leaves (Figure 2A). Each leaflet type can be determined on the basis of its position on the leaf and is differentiated from lobes by having a petiolule, the petiole of the leaflet (Figure 1B). Primary leaflets initiate first and arise on the flanks of the developing leaf primordium (Figure 1A). In the mature leaf they are attached to the rachis via a petiolule. Intercalary leaflets are initiated on the flanks of the leaf primordium between the previously established primary leaflets and are attached directly to the rachis in the mature leaf where they are flanked by the larger primary leaflets. Secondary leaflets are initiated on the flanks of primary leaflets, and tertiary leaflets are initiated on the flanks of the secondary leaflets.

L. pennellii **leaf morphology:** All aspects of leaf dissection are reduced in *L. pennellii* relative to M82, with decreased lobing, reduced number of primary leaflets,
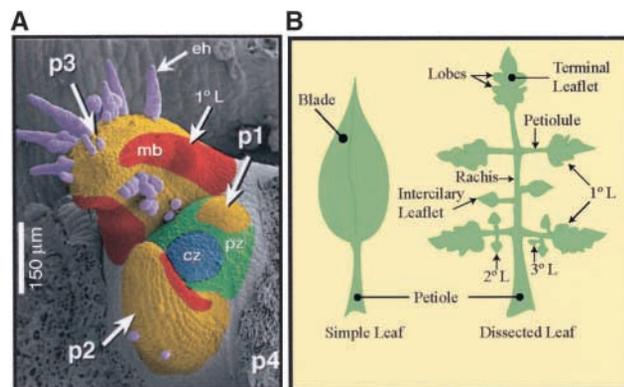

FIGURE 1.—Leaf initiation and adult leaf structure. (A) Scanning electron micrograph of a wild-type tomato shoot apex, showing the leaves arising from the peripheral zone of the shoot apical meristem. The peripheral zone surrounds the central zone, which functions as a reservoir of dividing cells, allowing the meristem to be indeterminate. The leaf primordia are labeled from youngest to oldest as p1–p3. The emergence of the first primary leaflets can be seen along the marginal blastozone of the p3 leaf. cz, central zone; eh, epidermal hair; mb, marginal blastozone; pz, peripheral zone. (B) The parts of a dissected leaf.

and a complete absence of secondary, tertiary, and intercalary leaflets (Figure 2F). M82 leaves always have a number of secondary, and occasionally a few tertiary, leaflets; *L. pennellii*, on the other hand, has not been observed to ever bear secondary or tertiary leaflets. The leaves of *L. pennellii* are also much more densely covered in epidermal hairs than those of M82, and these hairs are much longer. This trait was deemed to be unrelated to leaf dissection and was not included in this study. In both species, a heteroblastic leaf series culminates in mature leaf morphology in the fifth or sixth postembryonic leaf.

Phenotypic measurements

Leaf width and leaf length: Leaves of M82 are significantly larger than those of *L. pennellii* at 40 × 45 cm and 14 × 11 cm, respectively. Nine IL are significantly reduced in LL compared to M82, 14 are reduced in LW, and 7 IL are reduced in both length and width (Figure 3).

A slight to low degree of covariance was found between leaf size (width and length) and all leaf dissection measurements (see below). Secondary leaflets and tertiary leaflets have the greatest level of correlation with leaf size. A Pearson test for covariance over the whole set of introgression lines gave $r^2 \cong 0.1$ for both these characters when compared to leaf length and width. This number implies that ∼10% of the variance was shared. Finding such a low degree of covariance indicates that the variance in leaf dissection is predominantly independent of variance in leaf size.

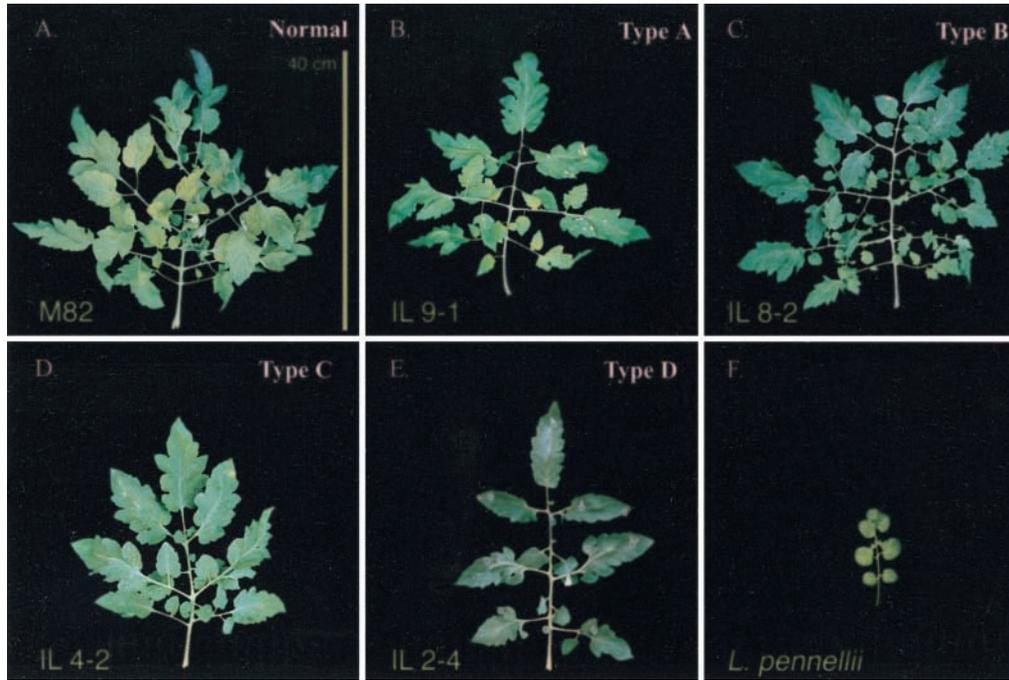**Primary leaflets:** The first structures to be initiated are

FIGURE 2.—Leaf phenotypes representative of the parental lines and the four types of QTL. The sixth postembryonic mature leaf representing the parental types *L. esculentum cv.* M82 (A) and *L. pennellii* (F) as well as the four different types of QTL affecting leaf dissection (B–E) are shown here at equal magnification. Type A affects primary and intercalary leaflets, type B affects secondary and tertiary leaflets (increase shown here), type C affects lobing, and type D affects all aspects of leaf dissection.

the primary leaflets (1°L). A marked difference between M82 and *L. pennellii* exists for this character, with mean values of 7.38 and 4.25, respectively (Figure 3). This range of mean values is quite small but the variance in the raw measurements is also low, averaging only 1.0 across the introgression lines. Therefore it was easy to distinguish IL with statistically significant reduction in this character. Six introgression lines show statistically significant reduction compared with M82 (Figure 3). The most severe reduction in this character is seen in IL 9-1 (and similarly in the sublines that overlap IL 9-1) and produce only 5.7 primary leaflets on average (Figure 3). In total there are a minimum of four QTL for this trait (Figure 4). All four of these QTL also show reduction in the number of IL. No lines are significantly increased in the number of primary leaflets relative to M82.

Intercalary leaflets: *L. pennellii* leaves do not develop InL, 2°L, or 3°L. M82 has an average of 10, 14, and 0.75 of each of these leaflets, respectively. Eighteen lines are significantly reduced in the number of intercalary leaflets (Figure 3), corresponding to a minimum of 14 QTL (Figure 4A). IL 2-6 is increased relative to both parental lines with an average of 13.9 intercalary leaflets, which corresponds to 139% of that seen in M82.

Four of the QTL that affect intercalary leaflets also showed reduced numbers of primary leaflets (Figure 4A). Because intercalary leaflets occur, by definition, between primary leaflets, we considered the possibility that reduction in the number of intercalary leaflets is a consequence of the reduced number of intercalary regions found in a leaf with fewer primary leaflets. We tested this possibility by determining if the ratio of InL/1°L also showed a significant decrease. All the lines with any significant reduction of intercalary leaflets in fact did show a reduced InL/1°L ratio (data not shown),

demonstrating that intercalary leaflets are directly affected by these QTL and not due to reduced intercalary domains in the leaf.

Secondary and tertiary leaflets: Three lines representing a minimum of two QTL have reduced numbers of secondary leaflets (Figure 3), both of which also have reduced numbers of intercalary leaflets. All IL with reduced numbers of secondary leaflets also have a reduced ratio of secondary to primary leaflets; thus we conclude that the reduction is not simply a consequence of a reduced availability of primary leaflets from which to initiate secondary leaflets. Five lines show an increase in tertiary leaflets and in some cases in secondary leaflets.

Total leaflet count: A total leaflet count (1°L + 2°L + 3°L + InL) was calculated and analyzed to discover any lines that are not significantly altered for a single parameter but overall show a significant change. No new QTL were discovered in this analysis, but two QTL previously identified in this study did show greater significance. The QTL found on IL 4-3 and IL 4-4 causes significant reduction of intercalary and secondary leaflets. Because this QTL affects more than one leaflet count, it shows twofold greater significance in the reduction of total leaflets compared to any of the individual characters (Figure 3).

More surprising is the QTL on IL 2-1, which causes a mild reduction in intercalary leaflets and is not significantly altered in any other leaflet counts. In this line, the significance of the total leaflet count ($P = 0.004$) is five times greater than that indicated by intercalary leaflets alone ($P = 0.021$), indicating that this QTL regulates more than just the intercalary leaflet development, but that the effect on the other characters individually is below our significance threshold.

Lobing: We assigned the lobing score averages for

Parameter	Line	No. of Leaves	p-value	Average	Median	Stdev.	%	Parameter	Line	No. of Leaves	p-value	Average	Median	Stdev.	%
LL	M82	8	1.000	39.9	39.3	9.5	100%	2°L	M82	8	1.000	8.0	14.0	15.5	100%
	IL1-1-3	30	**0.002**	30.6	30.8	4.9	77%		IL2-4	24	0.028	6.6	5.0	5.6	83%
	IL3-3	12	0.011	32.0	28.5	3.7	80%		IL3-1	23	0.019	22.0	22.0	5.2	276%
	IL3-4	12	**0.003**	28.0	26.3	5.3	70%		IL4-3	24	0.011	5.2	5.0	1.8	65%
	IL5-2	4	0.034	27.5	27.5	6.5	69%		IL4-4	10	0.029	5.1	5.0	1.9	64%
	IL5-3	8	0.046	32.5	33.8	4.9	81%		IL8-2	23	**1.0E-04**	33.3	34.0	6.9	416%
	IL7-3	12	0.037	32.8	31.8	5.1	82%		IL9-2	12	0.014	24.3	23.5	6.2	303%
	IL9-1-3	10	0.026	31.9	30.0	5.9	80%		*pennellii*	12	**2.5E-04**	0.0	0.0	0.0	0%
	IL9-2-5	31	**1.1E-04**	27.6	27.5	2.9	69%	2°L/1°L	M82	8	1.000	1.8	1.9	1.0	100%
	IL11-3	11	0.048	31.9	31.0	6.8	80%		IL2-4	24	0.031	1.0	0.8	0.7	53%
	pennellii	12	**2.5E-04**	13.9	13.8	1.5	31%		IL3-1	23	**0.009**	2.8	2.8	0.5	155%
LW	M82	8	1.000	44.6	44.3	13.7	100%		IL4-3	24	**0.004**	0.7	0.8	0.2	37%
	IL1-1-3	30	0.045	31.5	34.5	7.3	71%		IL4-4	10	0.033	0.8	0.8	0.3	44%
	IL2-5	12	0.023	32.0	29.5	8.7	72%		IL8-2	23	**1.0E-04**	4.2	4.3	0.9	228%
	IL3-3	12	0.049	30.7	31.0	5.4	69%		IL9-2	12	**0.006**	3.1	2.9	0.7	171%
	IL3-4	12	0.011	27.5	26.3	9.6	62%		*pennellii*	12	**0.00**	0.0	0.0	0.0	0%
	IL4-1	12	0.045	32.2	28.5	9.9	72%	3°L	M82	8	1.000	0.8	0.0	1.2	100%
	IL4-2	12	0.045	31.5	29.5	11.0	71%		IL3-1	23	**0.001**	4.7	4.0	3.8	626%
	IL5-2	4	0.034	24.6	23.3	6.9	55%		IL8-2	23	**1.0E-04**	11.2	10.0	6.3	1496%
	IL5-3	7	0.037	28.6	31.0	7.6	64%		IL8-3	13	0.033	5.0	4.0	4.5	667%
	IL6-1	12	0.017	31.3	28.8	10.5	70%		IL9-2	12	0.049	5.6	5.0	5.3	744%
	IL6-3	13	0.014	28.8	20.0	14.5	64%		IL9-3	8	0.031	2.6	3.0	1.6	350%
	IL7-2	12	0.049	31.6	29.5	9.9	71%		*pennellii*	12	0.247	0.0	0.0	0.0	0%
	IL7-4	12	0.037	36.3	34.5	8.0	81%	Lobing	M82	8	1.000	6.9	6.8	0.9	100%
	IL9-1-3	10	0.046	34.3	31.0	8.8	77%		IL2-4	24	**1.5E-04**	3.9	3.5	1.2	57%
	IL9-2-5	31	**0.002**	28.5	27.5	5.4	64%		IL3-3	12	**0.006**	5.5	5.0	0.8	81%
	pennellii	12	**2.5E-04**	10.7	9.5	3.1	24%		IL3-4	11	**0.005**	5.7	5.5	0.4	83%
1°L	M82	8	1.000	7.4	8.0	0.9	100%		IL4-3	24	**3.2E-05**	3.4	3.5	0.9	50%
	IL5-3	8	0.036	6.0	6.0	1.1	77%		IL4-4	10	**4.5E-04**	4.7	4.5	0.6	68%
	IL6-2	10	0.029	6.2	6.0	0.8	84%		IL7-2	12	**0.005**	5.3	5.3	1.0	77%
	IL9-1	22	**0.003**	5.7	6.0	1.2	77%		IL7-4	12	**0.005**	5.3	5.5	1.0	77%
	IL9-1-2	22	**0.005**	6.1	6.0	0.6	83%		IL11-1	12	**0.005**	5.5	5.5	0.7	81%
	IL9-1-3	10	0.026	6.2	6.0	0.6	84%		IL11-2	12	**0.005**	5.5	5.5	0.8	81%
	IL9-2-5	31	0.037	6.5	6.0	0.9	88%		*pennellii*	12	**2.5E-04**	1.0	1.0	0.0	15%
	pennellii	12	**2.5E-04**	4.3	4.0	0.5	58%	Total L	M82	8	1.000	35.6	40.0	11.6	100%
InL	M82	8	1.000	10.0	9.5	3.9	100%		IL2-1	20	**0.004**	19.9	20.0	10.4	56%
	IL2-1	20	0.021	5.7	5.5	3.6	57%		IL2-4	24	**0.003**	20.3	18.0	10.3	57%
	IL2-4	24	**0.009**	5.8	5.5	3.4	58%		IL4-3	24	**3.0E-04**	19.2	19.0	2.3	54%
	IL2-6	12	0.041	13.9	13.0	3.5	139%		IL4-4	10	0.016	20.6	20.5	6.1	58%
	IL3-5	12	0.031	6.4	6.0	1.8	64%		IL5-3	8	0.012	20.8	17.5	8.2	58%
	IL4-3	24	**0.002**	5.4	6.0	1.4	54%		IL6-2	10	**0.007**	19.5	18.5	5.9	55%
	IL4-4	10	0.037	6.5	6.5	1.6	65%		IL6-5	16	0.030	25.4	26.0	10.8	71%
	IL5-3	8	**0.007**	4.5	4.0	1.9	45%		IL7-2	12	0.045	24.1	23.5	7.9	68%
	IL6-2	10	**0.001**	3.5	3.0	1.1	35%		IL8-2	24	**0.001**	59.1	61.5	17.7	166%
	IL6-3	13	0.025	6.3	6.3	2.3	63%		IL9-1	22	**0.002**	19.4	18.5	6.8	54%
	IL7-2	12	0.034	6.4	6.5	2.1	64%		IL9-1-2	22	**0.009**	22.5	21.0	5.7	63%
	IL8-1	12	0.031	6.6	6.0	1.6	66%		IL9-1-3	10	0.029	22.5	22.5	5.5	63%
	IL9-1	22	**0.002**	4.9	5.0	2.7	49%		IL9-2-5	31	0.023	24.3	25.0	8.6	68%
	IL9-1-2	22	**0.003**	5.6	5.0	2.4	56%		IL9-2	12	0.021	50.3	49.0	11.1	141%
	IL9-1-3	10	**0.001**	3.6	4.0	0.8	36%		*pennellii*	12	**1.2E-04**	4.3	4.0	0.5	12%
	IL9-2-5	31	**0.002**	5.8	6.0	2.3	58%								
	IL12-2	9	**0.005**	4.8	5.0	1.6	48%								
	IL12-3	12	0.014	5.8	5.0	1.5	58%								
	IL12-4	12	0.023	5.8	5.0	2.4	58%								
	pennellii	12	**2.5E-04**	0.0	0.0	0.0	0%								

Figure 3.—ILs with divergent leaf phenotypes. All ILs with statistically significant divergence from M82 are listed for each trait that was analyzed. The number of leaves measured, *P*-values, average, median, standard deviations, and percentage relative to M82 are shown. Highly significant lines with $P < 0.01$ are in boldface type.

lobing frequency and lobing acuteness by eye. Because of the subjective nature of these scores, only values of $P < 0.01$ were considered significant for this parameter. The mild serration seen in *L. pennellii* was given a lobing score of 1 and is invariant. The most extremely lobed individuals were given a score of 10. Within M82 the average score is 6.9 with a standard deviation of 0.9. Nine lines reflecting a minimum of six QTL have marked reductions in lobing (Figures 3 and 4). Two QTL, one described by IL 2-4 and the other by the intersection

	IL	BIN #	QTL	LL	LW	1°L	InL	2°L	3°L	Lobing	Type
A.	2-1	2-B	A2.1				<				A
	2-6	2-I	A2.2				>				
	3-5	3-I	A3				<				
	5-3	5-E	A5			<	<<				
	6-2	6-CD	A6.1			<	<<<				
	6-3	6-EF	A6.2				<				
	7-2	7-G	A7		<		<			<<<	
	8-1	8-ABC	A8				<				
	9-1, 9-1-2, 9-1-3	9-AB	A9.1			<<<	<<<				
	9-1, 9-1-3, 9-2-5	9-D	A9.2	<<<<	<<<	<	<<<				
	12-2, 12-3	12-DE	A12.1				<				
	12-3, 12-4	12-G	A12.2				<				
B.	3-1	3-A	B3					>	>>>		B
	8-2	8-F	B8.1					>>>>	>>>>		
	8-3	8-GH	B8.2					<	<		
	9-2	9-FG	B9.1					>	>		
	9-3	9-GHIJK	B9.2						>		
C.	3-3, 3-4	3-F	C3	<<<	<<<					<<<	C
	7-4	7-AD	C7		<					<<<	
	11-1, 11-2	11-B	C11							<<<	
D.	2-4	2-G	D2				<<	<		<<<<	D
	4-3, 4-4	4-H	D4				<<<	<		<<<<	
E.	1-1-3	1-B	S1	<<	<						S
	2-5	2-H	S2		<						(size)
	4-1, 4-2	4-C	S4		<						
	5-2, 5-3	5-D	S5	<	<						
	6-1	6-A	S6.1		<						
	6-3	6-G	S6.2		<						
	7-3	7-E	S7	<							
	11-3	11-EF	S11.2	<							

FIGURE 4.—All QTL grouped by type. QTL are assigned to bins and grouped on the basis of which traits are affected. As indicated in the last column, four types of QTL affect leaf dissection (A–D) and one type affects only leaf size (S). The number and orientation of the triangles indicate the nature of the change. < indicates a decrease relative to M82; > indicates an increase. The significance of the change is indicated as follows: <, $0.05 > P \geq 0.01$; <<, $0.01 > P \geq 0.005$; <<<, $0.005 > P \geq 0.001$; <<<<, $0.001 > P$.

of IL 4-3 and IL 4-4, show an especially strong reduction in this character. These three lines were also reduced in InL and 2°L leaflets.

Analysis of QTL

Heteroblasty: Like most species with dissected leaves, tomato progresses through a heteroblastic series of leaf morphologies as the plant matures. The first leaves are less dissected and become progressively more dissected until leaf five or six when a mature leaf morphology is obtained. To discover QTL that might be involved in controlling this process, we initially included all seven of the first leaves in our analysis. These seven leaves encompass the heteroblastic series seen in both *L. esculentum* and *L. pennellii*. Preliminary analysis of the data collected from the first planting showed that there were no IL with a significant change in the heteroblastic series (data not shown). IL showing significant change of any character in the juvenile leaves were also seen by looking at leaf five and six alone. Therefore, measurements of juvenile leaves were not collected for the remainder of the study.

Number and location of QTL in overlapping ILs: To more precisely map the QTL, we utilized the overlapping portions of the IL to delineate smaller bin sizes (Figure 5). In other words, the unique portions of an introgression segment can be differentiated from the portion(s) that overlap the introgression segment in another IL. In doing this we make the two following

assumptions: First, a QTL can be confined to a region of overlap between two lines if the relevant traits in both lines are significantly different from M82 and if they are not significantly different from each other. Second, a QTL can be excluded from a region if it overlaps a line that is not significantly different from M82 for the traits in question.

For most QTL it was a simple process to use these rules to determine the bin location of the QTL. For example, IL 2-1 has a reduced number of intercalary leaflets (Figure 3). IL 2-1-1 does not differ from M82 and neither does IL 2-2; therefore we could place QTL *A2.1* in the remaining portion of IL 2-1, which defines bin 2-B (Figure 5). Of the 22 QTL affecting leaf dissection plus the 8 QTL affecting only leaf size, 21 QTL were easily placed to bin locations using these rules.

In 10 cases, the phenotypes of the overlapping IL do not resolve easily to a discrete placement of a QTL to a single bin. By postulating the existence of multiple QTL within a single IL we are able to explain the observed phenotypes. A striking example of this is seen on chromosome 2. IL 2-4 has a marked reduction in all aspects of leaf dissection, and it is completely overlapped on one end by IL 2-3 and on the other end by IL 2-5 (Figure 5). Because neither IL 2-3 nor IL 2-5 shows a reduced dissection phenotype, we suggest the presence of an antagonistic QTL on one of these two IL that prevents the manifestation of reduced leaf dissection. A candidate for such a QTL is revealed by IL 2-6, which shares a large overlap with IL 2-5 and causes a mild

FIGURE 5.—Chromosomal location of introgression lines, bins, and QTL. ILs with significant ($P \le 0.05$) changes in leaf dissection are indicated as reduced or increased by shading. QTL are named on the basis of type (Figure 6) and chromosome location. A number is appended if necessary to make each name unique. *, IL 2-5 was shown to be normal, but, as is explained in the text, it is not used to exclude QTL from bins corresponding to its *L. pennellii* content. **, QTL C7 is shown to reside at either end of IL 7-4, but is excluded from the center by the overlap of IL 7-5, which displays a normal phenotype.

increase in dissection. Therefore, we suggest that QTL *A2.2* is located in bin 2-I, that QTL *D2* is localized to bin 2-G (Figure 5), and that these two opposing QTL prevent the manifestation of altered leaf dissection in IL 2-5.

On chromosome 6, IL 6-2 and IL 6-3 both show a reduction in leaflets on the rachis, but in this case there is too much difference in the degree of reduction to conclude that both lines share the same QTL. This means that QTL *A6.1* must be in bin 6-C or 6-D and that QTL *A6.2* can be unique to IL 6-3 in bin 6-F or overlap with IL 6-2 in bin 6-E.

Another case that requires individual explanation is seen on chromosome 8. Here two overlapping lines, IL 8-2 and IL 8-3, are both increased in tertiary, but IL 8-2 is increased significantly more and also shows an increase in secondary leaflets. Because IL 8-2-1 is not increased for these characters, it suggests that QTL on IL 8-2 are shared with IL 8-3. These observations suggest another QTL on IL 8-3, which reduces the dissection in this line.

The final case requiring individual discussion here concerns the QTL on chromosome 9. Here IL 9-1 overlaps at least partially with four other IL on this chromosome. IL 9-1, IL 9-1-2, IL 9-1-3, and IL 9-2-5 all have reduced numbers of primary and intercalary leaflets. Since IL 9-1-2 and IL 9-2-5 are subdivisions of IL 9-1, but do not overlap each other, it is clear that there are two QTL here: QTL *A9.1* is in bin 9-A or -B and QTL *A9.2* is in bin 9-D. Because the leaves in IL 9-1-3 and IL 9-2-5 are reduced in size, we conclude that *A9.2* also affects leaf length and width; however, this reduced leaf size is not seen in IL 9-1 as would be expected. The explanation for this finding could be that another QTL residing within IL 9-1 but outside of bin 9-D is epistatic to the effect of *A9.2* in this regard. Distal to this position on chromosome 9, both IL 9-2 and IL 9-3 are increased in the number of tertiary leaflets although IL 9-2 is significantly more severe. Furthermore, IL 9-2 shows an increase in secondary leaflets whereas IL 9-3 does not, demonstrating the existence of two separate QTL, *B9.1* and *B9.2*.

Complementation between a QTL and a mutant locus affecting leaf dissection: Many mutant loci that affect leaf dissection in tomato have been described (Goliber *et al.* 1999). Consequently, correlations can be found between map positions of the QTL described here and known mutants. An example of such a case is seen in IL 4-3 and IL 4-4, which are dramatically reduced in secondary leaflets and lobing, and span the locus of the *entire* (*e*) mutation. *entire* is severely reduced in all aspects of leaf dissection (Dengler 1984; Goliber *et al.* 1999). No other known mutants affecting leaf dissection map to this region. We crossed *entire* to IL 4-3 and IL 4-4 as well as to *L. pennellii*, and the F$_1$ progeny were measured and analyzed. Comparisons were also made of the F$_1$ hybrid between *L. pennellii* and *Ailsa Craig*, the isogenic background of the *e* allele (data not shown).

The analysis showed that the F$_1$ progeny of crosses between *e* and *L. pennellii*, as well as crosses between *e* and the introgression lines, were not reduced in any of the parameters compared to that of a cross between *L. pennellii* and *Ailsa Craig*. The fact that the F$_1$ hybrid between *e* and *L. pennellii* does fully complement the *e* phenotype suggests that this locus is not involved in the difference in leaf dissection seen between *L. esculentum* and *L. pennellii*.

DISCUSSION

In this study we set out to discover the loci that contribute to the differences in leaf dissection between *L. esculentum* and *L. pennellii*. Of 50 IL, 32 showed a change in at least one of the eight characters we measured. Twenty-four lines showed phenotypes that were intermediate relative to the parental lines, whereas 6 lines displayed increased dissection relative to both parents. We describe below how these partially overlapping IL harbor a minimum of 22 QTL affecting leaf dissection and 8 QTL affecting leaf size.

These IL are distributed across 11 of the 12 chromosomes, with only chromosome 10 containing no QTL affecting leaf size or dissection. The number of QTL identified from these results might underestimate the real number of loci regulating these characters in two ways. Multiple loci contributing similarly to a parameter on a single IL or on two overlapping IL would be diagnosed as a single QTL. Alternatively, two linked QTL with opposite effects could mask each other, resulting in one or both QTL being missed in the analysis. Examples of such events were discovered in this study when subdivision lines were analyzed. A more precise estimate of the number of relevant QTL will be achieved when these QTL are mapped more finely. With this in mind, the following estimates were made concerning the number of QTL controlling each trait. At least 22 QTL control leaf dissection, and a minimum of 8 QTL control the difference in leaf size between *L. esculentum* and *L. pennellii*. Of the QTL that contribute to dissection, 17 cause a reduction in dissection and 5 cause an increase beyond that seen in M82. Three sites contribute to lobing but no other aspects of dissection, leaving 19 QTL that change the number of leaflets. Of these, QTL *D2* and *D4* cause a reduction in the broadest number of characters and are found in bin 2-F or 2-G and 4-H, respectively.

Interestingly, the *KNOX* gene *LeT6*, which has been suggested to play a role in leaf dissection (Janssen *et al.* 1998), maps to bin 2-G. Rearrangements of *LeT6* increase leaf dissection and cause the gene to be ectopically expressed (Chen *et al.* 1997). No other mutants affecting leaf dissection map to this region. It is possible that QTL *D2* corresponds to *LeT6*, supporting a role for *LeT6* in controlling the level of leaf dissection. Further analysis of its sequence and regulation in *L. pennellii* and IL 2-4 will make it possible to determine with cer-

tainty what role *LeT6* plays in the dissection identified by our QTL studies.

The low mean value of tertiary leaflets in M82 precludes a determination of statistical difference between M82 and *L. pennellii* in this data set (Figure 3). However, on the basis of our observations that *L. pennellii* never display tertiary leaflets, we expect that with a larger sample size we would see a statistically significant difference. Although this study is not sensitive enough to discover QTL causing a decrease in tertiary leaflets, there are four QTL that cause an increase in the number of secondary leaflets and tertiary leaflets (Figure 4B). The numbers of secondary and tertiary leaflets in these IL are increased relative to both of the parental lines. This phenomenon is known as transgression and is often observed in the progeny of interspecies crosses (RICK and HARRISON 1959; DEVICENTE and TANKSLEY 1993). Only QTL *B9.2* caused a change in tertiary leaflet number without also causing a similar change in secondary leaflet number, indicating that these characters are usually coregulated.

As was described above, only a slight covariance was seen between leaf size and dissection when the population was viewed as a whole. If we analyze the correlation between size and dissection within the QTL described in this study we find that 4 QTL (10%) affect both size and dissection, 8 affect size only and 18 affect dissection only. This demonstrates again that leaf dissection is primarily, but not absolutely, independent of leaf size.

Classification of QTL types: To differentiate the functions of the QTL we used the characters affected by each QTL to assign them to one of five classes (Figure 4). Type A QTL affect only the leaflets that initiate directly from the leaf primordium, namely the primary leaflets and intercalary leaflets (Figures 2B and 6A). There are 12 QTL of this type. Type B QTL affect only the leaflets that initiate from other leaflets, namely the secondary leaflets and tertiary leaflets (Figures 2C and 6B). There are 5 QTL of this type. Type C QTL show no effect on the number of leaflets but affect the degree of lobing (Figures 2D and 6C). Two QTL define type D and affect leaflets along the rachis as well as secondary leaflets and lobing (Figures 2E and 6D). Type S QTL affect only size.

This classification assumes that QTL affecting multiple characters are in fact single pleiotropic QTL and not a cluster of multiple linked loci affecting different characters. The distinction of these classes is also seen in mutations that affect leaf dissection in tomato, which in many cases are pleiotropic for the characters analyzed here. Type B QTL are similar to such mutants as *solanifolia* (*sf*), *tripinate* (*tp*), and *clausa* (*clau*), which affect the number of secondary and tertiary leaflets without a change in the number of primary leaflets (SEKHAR and SAWHNEY 1991; GOLIBER *et al.* 1999). Other mutants such as *e*, *potato leaf* (*c*), and *Lanceolate* (*La*) affect all aspects of leaf dissection and are similar to type D QTL (DENGLER 1984; GOLIBER *et al.* 1999). Type A and type
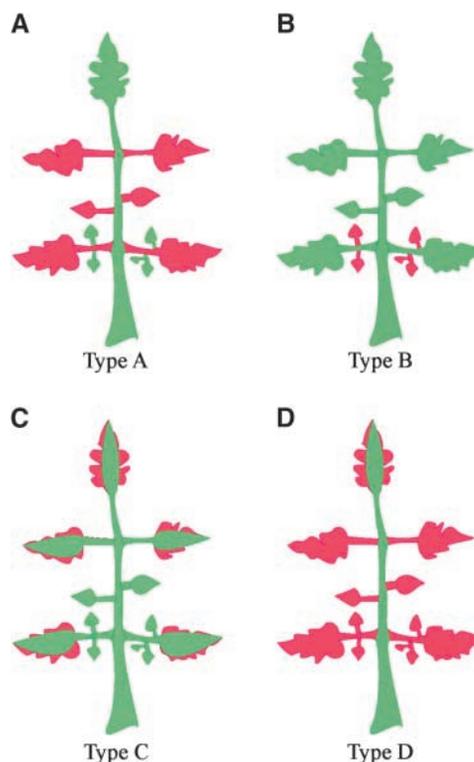

FIGURE 6.—The four types of QTL involved in leaf dissection. Type A affects 1°L and InL (A), type B affects 2°L and 3°L (B), type C affects lobing (C), and type D affects all aspects of leaf dissection (D).

C QTL are not like any known tomato mutants and the discovery of these QTL is indicative of this study's sensitivity.

Future studies: All the traits analyzed in this study are regulated by multiple QTL. Any analysis of leaf dissection can use the number of relevant QTL discovered in this study as an indicator for the complexity of this developmental process. These results and the great number of mutations that perturb leaf dissection in tomato should serve to temper the assessment of control attributed to any single gene.

As sequencing strategies rapidly improve, the full genomic sequence of tomato will soon be determined. With these tools, the QTL described here will facilitate rapid discovery of developmentally and evolutionarily important genes controlling morphological diversity.

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