Dissecting the Genetic Pathway to Extreme Fruit Size in Tomato Using a Cross Between the Small-Fruited Wild Species *Lycopersicon pimpinellifolium* and *L. esculentum* var. Giant Heirloom

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ABSTRACT

In an effort to determine the genetic basis of exceptionally large tomato fruits, QTL analysis was performed on a population derived from a cross between the wild species *Lycopersicon pimpinellifolium* (average fruit weight, 1 g) and the *L. esculentum* cultivar var. Giant Heirloom, which bears fruit in excess of 1000 g. QTL analysis revealed that the majority (67%) of phenotypic variation in fruit size could be attributed to six major loci localized on chromosomes 1–3 and 11. None of the QTL map to novel regions of the genome—all have been reported in previous studies involving moderately sized tomatoes. This result suggests that no major QTL beyond those already reported were involved in the evolution of extremely large fruit. However, this is the first time that all six QTL have emerged in a single population, suggesting that exceptionally large-fruited varieties, such as Giant Heirloom, are the result of a novel combination of preexisting QTL alleles. One of the detected QTL, *fw2.2*, has been cloned and exerts its effect on fruit size through global control of cell division early in carpel/fruit development. However, the most significant QTL detected in this study (*fw11.3*, *lcn11.1*) maps to the bottom of chromosome 11 and seems to exert its effect on fruit size through control of carpel/locule number. A second major locus, also affecting carpel number (and hence fruit size), was mapped to chromosome 2 (*fw2.1*, *lcn2.1*). We propose that these two carpel number QTL correspond to the loci described by early classical geneticists as *fasciated* (*f*) and *locule number* (*lc*), respectively.

A great improvement in tomato fruit size has been achieved in the centuries since cultivated tomatoes were first domesticated from their supposed wild progenitors, *Lycopersicon pimpinellifolium* and/or *L. esculentum* var. cerasiforme (LUCKWILL 1943; JENKINS 1948; RICK 1976). Classical breeders of ancient and modern times have searched for and exploited tomato germplasm in attempts to create larger-fruited varieties and attain higher crop yields. As a result, tremendous variability in fruit size exists within Lycopersicon from the extremely small-fruited wild species *L. pimpinellifolium* (fruit 1–2 g) to *L. esculentum* lines, some of which produce fruit that reach 1000 g.

While improvement in tomato fruit size has been relatively easy to achieve due to high heritability (KHALF-ALLAH and PIERCE 1963; KHALF-ALLAH and MOUSA 1972), inheritance studies reveal that this trait is quite complex and determined by multiple loci (MACARTHUR and BUTLER 1938; POWERS 1941; FOGLE and CURRENCE 1950; IBARBIA and LAMBETH 1969). Classical genetics have suggested that at least 5–6 genes and possibly as many as 10–20 genes govern the trait (POWERS 1941;
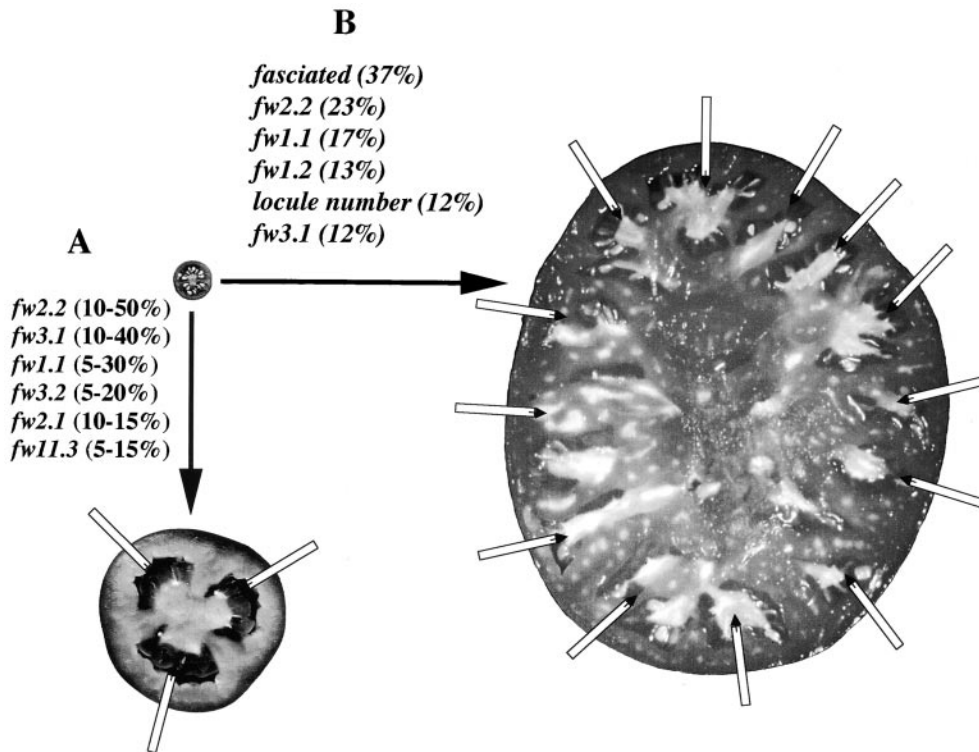
FOGLE and CURRENCE 1950; IBARBIA and LAMBETH 1969). It is likely that these genes are involved in a variety of distinct fruit developmental pathways, each contributing to final fruit size. For example, developmental studies have indicated that tomato size is a function of the number of cells within the ovary prior to fertilization, the number of successful fertilizations, the number of cell divisions that occur within the developing fruit following fertilization, and the extent of cell enlargement (BOHNER and BANGERTH 1988; GILLAPSY *et al.* 1993).

With the advent of molecular markers, such as restriction fragment length polymorphisms (RFLPs), plant geneticists have acquired the tools to break down quantitative traits, such as fruit size, into Mendelian factors to study their genetic basis (PATERSON *et al.* 1988; LANDER and BOTSTEIN 1989; KNAPP *et al.* 1990). In tomato, a high-density molecular linkage map was created to facilitate the mapping and identification of biologically and horticulturally significant genes underlying quantitative traits (TANKSLEY *et al.* 1992). This highly efficient tool has already served as a basis for quantitative trait loci (QTL) characterization in over 15 mapping studies involving many complex traits of *L. esculentum*, including fruit size and shape. A review by GRANDILLO *et al.* (1999) enumerates a total of 28 fruit weight QTL identified in these studies.

Until now, most QTL mapping studies have involved

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FIGURE 1.—Evolution of fruit size in tomato. (Left) A fruit from the small-fruited wild tomato species *L. pimpinellifolium.* Like most wild tomato species, *L. pimpinellifolium* bears fruit with only 2 locules. (Bottom) A fruit from a commercial processing variety of the domesticated species *L. esculentum,* which typically contain 2–4 locules (arrows). (Right) A fruit from *L. esculentum* var. Giant Heirloom Tomato, a fresh-market variety bred for extremely large size. Fruit from this variety and most extremely large-fruited varieties contain 10 or more locules (arrows). (A) Major QTL contributing to the evolution of processing-type fruit from wild forms. None of these loci have been shown to affect locule number (GRANDILLO *et al.* 1999). The percentage of phenotypic variance attributable to each locus is in parentheses (based on GRANDILLO *et al.* 1999). (B) Major QTL contributing to the evolution of exceptionally large-fruited tomatoes (detected together for the first time in this study; Table 1). Two of these loci, *fasciated* and *locule number,* are associated with an increase in locule number (Table 1; see RESULTS AND DISCUSSION).

crosses between very small-fruited wild tomatoes (1–2 g) and tomato cultivars producing medium-sized fruit (<100 g). As a result, we now know which loci are involved in the genetic pathway leading from very small wild tomato fruit to medium-sized domesticated fruit—an ~100-fold increase in size (Figure 1A; GRANDILLO *et al.* 1999). However, as mentioned previously, some tomato cultivars produce fruit up to 1000 g—another 10-fold increase in size beyond what is seen in medium-sized tomatoes (Figure 1B). As none of the QTL studies heretofore have involved crosses to such large-fruited cultivars, we lack knowledge of the genetic loci that have enabled modern varieties to reach extreme sizes. One hypothesis is that the size increase is the result of combining (through selection) previous QTL alleles already present in tomato germplasm. An alternative hypothesis is that one or more new mutations occurred at other loci in the genome, and it was due to these enabling new mutations that fruit were able to reach such extreme sizes.

To shed light on the issue of evolution and selection of extreme fruit size in tomato, we have studied, via QTL analysis, the inheritance of fruit size and associated traits in a cross between one of the smallest-fruited wild tomatoes (*L. pimpinellifolium* LA1589) and the largest-fruited (to our knowledge) cultivated tomato, *L. esculentum* cv. Giant Heirloom. These two accessions differ by as much 1000-fold in their fruit size (Figure 1B). The

objectives of this study were the following: (1) to identify the QTL responsible for the exceptionally large fruits of the Giant Heirloom tomato; (2) to compare the number, chromosome position, magnitude of effects, gene action, and gene interaction with previously reported fruit size and shape QTL; and (3) to use this information to hypothesize which evolutionary events contributed to the extremely large fruit size now observed in modern-day fresh-market tomatoes.

MATERIALS AND METHODS

Population development: The small-fruited wild tomato species *L. pimpinellifolium* (LA1589), native to Peru (hereafter designated as PM), was crossed as the pistillate parent to the large-fruited inbred *L. esculentum* var. Giant Heirloom (hereafter referred to as GHT). A single interspecific F_1 hybrid was selfed to produce an F_2 population suitable for molecular mapping. A total of 200 F_2 plants, 5 of each parental control, and 5 F_1 plants were transplanted to field plots in Ithaca, New York, in a completely randomized design on May 27, 1999.

Phenotypic analysis: Following fruit maturity, a minimum of 10 ripe tomatoes were harvested from each individual F_2 plant (except for 12 plants that did not produce enough fruit suitable for analysis) and were evaluated for a series of phenotypic traits related to fruit size. Average fruit weight, in grams, was determined from a sample of 10 representative fruits per plant. Five of the 10 harvested fruits were cut transversely to calculate the average locule number per fruit and average fruit. Fruit length was obtained by cutting the remaining 5 fruits longitudinally and measuring, in centimeters, from stem

to blossom end. Dividing the average fruit length by the average fruit width provided the values for fruit shape index. All the seeds were extracted from each set of 10 fruits and used to calculate hundred-seed weight, in grams, and average number of seeds per fruit. Each sample of 10 sliced fruits was scanned on a computer scanner and stored as a digital image.

Average values for each F_2 plant, for each trait described above, were used for plotting trait distributions and QTL analyses.

Genotypic analysis: Leaf tissue was used to extract total genomic DNA from each F_2 field-grown plant. F_2 plants representing the two extremes in fruit weight (smallest and largest fruits) were chosen to facilitate the identification of major fruit weight QTL based on the fruit weight distribution derived from 188 suitable F_2 plants. Thus, 114 phenotypic extremes were selected for molecular mapping.

To prepare filters for the mapping analysis, DNA was digested with one of seven restriction enzymes (*Bst*NI, *Dra*I, *Eco*RI, *Eco*RV, *Hin*dIII, *Sca*I, and *Xba*I) and subjected to Southern blot analysis as described by BERNATZKY and TANKSLEY (1986). The polymorphic markers used in this study were identified using marker data from previous studies involving the interspecific cross *L. esculentum* × *L. pimpinellifolium* (GRANDILLO and TANKSLEY 1996; A. FRARY, personal communication). Entire genome coverage was obtained by mapping a total of 90 segregating genetic markers (89 RFLP and 1 cleaved amplified polymorphism marker) on the 12 tomato chromosomes, which corresponded to an average spacing of 12 cM.

Statistical analysis: MAPMAKER V2.0 was used to create linkage maps from the 90 markers spanning the 12 tomato chromosomes (LANDER *et al.* 1987). Markers were included on the map only if the LOD value obtained from the ripple was >3 with the exception of four pairs of markers (CT50:TG500, TG174:TG183, CT92:CD40, and TG403:CT95) that were tightly linked. The Kosambi mapping function (KOSAMBI 1944) was used to convert recombination frequencies to map distances in centimorgans.

Pearson correlation coefficients were calculated for each trait using the program QGENE. The same program was used to identify putative fruit size and shape QTL using single-point linear regression models where the genetic markers served as independent variables and phenotypes served as dependent variables (NELSON 1997). To minimize the number of type-I errors leading to QTL false positives and to compensate for nonrandom selection of plants used for molecular mapping, we chose a strict probability level of $P < 0.001$ as the threshold to indicate a significant association of a QTL with a particular marker locus. The percentage of phenotypic variation explained (R^2) was also obtained from QGENE and used to show the relative contribution of particular loci to fruit size characters. In addition, multiple regression was used from the same software program to estimate the percentage of phenotypic variation accounted for by all significant QTL in each trait. Interval analysis was carried out using QGENE to confirm the presence of putative fruit size QTL on the framework map. A LOD score of >2.4, which corresponds to a significance level of 0.001, was chosen to indicate significant results in the interval analyses. Finally, using the program StatView, two-way ANOVAs were performed on all significant markers for each trait in pairwise combinations to determine interaction between loci.

RESULTS AND DISCUSSION

Phenotypic distributions of fruit size characters: A total of seven fruit-size-related traits were scored from 188 F_2 plants derived from the interspecific cross PM × GHT.

A tremendous difference in fruit weight was observed between the two parents where PM fruit averaged 1.1 g in weight as compared to an average fruit weight of ~500 g for GHT (Figure 1B). The F_1 hybrid produced fruit averaging only 10.5 g, and the average fruit weight for the F_2 population was 11.1 g (Figure 2A). These observations are consistent with classical studies in which F_1 hybrids resulting from a cross between a large- and small-fruited cultivar typically exhibited weights similar to that of the smaller-fruited parent (MACARTHUR and BUTLER 1938). Consequently, it has been postulated that small-fruit alleles are semidominant to large-fruit alleles, which may explain the skewed distribution in favor of small fruit size that was observed in this study (Figure 2A). Fruit weight QTL studies support this notion as most small-fruit alleles showed semidominance to large-fruit alleles (GRANDILLO *et al.* 1999). A similarly skewed distribution was obtained for locule number, which is likely due to semidominance of few-loculed fruit over many-loculed fruit (MACARTHUR and BUTLER 1938). Fruit length, fruit diameter, fruit shape, number of seeds per fruit, and seed weight were all distributed normally (data not shown), which is consistent with previous findings (GRANDILLO and TANKSLEY 1996).

According to LANDER and BOTSTEIN (1989), selective genotyping of the extreme progeny in a population can increase the power of QTL mapping. Therefore, to facilitate molecular mapping and to more clearly define the major genomic regions contributing to large fruit size, 114 plants representing the extremes in fruit weight were selected for genotypic analysis. As a result of this modest selection, fruit weight exhibited a bimodal distribution in the mapping population and the average fruit weight of F_2 changed from 11.1 to 11.9 g (Figure 2B). Fruit length and fruit diameter showed similar changes in their distributions (*i.e.*, from continuous to bimodal), while locule number, fruit shape, number of seeds per fruit, and seed weight maintained distributions comparable to those that were observed prior to selection (data not shown).

Correlations between traits: Nearly all fruit size characters measured in this study showed significant correlation with one another ($P < 0.001$; Figure 3). The most highly correlated were fruit weight, fruit length, and fruit diameter ($r > 0.90$ for all pairwise combinations). This result was expected because as fruit length and fruit diameter increase, there will obviously be a corresponding increase in overall fruit size. Fruit weight and locule number were also positively correlated ($r = 0.73$). Previous studies have shown that locule number can exert significant effects on fruit size, and is therefore likely to be a major factor contributing to large-fruited tomato varieties (HOUGHTALING 1935; YEAGER 1937). An additional, but smaller, correlation was found for fruit weight and number of seeds per fruit (NSF; $r = 0.36$). These results can be explained in part by developmental studies, which suggest that the total number of
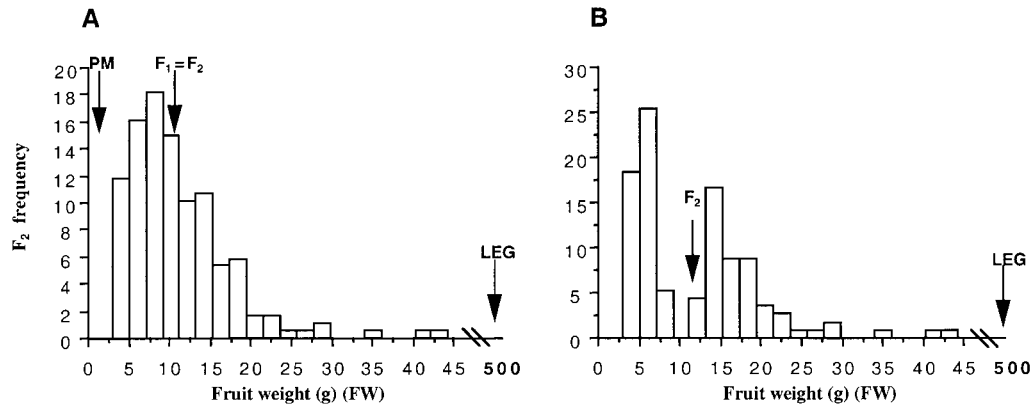
FIGURE 2.—Frequency distributions for fruit weight in (A) the F_2 progeny prior to selection and (B) the F_2 progeny following selection. Fruit weight means for parentals are designated as *L. esculentum* (LEG) and PM, and the F_1 hybrid mean is designated as F_1.

developing seeds influence final fruit size and weight (NITSCH 1970). Seeds produce and act as sinks for hormones such as cytokinin and auxin, which induce rapid growth of the developing ovary by increasing cell division and cell expansion (BOHNER and BANGERTH 1988). Hence, the greater the number of seeds, the larger the fruit. However, we cannot rule out the possibility that this correlation is due to linkage of separate genes controlling fruit weight and NSF. Given the large number of QTL for both traits, this scenario is quite likely. Two highly significant negative correlations were observed between fruit weight and fruit shape index ($r = -0.38$) and locule number and fruit shape ($r = -0.7$). Fruit shape is calculated by dividing fruit length by fruit diameter. The negative correlations observed here might be due to an increase in locule number, which changes width more than length and translates into a reduction in the fruit shape index.

Genetic map: The linkage maps generated in this study are the result of scoring 90 genetic markers (89 RFLP and 1 CAPs marker) spanning the 12 tomato chromosomes at an average spacing of 12 cM (Figure

FW

	FW	LCN	FL	FD	FS	NSF	SW
LCN	0.73	LCN					
FL	0.92	0.54	FL				
FD	0.96	0.75	0.93	FD			
FS	-0.38	-0.7	NS	-0.49	FS		
NSF	0.36	0.33	0.36	0.44	-0.35	NSF	
SW	0.62	0.41	0.59	0.63	-0.3	NS	SW

FIGURE 3.—Correlations (R) between all fruit size characters in the selected F_2 population ($P < 0.001$). NS, not significant; FW, fruit weight; LCN, locule number; FL, fruit length; FD, fruit diameter; FS, fruit shape index; NSF, number of seeds per fruit; SW, hundred-seed weight.

4). The general order of the markers agreed with the previously published high-density tomato linkage map (TANKSLEY *et al.* 1992). An exception was TG260, which mapped to chromosome 1 despite having been originally mapped to chromosome 4. These results were consistent with previous studies, which showed that TG260 is multiple copy and, therefore, maps to two distinct loci (FULTON *et al.* 1997).

In addition, the linear order and genetic distances presented here correspond to mapping results involving a similar interspecific cross between a processing *esculentum* variety and PM (GRANDILLO and TANKSLEY 1996). Five low-density marker regions ranging in size from 30 to 45 cM were distributed on chromosomes 1, 4, 7, 10, and 12 and correspond to previous marker linkage gaps involving *esculentum* × *pimpinellifolium* crosses (GRANDILLO and TANKSLEY 1996). Two large regions on the tops of chromosomes 7 (TG183–CT52) and 11 (TG384–TG497) deviated significantly from the expected 1:2:1 allele frequency ($P < 0.05$). In both cases, the markers involved became progressively more skewed toward the PM alleles (*i.e.*, a greater number of heterozygous and homozygous PM genotypes) upon moving north (toward the telomeres) on the chromosomes. Two additional markers, TG421 and TG1A, on chromosomes 9 and 10, respectively, diverged from the expected segregation ratios, again in favor of the PM alleles. Such skewed segregation in favor of alleles originating from the wild parent are consistent with previous analyses of interspecific crosses (ZAMIR and TADMOR 1986) and has been detected in the same region of chromosome 7 in another *esculentum* × PM cross (E. VAN DER KNAAP, personal communication).

QTL analysis: Thirty highly significant fruit-size-related QTL ($P < 0.001$) were detected on the basis of single-point linear regression analyses (Table 1). It is important to recognize that the selection of extreme plant progeny based on fruit weight may have resulted in QTL with slightly reduced significance levels and increased R^2 values. This effect is due to changes in gene frequency and the corresponding overestimation of phenotypic effects (LANDER and BOTSTEIN 1989). In
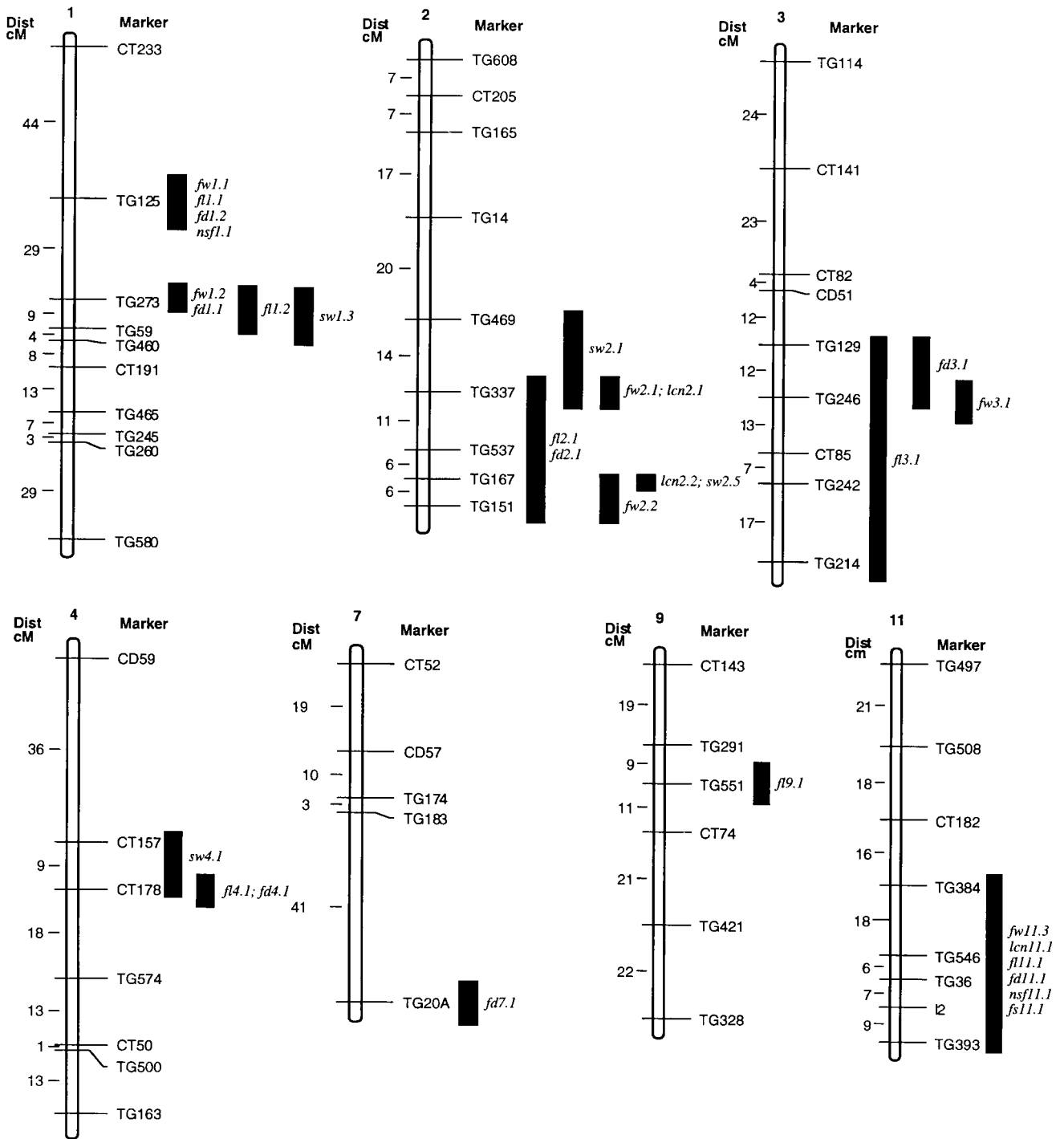
FIGURE 4.—Linkage map derived from the F_2 population resulting from the interspecific cross *L. pimpinellifolium* × *L. esculentum* var. Giant Heirloom. Only those chromosomes with QTL are shown. The numbers on the left side of each chromosome indicate the map distances (in centimorgans) between linked markers. Solid bars indicate marker-trait associations ($P \leq 0.001$) based on single-point regression analyses (see Table 1 and text for details).

our case, it is unlikely that progeny selection significantly affected the QTL analyses for two reasons: (1) selection was minor (*i.e.*, only 73 plants were eliminated out of 188 available for QTL analysis) and (2) significant deviations ($P < 0.05$) from the expected gene frequency of 1:2:1 were not found in any regions where major QTL

were detected. In addition, even if progeny selection resulted in the overestimation of QTL R^2 values, it did not affect the identification and ranking of major fruit size QTL, which were the overall goals of the study. However, to compensate for plant selection, a stringent significance level of $P < 0.001$ was adopted for single-

point analysis to avoid false positives. Interval analyses based on the maximum likelihood method were performed and yielded significance levels and R^2 values similar to those obtained for single-point analyses. For this reason, only the results from single-point analyses are presented.

Fruit weight (fw): Six QTL for fruit weight were identified on chromosome 1 (*fw1.1* and *fw1.2*), chromosome 2 (*fw2.1* and *fw2.2*), chromosome 3 (*fw3.1*), and chromosome 11 (*fw11.3*). The most significant QTL were *fw1.1, fw2.2,* and *fw11.3,* which exhibited R^2 values of 17, 22, and 37%, respectively. The remaining fruit weight QTL showed R^2 values of ~12%. When fit simultaneously, the six QTL explained 67% of the phenotypic variation. Each fruit weight QTL was attributable to the GHT alleles, which served to increase fruit weight. Gene action for the GHT alleles ranged from largely recessive for *fw1.2* ($d/a = -0.9$) and *fw2.2* ($d/a = -0.7$) to largely additive for the remaining fruit weight QTL. The most significant fruit weight QTL, *fw11.3,* exhibited additive gene action ($d/a = -0.1$).

The map positions of the six fruit weight QTL detected in this study correspond to map positions of major fruit weight QTL detected in past studies involving crosses between small-fruited wild tomatoes and medium-sized domesticated types (GRANDILLO *et al.* 1999). In previous studies, the corresponding QTL also accounted for a relatively large proportion of the phenotypic variance (>10%), as observed in this study. However, two aspects of the results presented here are novel. First, this study is the first in which all six of these major fruit weight QTL have been found to be segregating in a single population. Second, two of these loci (*fw2.1* and *fw11.3*) appear to exert their effect on fruit size through modulation of carpel/locule number (see the following sections).

Fruit length (fl) and *fruit diameter (fd):* Seven QTL for fruit length were distributed on chromosomes 1–4, 9, and 11 and had R^2 values ranging from 13 to 30%. The most significant QTL were detected on chromosomes 2 (*fl2.1*) and 11 (*fl11.1*), which explained 25 and 30% of the phenotypic variation, respectively. The simultaneous fit of the seven QTL explained 70% of the phenotypic variation. The increases in fruit length were all due to GHT alleles. A gene action value of 3.8 was calculated for *fl4.1,* which indicated that this QTL might be overdominant in effect. Recessive gene action was identified for *fl1.2* ($d/a = -1.2$), *fl2.1* ($d/a = -0.7$), and *fl9.1* ($d/a = -0.7$), while the remaining GHT QTL alleles were more additive in effect.

Seven QTL were detected for fruit diameter, which, as expected, were coincidental with QTL detected for fruit length, except for *fd7.1.* The GHT alleles were responsible for an increase in fruit diameters at each QTL. Again, the largest QTL were found on chromosomes 2 and 11; *fd2.1* and *fd11.1* explained 24 and 40% of the phenotypic variation, respectively. Simultaneous

fit of the seven QTL explained 78% of the phenotypic variation. All seven fruit diameter QTL exhibited gene action values corresponding to their fruit length counterparts. Five of the seven fruit diameter QTL occupied similar positions as the QTL mapped for fruit weight.

Since nearly all of the fruit length and fruit diameter QTL localized to regions containing major fruit weight QTL, it is likely that their effects are pleiotropic. Such reasoning is logical because as fruit weight increases, there is a corresponding increase in fruit length and fruit diameter. It should be noted that despite lacking a major fruit weight QTL corresponding to *fl4.1* and *fd4.1* in our study, previous results revealed a relatively large QTL for fruit weight (*fw4.1*) in the same region (GRANDILLO *et al.* 1999). In our study, the region of chromosome 4 corresponding to *fl4.1* and *fd4.1* was also associated with a change in fruit weight, but at a significance ($P < 0.002$) slightly less than the established threshold ($P < 0.001$). A similar situation exists for *fl9.1* and *fd7.1.* Both correspond to chromosomal positions previously reported to contain fruit weight QTL (GRANDILLO *et al.* 1999) and both were associated with changes in fruit weight in this study at significance levels just below the declared threshold. Given the prior mapping of fruit weight QTL to these chromosomal regions and the borderline significance observed in our study, it seems likely that these chromosomal regions are involved in modulating fruit size in the GHT × PM population. However, the allelic effects of these three loci are not as great as the other major fruit weight QTL described previously. In fact, when these three threshold QTL were fitted simultaneously with the six major fruit weight QTL, the R^2 value increased modestly to 72%.

Fruit shape index (fs): A single QTL with a large effect on fruit shape that has not been detected previously was found on chromosome 11. *fs11.1* explained 30% of the phenotypic variation and exhibited a partially recessive PM allele ($d/a = -0.6$), which is associated with the formation of more spherical fruit and, therefore, higher fruit shape (fruit length/fruit diameter) values. In contrast, the GHT allele, when homozygous, was associated with much wider fruits without an equivalent increase in fruit length. The result is a fruit that is less rounded and more flattened, like those produced by the GHT parent (Figure 1).

Number of seeds per fruit (nsf): Two QTL located on chromosomes 1 and 11 affected NSF. *nsf1.1,* controlled by the GHT allele, explained 14% of the phenotypic variation and exhibited recessive gene action ($d/a = -1.1$). The GHT allele controlling *nsf11.1* was larger in magnitude of effect ($R^2 = 19\%$) and more additive in gene action ($d/a = 0.16$). Simultaneous fit of the two QTL explained 20% of the phenotypic variation. It should be noted that both of these QTL were coincidental with *fw1.1* and *fw11.3,* indicating that they may be associated with increases in fruit size.

Seed weight (sw): A total of four highly significant seed

weight QTL were identified on chromosome 1 (*sw1.3*), chromosome 2 (*sw2.1* and *sw2.5*), and chromosome 4 (*sw4.1*). Among the QTL, sw4.1 had the greatest effect on the trait, accounting for 23% of the phenotypic variation. Previous studies involving seed weight support this result (DOGANLAR *et al.* 2000). The four QTL fitted simultaneously explained 55% of the phenotypic variation. It has been reported that many seed weight QTL colocalize with fruit weight QTL (DOGANLAR *et al.* 2000). In this study *sw1.3*, *sw2.1*, and *sw2.5* were found in the vicinity of corresponding fruit weight QTL, which is consistent with the high correlation seen between fruit weight and seed weight. However, it has not yet been determined if these associations are the result of pleiotropy or QTL linkage.

Locule number: Three QTL were detected for locule number (lcn). Two were identified on chromosome 2 (*lcn2.1* and *lcn2.2*) and one on chromosome 11 (*lcn11.1*). By far the most significant of the three was *lcn11.1*, which accounted for 65% of the phenotypic variation and was partially recessive in nature ($d/a = -0.5$). *lcn2.1* and *lcn2.2* had R^2 values of 13 and 12%, respectively, and both exhibited partially recessive gene action. When the three QTL were fitted simultaneously they explained up to 66% of the phenotypic variation. Map positions for the three locule number QTL coincide with fruit length, fruit diameter, and fruit weight QTL. All three QTL were explained by the GHT alleles, which served to increase the number of locules per fruit.

lcn2.1 and *lcn11.1* map to regions of the genome where early tomato geneticists described two fruit mutations, *fasciated* (*f;* chromosome 11; MACARTHUR 1934) and *locule number* (*lc;* chromosome 2; YEAGER 1937). Both loci are reported to affect locule number as well as fruit weight, with the largest effect being ascribed to *fasciated*. Given the similar location and phenotypic effects, we propose that *lcn2.1* in this study is the same as *locule number* reported by YEAGER (1937) and that *lcn11.1* is the same as *fasciated* reported by MACARTHUR (1934). Moreover, because of the large effect both loci have on fruit weight in our population (12 and 37%, respectively), we propose that the increase in carpel/locule number associated with GHT alleles of these loci was essential to the evolution of the extreme fruit size now manifest in large tomato varieties such as GHT.

The chromosomal locations for *lcn2.1* and *lcn11.1* are associated with QTL that influence a wide range of other fruit traits. In addition to increasing locule number, the GHT allele for the *lcn2.1* region of chromosome 2 is associated with increased fruit weight, length, and diameter (Figure 4). If the primary effect of *lcn2.1* is to specify more locules (carpels), then the secondary effect of increased fruit size might be expected. Likewise, the GHT allele for the *lcn11.1* region of chromosome 11 was associated with an increase in these same characters. In addition, the GHT allele of *lcn11.1* was associated with an increase in seed number and a decrease in

the fruit shape index (fruit length/fruit diameter; see previous section). A greater increase in fruit diameter compared to fruit length might be expected if the primary effect of this locus was to specify more locules (or carpels). In a similar manner the increased seed production associated with the GHT allele would be expected as a secondary effect of greater locule number. Thus, all the QTL effects ascribed to the *lcn2*.1 and *lcn11.1* regions of the genome could be explained as a direct result of the modulation of carpel number. Hence, all the evidence is consistent with the identification of *lcn2.1* and *lcn11.1* as *locule number* and *fasciated*, respectively.

Finally, as mentioned earlier, fruit weight QTL mapping to the same chromosomal regions as *fw2.1* and *fw11.3* have been reported in crosses between wild tomato species and processing varieties (GRANDILLO *et al.* 1999). However, this is the first time that "alleles" of these QTL have shown an effect on both fruit weight and locule number. The following possibilities therefore exist: (1) multiple alleles exist for both loci, some of which affect locule number and others of which do not; or (2) one or both of these QTL correspond to two or more tightly linked genes with different effects on fruit weight and locule number. Data from this study cannot distinguish between these two hypotheses.

Epistatic interactions involving *lcn2.1* and *lcn11.1*: To determine whether the QTL detected in this study interacted epistatically with each other, two-way ANOVAs were performed among all significant markers within each trait (excluding seed weight) for a total of 56 two-way tests. A probability threshold of $P < 0.01$ was used for declaring an interaction significant. A single highly significant ($P < 0.005$) interaction was detected between *lcn2.1* (TG337) and *lcn11.1* (I2) for the control of locule number. Figure 5 graphically depicts the interaction between these two loci. While either locus alone can increase locule number, a disproportionate increase in locule number is seen when both loci are homozygous for GHT alleles. Such results would be expected if *lcn2.1* and *lcn11.1* code for genes with a similar function in carpel development. A similar type of epistasis was recently reported for the *sepallata 1/2/3* genes, which encode redundant functions in formation of floral organ identity (PELAZ *et al.* 2000). However, in this case, all three genes needed to be fixed for mutant alleles before a modified phenotype was observed.

The finding that *lcn2.1* and *lcn11.1* interact in an epistatic manner in determining locule number has implications for the manner in which the mutant forms of these genes (*i.e.*, those alleles that result in increased locule number) might have been selected following domestication. In the absence of mutant alleles at *lcn11.1*, *lcn2.1* has only a marginal effect on increasing carpel number (Figure 5). However, *lcn2.1* has a much larger effect on increasing carpel number in a background already fixed for mutant alleles at *lcn11.1* (Figure 5).
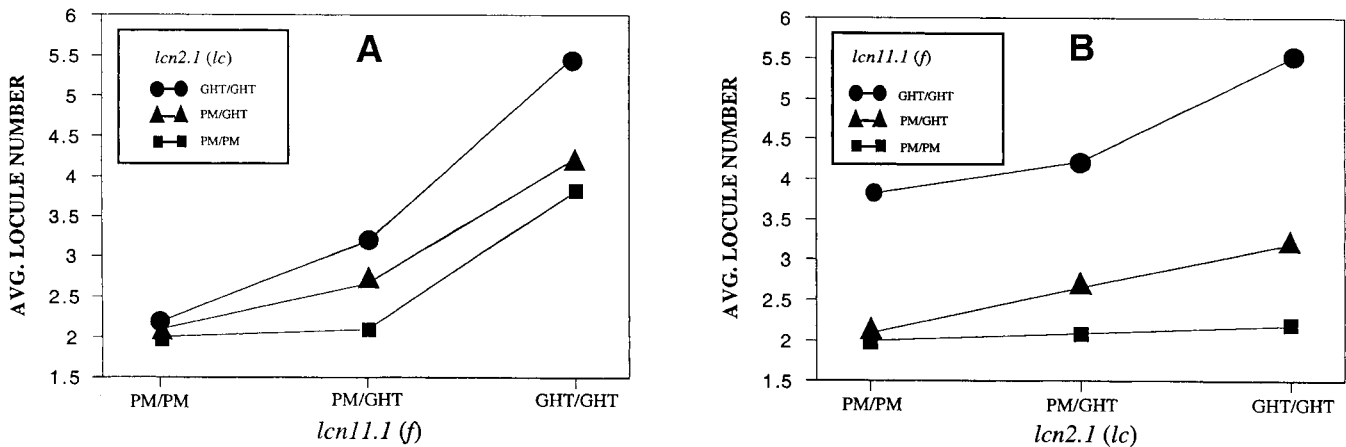
FIGURE 5.—(A) *lcn2.1* (*lc*) × *lcn11.1* (*f*) locule number interaction plot. (B) *lcn11.1* (*f*) × *lcn2.1* (*lc*) locule number interaction plot. PM/PM, homozygous for *pimpinellifolium* alleles; GHT/GHT, homozygous for *esculentum* alleles; PM/GHT, heterozygous; *lc*, *locule number* (TG337); *f*, *fasciated* (I2).

This raises the possibility that humans first selected for mutations at *lcn11.1*, followed by selection at *lcn2.1*, which would produce a mutant phenotype with a larger visible effect on locule number (Figure 5). However, if this scenario is correct, selection would have been primarily for a multilocular phenotype rather than fruit size, since no epistasis was observed for *lcn11.1* and *lcn2.1* with respect to fruit weight (*fw11.1* and *fw2.1*).

CONCLUSION

A unique set of six major fruit weight QTL is responsible for conditioning large-fruited tomato varieties: *L. pimpinellifolium* is the closest living wild relative of the cultivated tomato, and it is thought that large-fruited tomatoes evolved through the accumulation of numerous mutations in the genome of this small-fruited ancestor (LUCKWILL 1943; RICK 1976). This study has further elucidated the major genetic mutations that account for the evolution of very large-fruited, cultivated tomatoes. Previous studies have presented QTL results revealing fruit weight evolution from small wild tomato ancestors to medium-sized processing types only. This study is the first to address the primary set of fruit size and shape QTL that have given rise to the extreme phenotype seen in large fresh-market tomato types. In so doing, we detected six major fruit weight QTL dispersed on chromosomes 1–3, and 11. The unique combination and order of magnitude of this group is key in understanding the evolution and selection of large fresh-market tomato varieties such as Giant Heirloom. Our results show that these six major fruit weight QTL, while reported in previous literature, have emerged together for the first time in a single interspecific tomato cross. Therefore, it is unlikely that any of the fruit weight QTL we detected were due to mutations at previously unreported loci. Rather, we propose that preexisting alleles of *fw1.1*, *fw1.2*, *fw2.1*, *fw2.2*, *fw3.1*, and *fw11.3* came together in unique combination through human

selection relatively recently and have been the crucial genetic determinants increasing fruit size, particularly in the case of the exceptionally large fresh-market esculentum variety Giant Heirloom.

The largest QTL to date has been *fw2.2*, which in early tomato mapping work appeared often and explained as much as 30% of total fruit weight variation (GRANDILLO *et al.* 1999). The current study confirms *fw2.2* to be a significant factor in the generation of large-fruited tomatoes as well as processing types. However, for the first time, *fw2.2* does not have the largest effect on fruit weight. Rather, the QTL with the greatest effect was *fw11.3*. The majority of the effect of this QTL is likely a result of the *fasciated* gene, which has been mapped to the same region as *fw11.3* on chromosome 11.

Role of changes in locule number in permitting evolution of extreme-sized fruit: This is the first QTL study in which changes in carpel/locule number have been implicated as major contributors to fruit size. Classical studies have shown that genes that modify locule number in tomato influence final fruit weight (YEAGER 1937; MACARTHUR and BUTLER 1938). In particular, two loci, *fasciated* (*f*) and *locule number* (*lc*) appear to play crucial roles in specifying the number of carpels/locules and, hence, overall fruit size. The phenotypic effects and chromosomal location of several QTL, *fw11.3*, *fl11.1*, *fd11.1*, *lcn11.1*, *fs11.1*, and *nsf11.1*, are consistent with *fasciated*, and therefore these effects may be the result of the *fasciated* locus. For example, *fasciated* is associated with a change in fruit shape whereby fruits with multiple locules assume a lower value for fruit shape index (fruit length/fruit diameter). The second QTL affecting locule number, corresponding to *lc*, also carried with it an additional set of fruit-size-related QTL (*fw2.1*, *fl2.1*, *fd2.1*, and *lcn2.1*). *fw2.2* was also associated with a change in locule number, but the primary effect of this locus was on fruit weight rather than locule number (Table 1).

The gene responsible for the *fw2.2* QTL has been

TABLE 1

List of major fruit-size-related QTL detected for each trait (*P* < 0.001)

Trait	QTL designation	Marker	Chromosome	Source	% PVE (R^2)	P value	N	AA mean	N	Aa mean	N	aa mean	d/a
Fruit weight (FW)	*fw1.1*	TG125	1	LEG	17	0	26	17.4	56	11.6	32	8.1	−0.3
	fw1.2	TG273	1	LEG	13	0.0006	33	16.4	53	10.3	28	10.0	−0.9
	fw2.1	TG337	2	LEG	12	0.0009	28	16.1	58	11.5	25	8.2	−0.2
	fw2.2	TG167-TG151	2	LEG	23	0	35	17.5	56	10.0	23	8.4	−0.7
	fw3.1	TG246	3	LEG	12	0.0008	19	16.5	67	12.4	28	7.9	0.1
	fw11.3	TG384-TG36-TG393	11	LEG	37	0	25	20.0	44	12.2	32	6.2	−0.1
Locule number (LCN)	*lcn2.1*	TG337	2	LEG	13	0.0004	28	3.6	58	2.8	25	2.4	−0.3
	lcn2.2	TG167	2	LEG	13	0.001	35	3.5	56	2.7	23	2.5	−0.6
	lcn11.1	TG384-I2-TG393	11	LEG	65	0	26	4.6	44	2.7	36	2.1	−0.5
Fruit length (FL)	*fl1.1*	TG125	1	LEG	17	0	26	2.9	56	2.6	32	2.3	−0.03
	fl1.2	TG273-TG59	1	LEG	13	0.0004	33	2.9	53	2.5	28	2.5	−1.2
	fl2.1	TG337-TG167-TG151	2	LEG	25	0	35	3	56	2.5	23	2.3	−0.7
	fl3.1	TG129-TG246-TG214	3	LEG	22	0	19	3	67	2.6	28	2.2	0
	fl4.1	CT178	4	LEG	14	0.0003	23	2.49	61	2.75	29	2.3	3.8
	fl9.1	TG551	9	LEG	14	0.0002	30	2.9	58	2.5	26	2.4	−0.7
	fl11.1	TG384-TG36-TG393	11	LEG	30	0	25	3.0	44	2.6	32	2.2	0.05
Fruit diameter (FD)	*fd1.1*	TG273	1	LEG	13	0.0005	33	3	53	2.5	28	2.5	−0.9
	fd1.2	TG125	1	LEG	21	0	26	3.1	56	2.6	32	2.3	−0.3
	fd2.1	TG337-TG167-TG151	2	LEG	24	0	35	3.1	56	2.5	23	2.4	−0.7
	fd3.1	TG129-TG246	3	LEG	13	0.0004	19	3	67	2.7	28	2.3	0.06
	fd4.1	CT178	4	LEG	12	0.0008	23	2.6	61	2.8	29	2.3	2.8
	fd7.1	TG20A	7	LEG	13	0.0006	28	2.9	64	2.7	21	2.3	0.3
	fd11.1	TG384-I2-TG393	11	LEG	40	0	26	3.3	44	2.6	36	2.2	−0.1
Fruit shape index (FSI)	*fs11.1*	TG546-I2-TG393	11	PM	30	0	26	0.9	56	1	36	1	−0.6
No. of seeds per fruit (NSF)	*nsf1.1*	TG125	1	LEG	14	0.0002	26	66	56	49	32	50	−1.1
	nsf11.1	TG384-TG36-TG393	11	LEG	19	0	25	64	44	55	32	42	0.16
Hundred-seed weight (SW)	*sw1.3*	TG273-TG59-TG460	1	LEG	15	0.0001	33	0.21	50	0.19	31	0.17	0
	sw2.1	TG469-TG337	2	LEG	16	0.0001	27	0.22	53	0.18	31	0.17	−0.5
	sw2.5	TG167	2	LEG	14	0.0003	35	0.21	56	0.18	23	0.18	−2
	sw4.1	CT157-CT178	4	LEG	23	0	21	0.23	60	0.19	33	0.16	0

QTLs are named according to trait abbreviations. The first number following each abbreviation indicates the chromosome number, and the second number distinguishes the QTL mapping to the same chromosome and affecting the same trait (*e.g.*, *fw1.1*). For QTL that were significant for more than one adjacent marker, the two flanking markers are given with the most significant marker underlined. Sources: LEG, *L. esculentum*; PM, *L. pimpinellifolium*. % PVE (R^2), percentage phenotypic variation explained; N, number of plants; mean = average phenotypic value for plants with the following genotypes: AA, homozygous *esculentum*; Aa, heterozygous; aa, homozygous *pimpinellifolium*; *d/a*, gene action for each QTL.

cloned and appears to control cell division early in carpel/fruit development rather than specifying carpel number (FRARY *et al.* 2000). Thus far, the large-fruited allele of *fw2.2* has been present in all medium- and large-fruited tomato cultivars examined, regardless of their size. *fasciated* and *locule number*, however, are not normally found in medium-sized bilocular tomato varieties, which indicates that they may be the key genetic mutations that were selected for following domestication to give rise to large-fruited tomatoes (GRANDILLO *et al.* 1999). In fact, this study suggests that *fasciated* is acting in concert with *locule number* to condition extreme fruit size as is seen in the variety Giant Heirloom. Further genetic analysis of these regions will help elucidate the genetic effects of these QTL within other large-fruited multilocular varieties and will ultimately lead to the molecular cloning of these loci. This knowledge may then enable plant molecular geneticists to identify and isolate similar key QTL/genes that have influenced fruit weight in other cultivated plants such as pepper, eggplant, melon, and citrus, thus further increasing our understanding of fruit evolution in other crop species.

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