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ORIGINAL PAPER

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Evaluating the genetic basis of multiple-locule fruit in a broad cross section of tomato cultivars

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Abstract *Lycopersicon esculentum* accessions bearing fasciated (multiloculed) fruit were characterized based on their flower organ and locule number phenotypes. Greenhouse and field evaluations indicate that increases in locule number are associated with increases in the number of other floral organs (e.g., sepals, petals, stamens) in all stocks. F_1 complementation, F_2 segregation analysis, and genetic mapping indicate that at least four loci account for increases in the number of carpels/locules in these stocks. The most significant of these map to the bottoms of chromosomes 2 and 11 and correspond to the *locule number* and *fasciated* loci. All stocks tested were fixed for mutations at the *fasciated* locus, which maps to the 0.5-cM interval between the markers T302 and cLET24J2A and occurs in at least three allelic forms (wild type and two mutants). One of the *fasciated* mutant alleles is associated with nonfused carpels and repressed recombination and may be due to a small inversion or deletion. The other two loci controlling locule number correspond to the *lcn1.1* and *lcn2.2* loci located on chromosomes 1 and 2, respectively.

Introduction

Tomatoes (and most other fruit-bearing crop species) underwent a major increase in fruit size during domestication. The transition in tomato was especially dramatic, and now some cultivated tomatoes produce fruit a thousand times larger than their wild counterparts (Lippman and Tanksley 2001). Genetic research conducted by means of classical genetic approaches showed that these differences in fruit size are largely quantitatively inherited (Powers 1941; Fogle and Currence 1950; Ibarbia and Lambeth 1969). More recent quantitative trait mapping studies have verified this notion and resulted in the identification of 28 QTLs governing fruit size, with the majority of the variation being attributable to a subset of six major QTLs (Grandillo et al. 1999).

One manner in which tomato fruit size increased during domestication was through mutations in genes controlling cell division. The most dramatic change in cell division is attributed to the *fw2.2* QTL. The gene underlying *fw2.2* was recently cloned and encodes a negative regulator of cell division especially active in cortical tissue (Frary et al. 2000; Nesbitt and Tanksley 2001; Cong et al. 2002; Liu et al. 2003). *fw2.2* exerts its effects in fruit size without major changes of the overall structural organization or shape of fruit. All modern and heirloom tomatoes tested thus far contain the large-fruited allele for *fw2.2* and, based on molecular evolutionary studies, the large-fruited allele originated in wild tomatoes well before domestication (Nesbitt and Tanksley 2002).

Another way in which fruit size can be modulated is through changes in the number of carpels/locules that comprise a fruit. Most wild tomatoes produce bilocular fruit, whereas large-fruited cultivated tomatoes can bear fruit containing ten or more locules (MacArthur 1926; Zielinski 1945; Zielinski 1948; Young and MacArthur 1947; Powers et al. 1950; Lippman and Tanksley 2001). Loci modulating locule number not only affect fruit size, but also seed number and fruit shape (Yeager 1937; MacArthur and Butler 1938; Lippman and Tanksley 2001; van der Knaap and Tanksley 2003).

Several locule number-determining loci have been reported in tomato, the most prominent being *fasciated* (also *f* or *lcn11.1*), which maps at the end of the long arm of chromosome 11 (MacArthur 1928, 1934; Young and MacArthur 1947; Butler 1952; Lippman and Tanksley

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2001). A second locus on chromosome 2, termed *locule number* (also *lc* or *lcn2.1*), is also a major contributor to locule number, especially in tomato varieties that produce exceptionally larger fruit (Yeager 1937; Lippman and Tanksley 2001; van der Knaap and Tanksley 2003). Other locule number-controlling genes with more minor effects have also been proposed (Powers et al. 1950; Dennet and Larson 1953; Rick and Butler 1956; Ahuja 1968; Chaudhary and Khanna 1972; van der Knaap and Tanksley 2003).

While mutant alleles at *fasciated* and *locule number* have been shown to cause increased locule number (and hence, increased fruit size) in selected large-fruited cultivars, it is currently unknown whether all large-fruited, multiloculed varieties can attribute their multilocularity to one or both of these genes or whether additional loci are involved. In an attempt to shed light on this question, we have assembled a collection of large-fruited, multiple-locular accessions and subjected them to phenotypic evaluation, complementation analysis and genetic mapping experiments.

Materials and methods

Phenotypic characterization of *Lycopersicon esculentum* multilocular accessions

Seventeen diverse *L. esculentum* cultivars, reported to bear large fasciated (many-loculed) fruit, were assembled in 1999 from the Tomato Genetics Resource Center at the University of California–Davis (http://tgrc.ucdavis.edu; Table 1). Three plants from each accession were grown in the greenhouse in 1999 and field in 2000 for phenotypic evaluations in Ithaca, New York, USA. Additionally, two plants from each accession were also grown in the field in 1999. For all experiments the plants were arranged in a completely randomized design. A minimum of five mature flowers (anthesis) and/or five mature fruit per plant was analyzed for average floral-organ and locule number. Sepals, petals, and stamens were separated from each flower and counted. Carpels and fruit were dissected transversally and digitally imaged using a Zeiss dissecting microscope and SCION software (Scion, Frederik, Md., USA) and a computer scanner and VISTASCAN software (UMAX Technologies, Dallas, Tex., USA) for carpels and fruit, respectively.

Phenotypic data from the greenhouse and field of 1999 and 2000 were subjected to *t*-tests to determine whether the average locule number (ALN) per accession was significantly >3 ($P<0.05$), using MINITAB software. Only accessions with an ALN >3 were subjected to further evaluation (see next section; Table 1). Correlations between floral organ number and locule number traits per accession were calculated between traits measured in the greenhouse (1999) and field (1999 and 2000) using MINITAB software. Principal component analysis-multidimensional scaling (PCA-MDS) was used with the same data to further assess correlations between traits using the statistical program SAS.

Table 1 *Lycopersicon esculentum* accessions analyzed for floral organ and locule number traits. *(SD)* (Standard deviation)

Accession[a]	Other ID (reference)	Average number of carpels/locules (SD)		Average number of other floral organs (SD)			Deduced locule-number loci from current study[b]
		Anthesis	Mature fruit	Sepal	Petal	Stamen	
LA0014	MacArthur's Stock2	6.3 (1.1)	6.7 (2.2)	7.5 (0.5)	7.2 (0.7)	7.3 (0.8)	*f*[c]
LA0020	Pennheart-cultivar (Myers 1943)	8.7 (2.1)	8.6 (2.7)	7.5 (2.2)	7.4 (2.2)	7.5 (2.2)	*f*[c,d]
LA0312	Stock11	3.2 (0.9)	3.2 (0.9)	5.2 (0.8)	5.5 (0.7)	5.3 (0.6)	–
LA0517	Early Santa Clara-cultivar	12.9 (4.7)	12.4 (3.3)	8.0 (2.5)	8.3 (2.8)	9.6 (3.4)	*f*[c]
LA0767	Primitive cultivar (Rick 1965)	12.5 (5.0)	11.9 (4.0)	8.2 (2.9)	8.5 (3.2)	8.9 (3.3)	*f*[c,d, e]
LA0925		11.9 (2.1)	16.5 (4.9)	8.9 (1.3)	9.0 (1.7)	9.4 (1.7)	*f, lc, lcn2.2, lcn1.1*[e]
LA1113		11.7 (3.9)	13.3 (6.9)	7.8 (2.3)	7.9 (2.3)	8.2 (2.6)	*f*[c]
LA1786		11.1 (2.9)	9.1 (4.3)	7.5 (2.1)	8.3 (2.6)	9.7 (3.1)	*f*[c]
LA2349	PI193400-cultivar	12.2 (4.1)	11.1 (3.8)	8.6 (3.1)	8.7 (3.1)	9.2 (3.5)	*f*[c]
LA2352	PI193405-cultivar	7.1 (2.7)	3.8 (1.5)	6.3 (1.5)	6.2 (1.7)	6.1 (1.6)	*f, lc*[c, d, e]
LA2364	PI212428	5.7 (3.1)	4.6 (1.1)	5.8 (1.8)	6.1 (2.0)	6.0 (2.1)	*f*[c]
LA2367	PI224575-cultivar	14.7 (4.9)	12.1 (5.1)	10.7 (3.5)	11.1 (3.8)	13.5 (6.1)	*f*[c]
LA2371	PI254650-cultivar	12.4 (4.1)	11.5 (3.1)	9.9 (3.2)	10.1 (3.4)	12.1 (4.3)	*f*[c, d, e]
LA2452	K30	14.2 (3.5)	14.8 (5.5)	8.4 (2.6)	9.1 (2.8)	10.1 (3.6)	*f*[c, d]
LA2595	K503	5.7 (1.5)	5.3 (1.37)	7.1 (1.9)	7.0 (1.9)	7.0 (1.9)	*f*[c]
LA2798	PI212427	5.1 (1.4)	ND[f]	6.7 (1.1)	7.1 (0.9)	8.1 (0.9)	–
LA2799	PI212433	11.7 (2.9)	10.9 (4.0)	8.4 (2.3)	9.0 (2.5)	8.9 (2.4)	*f*[c]

[a]All accessions but LA2352 were deemed multilocular [average locule number (ALN)>3, $P<0.05$]
[b]*f fasciated* locus, *lc locule number* locus
[c]Loci inferred by complementation
[d]Loci inferred by segregation
[e]Loci inferred by mapping
[f]*ND* Not determined

Complementation and segregation tests

Accessions verified by phenotypic analysis to produce multiloculed fruit (ALN>3) were intercrossed and F_1 hybrids were obtained for 35 of the 120 possible F_1 combinations. For complementation testing, three F_1s and their corresponding parents for each cross were grown in the greenhouse in 1999. The same experiment was repeated in the field in 2001, but this time with five plants of each F_1 and parent. In addition, 50 F_2 progeny from a selected set of hybrids were grown along with parents in the 2000 field trial. For all experiments the plants were arranged in a completely randomized design. The decision to analyze 50 F_2 progeny was based on a 95% probability of observing at least one wild-type plant in the event the multiple-locule phenotype of the parents was due to recessive alleles at two unlinked genes [$n = -\ln(\alpha)(1/P-1/2)$, n= number F_2, P= probability double recessive genotype (1/16), α=0.95]. Due to technical reasons, only 34 plants were obtained from the LA0020 × LA2352 F_2 population. For F_1 and F_2 analysis, a minimum of five flowers and/or fruit per plant at anthesis and/or fruit maturity was analyzed for ALN. A *t*-test was performed to determine if the ALN per F_1 and F_2 plant was significantly >3 (P<0.05); hence, the plant was deemed mutant; otherwise, it was deemed wild type.

Genetic mapping

Four stocks—LA0767, LA2371, LA2352, and LA0925—were subjected to genetic mapping experiments using molecular markers from the tomato high-density genetic map (Tanksley et al 1992, http://www.sgn.cornell.edu). LA0767 and LA2371 were crossed to introgression lines IL11-3 and IL11-4 containing *L. pennellii* DNA for the segment of chromosome 11 known to encompass the *fasciated* locus (Eshed and Zamir 1995; Lippman and Tanksley 2001). One hundred and ten F_2 seedlings for the IL11-3 × LA0767, 92 for the IL11-4 × LA0767, 114 for the IL11-3 × LA2371, and 107 for the IL11-4 × LA2371 cross were analyzed for recombination events among markers TG546, TG105A, and TG393 which span the introgressed region containing *fasciated*. Plants with recombinant break points in these intervals were transplanted to the 2000 greenhouse in Ithaca for further phenotypic evaluation along with nonrecombinant controls (a minimum of three of each parental homozygote, three heterozygotes). LA2352 was crossed to *L. pimpinellifolium* LA1589; 85 F_2 plants from this cross were transplanted to the 2001 summer field in Ithaca and analyzed with markers corresponding to the *fasciated*- and *locule number*-containing regions of chromosomes 2 and 11, respectively (Lippman and Tanksley 2001). LA0925 was crossed to *L. pennellii* LA0716; a mapping population of 83 F_2 individuals from this cross were transplanted to the 2000 field in Ithaca and subjected to a whole

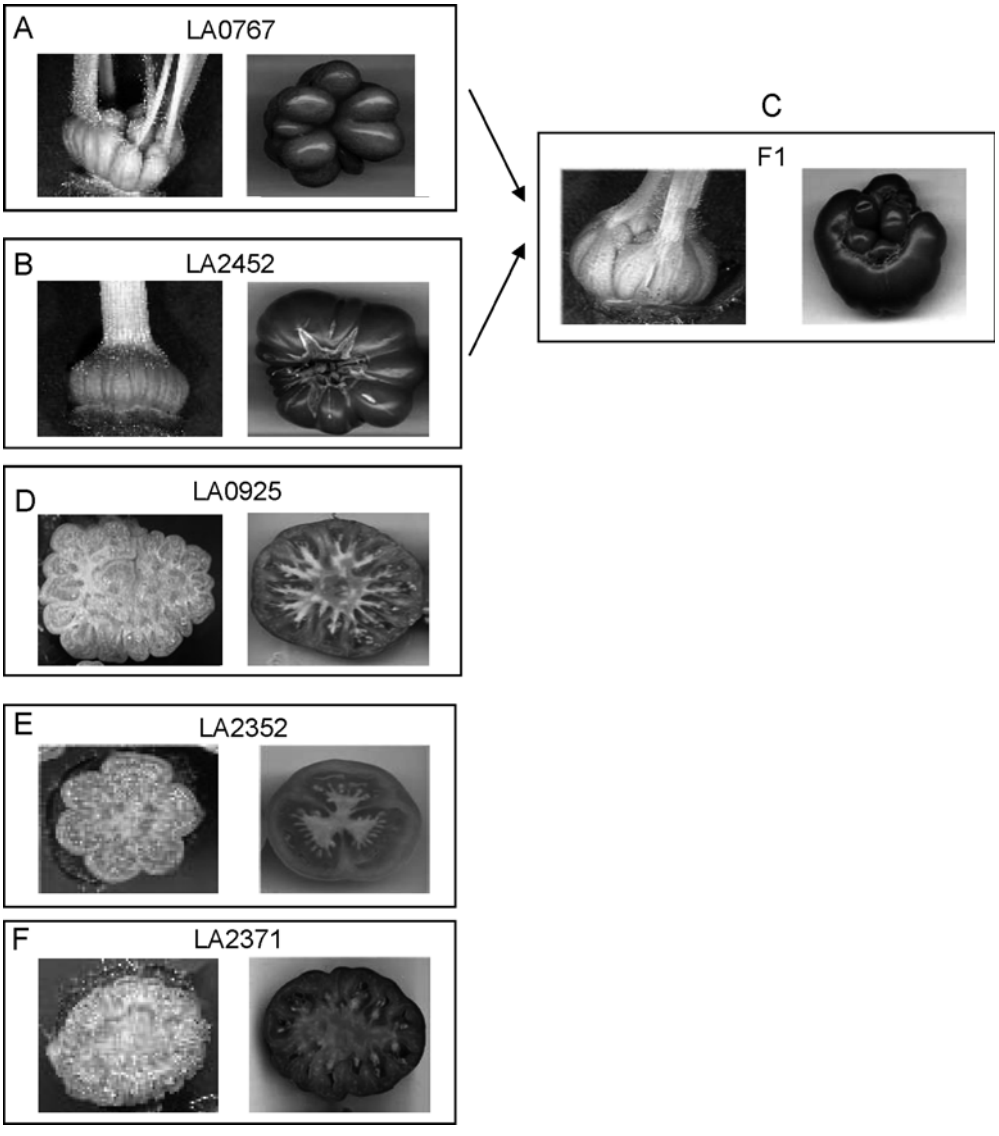

Fig. 1a–f Phenotypes of *Lycopersicon esculentum* accessions subjected to segregation/mapping. Photos were taken at anthesis (*left*) and mature fruit (*right*). **a** LA0767, **b** LA2452, **c** F1, **d** LA0925 (transverse section), **e** LA2352 (transverse section), **f** LA2371 (transverse section)

genome analysis with a set of markers covering the entire genome (http://www.sgn.cornell.edu). Of the over 1,000 markers genotyped in this population, 391 markers that mapped at LOD 3 and cover the 12 tomato chromosomes at an average of 4 cM were selected for QTL analysis.

For mapping experiments in the first three populations described above, the ALN per plant was determined from a minimum of five ovaries and/or five mature fruit per plant. For the LA0925 population, the ALN per plant was determined from a minimum of three ovaries and one mature fruit due to the reduced number of fruit produced for some individuals. For marker analysis, total genomic DNA from each plant was extracted as described by Fulton et al. (1995), and DNA was digested and subjected to Southern blot analysis as described by Bernatzky and Tanksley (1986).

Molecular markers known to map to the *fasciated* region of chromosome 11 were used to refine its location using the two F_2 recombinant populations derived from crosses between LA2371 and the IL lines. F_3 progeny tests from selected recombinants from the IL-derived populations were used to further refine the map position of the *fasciated* locus. These selected F_3 lines were progeny tested by comparing 4–6 homozygous recombinants and 4–6 homozygous nonrecombinant F_3 progeny. Significant differences in ALN in comparisons of recombinant versus nonrecombinant F_3 progeny were calculated using unpaired *t*-tests. Average locule number was estimated as described in the previous paragraph.

MAPMAKER, version 2.0, for Macintosh was used for linkage analysis (Lander et al. 1987). The parameter to include a locus in a linkage group was a minimum LOD of 3 obtained from the Ripple function (Fig. 4). The Kosambi mapping function was used to convert recombination frequencies in centiMorgans (Kosambi 1944). An association of a QTL with a particular marker was declared significant at $P<0.001$ for single-point regression and at LOD>3 for interval analysis using the program Q-GENE for Macintosh (Nelson 1997). The gene action (d/a) and the percentage of the total phenotypic variation explained by each marker (PVE) were also obtained from Q-GENE. Interactions between QTLs were performed via two-way ANOVA using the statistical program MINITAB.

Results and discussion

Characterization of *L. esculentum* accessions based on floral organ and locule number phenotypes

Wild-type tomato generally produces flowers with five to six sepals, petals and stamens, and two to four carpels. Varieties producing fruit with multiple locules often produce flowers with an increased number of floral organs (MacArthur 1926; Young and MacArthur 1947; Zielinski 1948; Szymoniack and Sussex 1992). Seventeen accessions reported to produce multiple locular fruit were characterized in the field and greenhouse with respect to the average number of sepals, petals, stamens, and carpels at anthesis as well as locule number in mature fruit. All but one accession (LA0312) produced fruit with multiple locules (>3). The other 16 accessions ranged widely with respect to carpel/locule and flower organ number (Table 1; Fig. 1). The highest number of locules (16.5) was recorded for LA0925 (Table 1; Fig. 1d).

The organ numbers of sepals, petals, stamens, carpels, and fruit locules were highly correlated both for greenhouse as well as field-derived data (r=0.70–0.98). Correlations amongst traits within a location (e.g., field or greenhouse) were higher than between traits in different locations as depicted by PCA (Fig. 2). This result suggests that while environmental conditions may alter organ number, these alterations affect all organs in a similar manner—a finding consistent with the notion of a common genetic mechanism exercising a global control on organ number. This result seems to apply to all accessions tested since similar correlations amongst traits were also observed on a per accession basis (data not shown).

Complementation testing

Complementation testing is useful for determining allelism and is most effective in crosses with recessive-gene mutations in isogenic backgrounds. In this regard, multiple locules has most often being described as a recessive or semi-recessive trait (MacArthur 1926; Yeager 1937; Young and MacArthur 1947; Zielinski 1948; Lippman and Tanksley 2001). However, we recognize that the current experiments do not involve isogenic backgrounds and that recessivity is not guaranteed. Nonetheless, complementation testing was still deemed to be potentially useful in identifying stocks that may carry mutations at different loci affecting locule number. Such stocks would then be the focus of further genetic testing for confirmation (see following section).

Thirty-five F_1 hybrids involving 14 of the 16 multiloculed accessions were analyzed for complementation testing (Fig. 3). Despite multiple attempts, no F_1s were obtained in crosses involving LA2798 and LA0925 (one of the parents used for genetic mapping, see below). Under 1999 greenhouse conditions, most crosses resulted in F_1s that produced multiple locules (ALN>3 at $P<0.05$) and were intermediate between the parents, suggesting that the corresponding parental stocks may share one or more common loci controlling locule number (Fig. 3). On the other hand, crosses involving parents LA2352 and LA2371 resulted in F_1s with much lower locule numbers

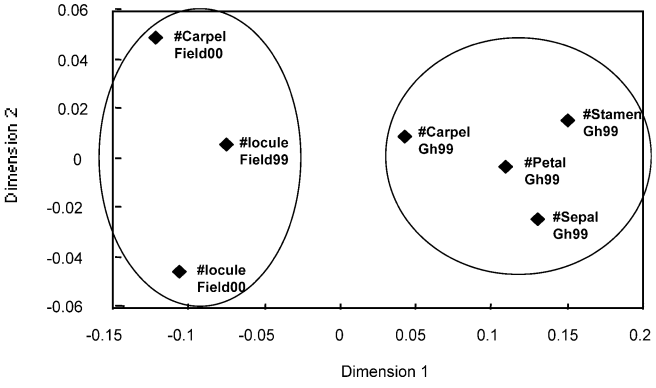

Fig. 2 Principal component analysis-multidimensional scaling depicting correlations amongst floral traits in tomato accessions (Table 1). (Note: a close distance between two points indicates higher correlation.) *#sepal* Average number of sepals, *#petal* average number of petals, *#stamen* average number of stamens, *#carpel* average number of carpels/locules, *#locule* average number of locules in mature fruit, *Gh99* Greenhouse 1999, *Field99* Field 999, *Field00* Field 2000. Traits more correlated are *encircled*

than the two parental stocks, suggesting that they may carry mutations at different loci (Fig. 3). Therefore, these results suggest that two or possibly three complementation groups for locule number may exist among the tested accessions. Most accessions belong to the first complementation group, LA2352 to a second complementation group, and LA2371 to a possible third complementation group.

Segregation tests

In order to shed additional light on the genetic control of multiple locules in the above stocks, segregation tests in selected F_2 progeny were performed. For these experiments five F_2 populations involving selected crosses amongst five stocks (LA0020, LA0767, LA2352, LA2371, LA2452) were tested. LA0767 was included since previous research has shown this stock to be a rare example in which the fasciated phenotype is associated with a nonfused carpel phenotype (Rick 1965; http://tgrc.ucdavis.edu). LA0020 and LA2452 were chosen as representatives of the first complementation group. LA2352 and LA2371 were chosen as representatives of the possible second and third complementation groups.

F_2 derived from LA0020 × LA2452, LA0767 × LA2452, and LA2371 × LA2452 all yielded high locule-number progeny, suggesting that LA0020, LA0767, LA2371, and LA2452 all likely share a common locus controlling locule number (Table 2). Two F_2 populations

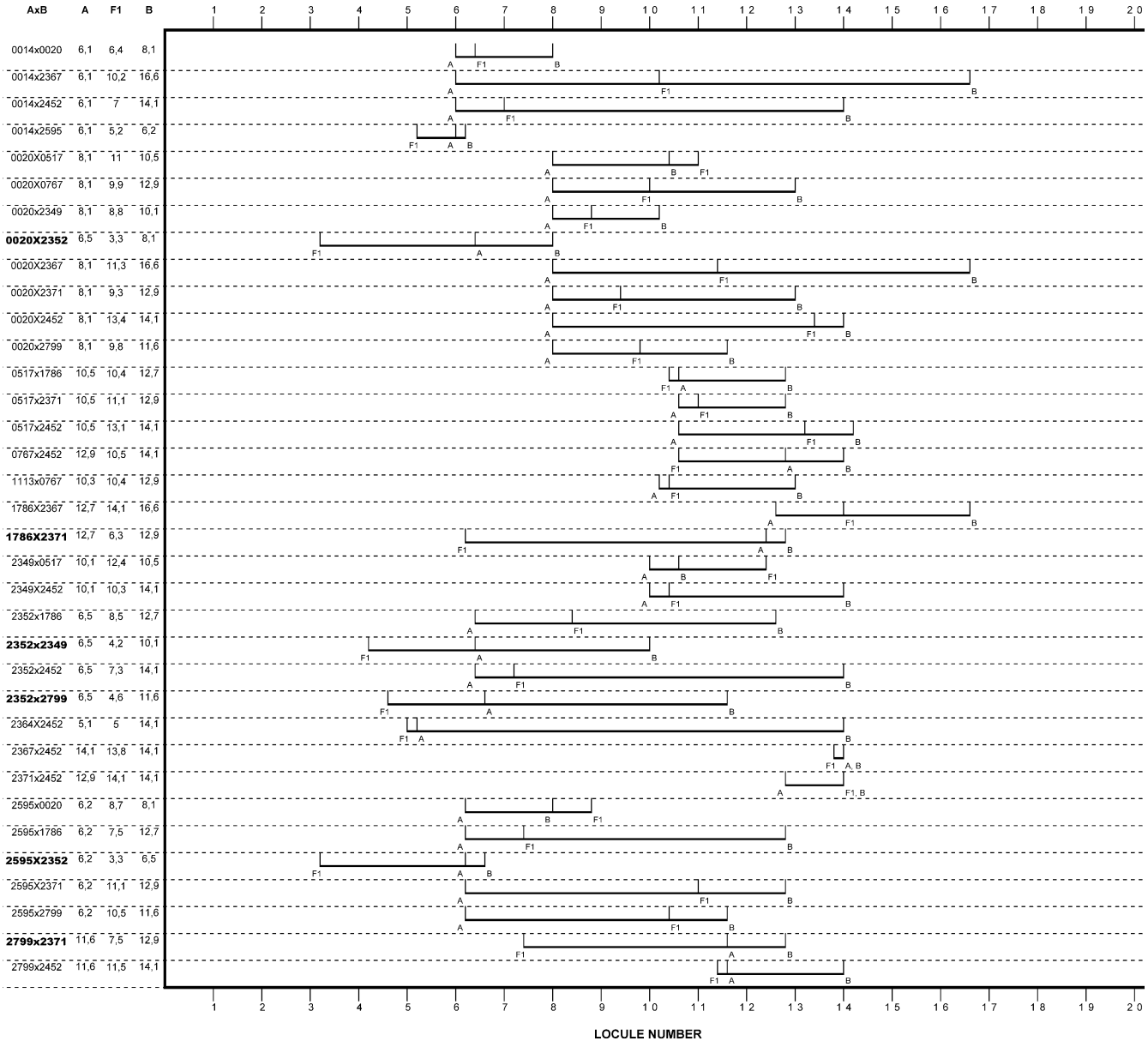

Fig. 3A, B Results from complementation tests in crosses between multilocular accessions. **A** Parent with the lowest average locule number in the cross. **B** Parent with the highest average locule number in the cross. Crosses that resulted in F_1s with much lower locule numbers than the two parental stocks are in *boldface*

involving LA2352 (LA0020 × LA2352 and LA2352 × LA2452) yielded segregants with both high locule numbers and low locule numbers, suggesting that LA2352 represents a second complementation group (Table 2). The segregation data do not support the existence of a third complementation group; rather, they indicate that LA2371 belongs to the first complementation group.

Genetic mapping

Genetic mapping using molecular markers was performed in an effort to determine whether either or both of the putative complementation groups described above correspond to the previously described locule-number loci/QTLs: *fasciated*, *locule number*, or *lcn2.2* (MacArthur 1928; Yeager 1937; Lippman and Tanksley 2001).

LA0767 and LA2371 populations F_2 segregation analysis indicated that LA0767 and LA2371 belong to the first complementation group. Crosses were thus set up to determine whether this complementation group corresponds to the previously described *fasciated* locus on chromosome 11. One hundred and ten F_2 plants derived from the cross IL11-3 × LA0767 and 92 F_2 plants from IL11-4 × LA0767 were screened with markers TG546, TG105A, and TG393, which cover the *fasciated*-containing region of chromosome 11 (Lippman and Tanksley 2001; Fig. 4). A single recombinant was observed between markers TG546 and TG105A in the IL11-3 population and none was observed between markers TG105A and TG393 in the IL11-4 population. Highly significant associations were observed for all three markers and the fasciated phenotype in both populations (Table 3). In the IL11-3 population, markers TG546 and TG105A explained 66% of the variation for locule number with partial recessive gene action for the multiple-locule allele (d/a=−0.87). In the IL11-4 population, markers TG105A and TG393 explained 79% of the variation for locule number, also with largely recessive gene action (d/a=−0.87, Table 3). The gene action observed here is contradictory to the early report suggesting that LA0767 carries a dominant allele at the *fasciated* locus (Rick 1965).

Table 2 Summary of F_2 segregation analysis in crosses between *fasciated* accessions

Genotype	Wild/mutant plants [a]	Allelic [b]
LA0020	0/3	
LA0767	0/3	
LA2352	0/3	
LA2371	0/3	
LA2452	0/3	
F2 LA0020 × LA2352	7/27	No
F2 LA0020 × LA 2452	0/50	Yes
F2 LA2352 × LA2452	8/42	No
F2 LA0767 × LA2452	0/50	Yes
F2 LA2371 × LA2452	0/50	Yes

[a]Plants were considered mutant when ALN>3 (P<0.05)
[b]Two genes were considered nonallelic when wild-type segregants were observed in the F_2 progeny (see Materials and methods)

In addition to bearing multilocular fruit, LA0767 also produces fruit with nonfused carpels, a trait unique to this stock. Unlike multilocularity, the nonfused carpel trait behaved in a dominant manner in F_1s with other *L. esculentum* accessions (Fig. 1). Moreover, in F_2 progeny derived from the cross LA0767 × LA2452, 42 out of 50 plants showed the nonfused carpel phenotype, a segregation consistent with a 3:1 ratio (χ^2=2.16), P<0.05). However, this dominance was not manifest in crosses to the small-fruited, wild species *L. pimpinellifolium* LA1589 since all F_1 bore nonfused carpels/locules (data not shown). The expression of this nonfused carpel characteristic must therefore be dependent upon the genetic background—perhaps not readily expressed in a small-fruited genotype as *L. pimpinellifolium*. The nonfused carpel phenotype was also associated with TG105A and TG546 in the IL11-3 population (χ^2=15.5, P<0.005), and with TG105A and TG393 in the IL11-4 population (χ^2=10.7) , P<0.005). Whether the nonfused carpel and multilocular phenotypes are caused by mutations in the same gene or two closely linked genes awaits further investigation.

The results involving the multilocular phenotype indicate that the complementation group represented by LA0767 corresponds to the *fasciated* locus. However, since only a single recombinant was obtained in both F_2 populations, it was not possible to precisely orient the *fasciated* locus with respect to the tested markers. LA2371 was also subjected to similar mapping in crosses with IL11-3 and IL11-4. Out of 114 F_2 plants derived from the IL11-3 × LA2371 cross, 33 recombinants between markers TG546 and TG105A and out of 107 F_2 derived from the IL11-4 × LA2371 cross, four recombinants between markers TG105A and TG393 were observed. Single-point analysis indicated that TG105A is highly associated with locule number in the IL11-3 × LA2371 recombinant population, explaining 79% of the variation with recessive gene action (d/a=−0.81, Table 3). This finding is consistent with the F_2 frequency distribution, which was skewed towards the low locule number phenotypes (data not shown). F_2 recombinants derived from IL11-4 × LA2371 also showed *fasciated* to be closest to TG105A (Table 4). We therefore conclude that both LA0767 and LA2371 contain mutant alleles at *fasciated*, and that this locus is located between TG546 and TG393 and is very close to TG105A.

As already mentioned, recombination in the *fasciated*-containing region of chromosome 11 was greatly reduced in the LA0767 population relative to the LA2371 population. For example, recombination between TG546 and TG105A markers in the IL11-3 × LA2371 F_2 population was 14.5% (33 out of 228 gametes) as compared to 0.45% (1 out of 220 gametes) for the IL11-3 × LA0767 F_2 population (significant difference, P<0.0001). The recom-

Table 3 Summary of single-point analysis for locule number in different F_2 populations

Population	Chromosome	QTL designation[a]	Marker [b]	Source	PVE[c]	AA[d]	N[e]	aa	N	Aa	N	*d*/*a*[f]
IL11-3 × LA0767	11	*f*	TG105A, TG546	*L. esculentum*	0.66	8.1	5	3.5	7	3.8	8	−0.87
IL11-4 × LA0767	11	*f*	TG105A, TG393	*L. esculentum*	0.79	7.9	3	3.0	8	3.3	4	−0.87
IL11-3 × LA2371	11	*f*	TG105A	*L. esculentum*	0.79	9.8	2	3.2	14	3.8	15	−0.81
LA1589 × LA2352	11	*f*	TG105A	*L. esculentum*	0.56	4.8	15	2.4	23	2.9	36	−0.56
	2	*lc*	TG469	*L. esculentum*	0.19	3.9	22	2.5	16	2.9	45	−0.37
LA0925 × LA0716	2	*lc*	TG337	*L. esculentum*	0.34	4.1	14	2.3	13	3.0	19	−0.23
	2	*lcn2.2*	T347	*L. esculentum*	0.28	4.1	12	2.3	9	2.9	24	−0.30
	11	*f*	TG105A	*L. esculentum*	0.23	4.7	10	2.7	25	3.1	44	−0.60
	2	*lc*	TG337	*L. esculentum*	0.32	4.5	16	2.4	24	3.1	38	−0.31
	2	*lcn2.2*	T347	*L. esculentum*	0.26	4.3	14	2.5	24	3.2	37	−0.30
	1	*lcn1.1*	CLET7E12	*L. esculentum*	0.24	4.7	9	2.9	24	2.9	44	−1.00

[a] *f fasciatedl*, *lc locule number*, *lcn2.2 locule number 2.2*, *lcn1.1 locule number 1.1*
[b] Marker most significantly linked to QTL
[c] The first seven rows indicate the *PVE* percentage of phenotypic variance explained for trait locule number in mature fruit. The last four rows indicate the PVE for trait locule number in carpels (anthesis)
[d] Average phenotypic value for plants with the following genotypes: *AA* homozygous *L. esculentum*, *aa* homozygous *L. pennellii* or *L. pimpinellifolium*, *Aa* heterozygous
[e] Number of F_2 plants for each genotype
[f] Gene action or degree of dominance for each QTL

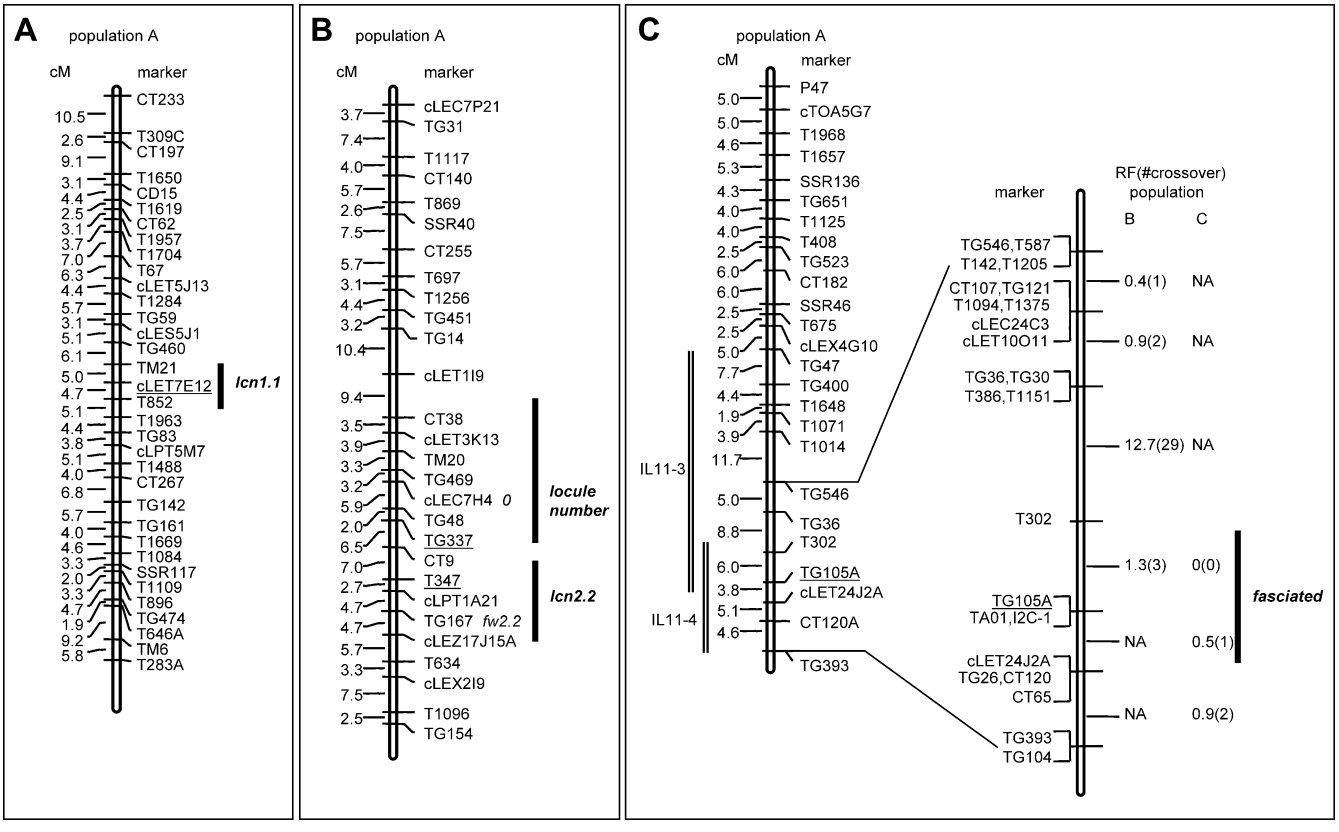

Fig. 4 Genetic map of tomato showing the position of carpel/locule number QTLs. Only chromosomes with QTLs are shown. Linkage map derived from *L. esculentum* LA0925 × *L. pennellii* LA716 population (**A**). Linkage map (bottom of chromosome 11) derived from *L. esculentum* LA2371 × IL11-3 (**B**), and from LA2371 × IL11-4 populations (**C**). *cM* CentiMorgan distances for population (**A**) represented to the left of chromosomes, *RF* recombination frequencies for populations (**B**), represented to the right of chromosome 11 (**C**), *NA* nonapplicable, i.e., no *L. pennellii* introgression in the corresponding IL parent. The most significant marker for each QTL is *underlined*. The positions of *o ovate*, *fw2.2 fruit weight* 2.2, *IL11-3*, and *IL11-4* are indicated. All markers were derived from http://www.sgn.cornell.edu except for markers I2C-1 (SL8D) and TAO1 derived from Ori et al. (1997a, b), respectively

Table 4 IL11-4 × *L. esculentum* LA2371 F_2 recombinant analysis

Pedigree F_2 plant	ALN	Marker[a]		
		TG105A	TG26	TG393
00T275-74	5.2	2	2	3
00T275-76	2.9	3	2	2
00T275-116	8.5	1	1	2

[a]Marker score: *1* homozygous for *L. esculentum* alleles, *3* homozygous for *L. pennellii* alleles, *2* heterozygous

bination frequency between TG105A and TG393 markers in the IL11-4 × LA2371 F_2 was 1.9% (4 out of 214 gametes) versus 0% (0 out of 184 gametes) for the IL11-4 × LA0767 cross (significant difference, $P<0.05$). The locule number allele in LA0767 is likely spontaneous in origin (Chetelat, personal communication) and may be associated with either an inversion or a deletion near the *fasciated* locus, both of which would reduce recombination. The reduced recombination and associated nonfused carpel characteristics are both novel to LA0767 and indicate that this stock carries a novel allele for *fasciated*.

To refine the map position of the *fasciated* locus, more markers were added to the populations generated with LA2371 (Fig. 4) and F_3 progeny tests were performed with selected recombinants (Table 5). These results indicate that the *fasciated* locus cosegregates with TG105A and is located in the 0.5-cM interval between markers T302 and cLET24 J2A.

LA2352 population Accession LA2352 was chosen for mapping the putative second complementation group. This was the only accession that produced both F_1 and F_2 progeny with wild-type locules in crosses with other multilocular accessions (Fig. 3; Table 2). Previous studies have shown that the *locule number* and *lcn2.2* loci—both on the distal portion of chromosome 2—also moderate locule number (Yeager 1937; Lippman and Tanksley 2001). In order to determine whether the second complementation group represented by LA2352, corresponds to either *locule number* or *lcn2.2*, an F_2 mapping population was generated from a cross between *L. pimpinellifolium* LA1589 × LA2352. F_2 progeny were analyzed for markers covering the *locule number*- and *lcn2.2*-containing regions of chromosome 2 and the *fasciated* region of chromosome 11 (Lippman and Tanksley 2001). Single-point analysis derived from this population indicated that both the *locule number* and *fasciated* loci segregate, contributing 19% and 56% of the variance for locule number, respectively. The multilocular alleles showed partially recessive gene action for both *fasciated* ($d/a=-0.56$) and *locule number* ($d/a=-0.37$, Table 3), which agrees with the F_2 frequency distribution for this population that was skewed towards the low locule-number parent (data not shown).

Significant epistatic interactions ($P=0.025$) as determined by a two-way ANOVA were observed between *fasciated* and *locule number* in this population. The interaction plot indicates that homozygosity for *L. esculentum* alleles at both loci results in a disproportionate increase in locule number (Fig. 5). The same interaction was also observed in the study of Lippman and Tanksley (2001) and suggests that *fasciated* and *locule number* may code for genes with a similar function in the control of locule number. Such redundant functions have also been described for the CLAVATA pathway in *Arabidopsis* (Clark et al. 1997; Mayer et al. 1998; Jeong et al. 1999; Fletcher et al. 1999; Schoof et al. 2000), whose carpel phenotypes are similar to *fasciated* and *locule number*.

As described above, the mapping results indicate that LA2352 contains a multilocular allele for the *fasciated* locus, despite the fact that wild-type F_1 and F_2 were observed in the complementation segregation experiments. One possible explanation for these seemingly contradictory results is that the genetic background in LA2352 is associated with variable expressivity, penetrance, and/or greater environmental sensitivity with respect to locule number. Consistent with this suggestion is the observation

Table 5 F_2/F_3 family analysis of selected recombinants in the *fasciated* region of chromosome 11

F_2 parent							F_3 family			
F_2 plant number	Parental pedigree	ALN	Marker				F_3 plant	Marker	ALN (SD)	*P*-value[a]
			TG36	T302	TG105A, *f*	CLET24 J2A				
00T274-19	IL11-3 × LA2371	9.9	2	1	1	1	TA3111	TG36=1	8.5 (2.4)	0.1194
								TG36=3	12.3 (4.9)	
00T274-22	IL11-3 × LA2371	9.8	2	1	1	1	TA3112	TG36=1	9.9 (4.5)	0.4978
								TG36=3	12.8 (7)	
00T274-32	IL11-3 × LA2371	2.3	1	2	3	3	TA3113	T302=1	3.1 (0.4)	0.3033
								T302=3	2.8 (0.2)	
00T274-64	IL11-3 × LA2371	5.1	3	3	2	1	TA3114	TG105A=1	12.2 (2.0)	0.0014
								TG105A=3	5.2 (1.6)	
00T275-76	IL11-4 × LA2371	2.9		3	3	2	TA3115	CLET24 J2A=1	3.9 (0.7)	0.0632
								CLET24 J2A=3	5.7 (2.0)	

[b]*P*-values of unpaired *t*-tests for comparisons between recombinants and nonrecombinant classes within each F_3 family

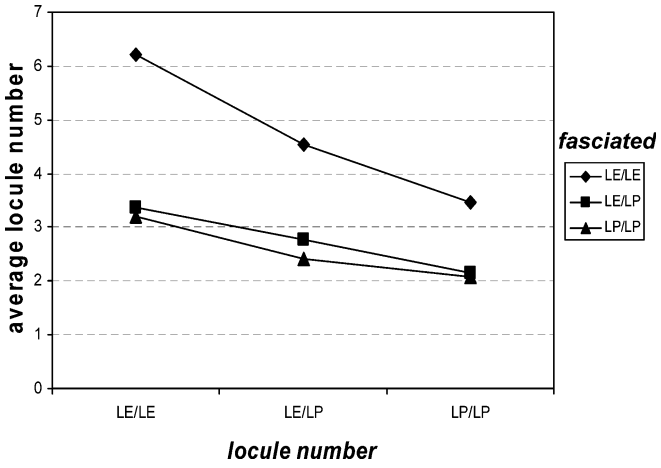

Fig. 5 Genotype–phenotype plot depicting epistatic interactions between *fasciated* × *locule number*. Data were derived from the *L. esculentum* LA2352 × *L. pimpinellifolium* LA1589 population. *LE/LE* homozygous for *L. esculentum* alleles, *LP/LP* homozygous for *L. pennellii* alleles, *LE/LP* heterozygous

that LA2352 shows different locule number phenotypes, depending on the developmental stage. For example, some plants displayed a medium locule number phenotype at anthesis (five or more locules) and a low locule number phenotype in mature fruit (two to four locules) in the 2000 field experiment (Fig. 1e). Additionally, some plants showed wild-type branches bearing flowers/fruit with two to four locules, as well as other branches bearing medium to high locule number flowers/fruit. Moreover, F_1 hybrids between LA2352 and other mutant accessions yielded variable locule-number phenotypes. For example, F_1 plants from LA0020 × LA2352 and LA2595 × LA2352 crosses bore wild-type (low-loculed) fruit in the 1999 greenhouse trial (Fig. 3). However, the same hybrids gave rise to plants with both wild-type and multilocular fruit in the 2001 field trial.

Different penetrance and expressivity have also been reported for the fasciated character in plant species including soybean (Leffel et al. 1993), pea (Gottschalk 1977), and chamomile (Das et al. 1999). Variable penetrance could be caused by epigenetic alleles associated with variable levels of methylation. The *SUP* (*SUPERMAN*) locus of *Arabidopsis* represents a good example of this phenomenon (Jacobsen and Meyerowitz 1997). Wild-type segregants in F_2 from crosses between *clk* (*clark kent*) and *sup* (*superman*) suggested that *clk* and *sup* were separate genes. However, later it was revealed that both are allelic and differ only by methylation (Jacobsen and Meyerowitz 1997).

LA0925 population LA0925 was unique in that produced the greatest number of locules in mature fruit (16.5 average) of any accession tested (Table 1). It was also the most difficult stock for production of F_1 hybrids. In order to examine the genetic content of this stock (with regard to locule-number control), an F_2 mapping population was generated using *L. pennellii* LA716 as the other parent. Despite being different species, *L. pennellii* and *L. esculentum* have the same chromosome number, near-normal meiosis in F_1 hybrids, and high levels of allelic variation for molecular markers. For these reasons, the high-density tomato linkage map and introgression lines—both of which are heavily used in tomato genetics—were derived from crosses between the two species (Tanksley et al. 1992; Eshed and Zamir 1995). *L. pennellii* also has wild-type (bilocular) fruit, making it suitable as a parent in crosses for mapping locule number loci. Eighty-three F_2 plants from an LA0925 × *L. pennellii* cross were analyzed for molecular markers throughout the genome (at intervals averaging approximately 4 cM) and subjected to phenotypic analysis for carpel/locule number—both at anthesis and mature fruit. However, due to sterility, mature fruit was obtained for only 46 individuals. Four loci were identified for locule number at anthesis ($P<0.001$): *fasciated, locule number*, *lcn2.2* (chromosome 2), and *lcn1.1* (chromosome 1) (Table 3; Fig. 4). However, in mature fruit, only *locule number* and *lcn2.2* were significant ($P<0.001$), probably due to the reduced population size. Because of the issue of reduced population size, the further discussions on locule number control in this population will be restricted to the carpel number data taken at anthesis.

Of the four loci detected, *locule number* was the most significant, accounting for 32% of the variation. The other loci each accounted for approximately 25% of the variation. However, because *locule number* and *lcn2.2* are linked on chromosome 2 (and hence not independent), the variance attributable to these loci is likely to be overestimated. Multiple regression with all four loci accounted for only 40% of the variation in locule number, suggesting epistatic interactions amongst these loci and/or residual environmental effects. However, when the four loci were subjected to pairwise two-way ANOVA, no significant interactions were detected. The lack of interaction between *fasciated* and *locule* number differs from what was observed in the LA2352 population (see earlier section) and in the cross reported by Lippman and Tanksley (2001). The different results might be attributed to the differences in genetic backgrounds or the differences in magnitude of effect per locus in each population, i.e., *fasciated* is the major QTL in the LA2352 and Lippman and Tanksley (2001) populations, and *locule number* is the major QTL in the LA0925 population.

Overall conclusions about the genetic control and evolution of multilocular tomato fruit

Of the 17 accessions included in the current study, 16 displayed the multilocular phenotype in either the field or greenhouse (Table 1). Based on a combination of complementation testing, segregation analysis and genetic mapping studies, it was possible to classify 15 of these stocks with respect to the loci that likely underlie their mutilocular phenotypes (one accession, LA2798, failed in crosses and could not be classified). The combined results indicate that all 15 accessions carry multilocule alleles at

the *fasciated* locus on chromosome 11. In cases where the phenotypic variation attributable to *fasciated* was measured in segregating populations (e.g., for LA0767 and LA2371), the locus accounted for up to 80% of the variance, suggesting that *fasciated* is the main cause of multiple locules in most of these stocks. Also, the fact that the *fasciated* locus is apparently a major contributor to increased locule number in all of the tested stocks suggests that selection for mutations at this locus may have been an early step in the generation of multilocular, large-fruited tomato cultivars. However, since only a small portion of all modern tomato cultivars are multilocular, the occurrence and/or selection for mutations at *fasciated* probably occurred relatively recently in tomato domestication. While the origins of the *fasciated* alleles in these stocks are unknown, it seems likely that there are at least two independent mutant alleles for *fasciated* that condition multilocular fruit. Both forms are largely recessive (d/a= −0.56 to −0.87, Table 3) and simultaneously condition carpel, stamen, petal, and sepal number (Table 1). One of the accessions (LA0767) is associated with both nonfused carpels and repressed recombination and might carry a deletion or inversion in the *fasciated* containing region of chromosome 11.

Two accessions (LA2352 and LA0925) were found to contain multiple-locule alleles at not only the *fasciated* locus, but also the *locule number* locus on chromosome 2. In the mapping population used to analyze the LA2352 accession, *fasciated* accounted for a much larger portion of the variation than did the *locule number* locus (56% vs 19%), which is consistent with the major role of *fasciated* in the evolution of large, multilocular fruit (Lippman and Tanksley 2001). In one population (LA0925), however, the *locule number* locus controlled a larger portion of the variation than did *fasciated* (32% vs 23%). However, this seemingly larger variance may be due to linkage of *locule number* to *lcn2.2*, which also affects locule number. Recent studies have also shown that *locule number* can also condition increased locule number in a stock not containing the *fasciated* mutation (van der Knaap and Tanksley 2003).

It is interesting to note that *locule number* and *fasciated* demonstrate positive epistatic interactions in some genetic backgrounds (LA2352, current study; Lippman and Tanksley 2001). The nature of the interaction is such that homozygosity for mutant alleles at both loci results in an increase in carpel number beyond what is predicted by the effects of either locus alone. The finding that mutant alleles of the *fasciated* locus occur in most multilocular accessions raises the possibility that *locule number* mutants were selected in stocks already fixed for mutations at the *fasciated* locus. Selection of *locule-number* mutations in such backgrounds may have been enhanced by positive epistatic interactions between the two loci.

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