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The genetic basis of pear-shaped tomato fruit

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Abstract Molecular-marker analysis of a cross between yellow pear, a tomato variety bearing small, pear-shaped fruit, and the round-fruited, wild species, *Lycopersicon pimpinellifolium* LA1589, revealed that pear-shaped fruit is determined largely by a major QTL on chromosome 2 and, to a lesser extent, a minor QTL on chromosome 10. The locus on chromosome 2 was also detected in a cross between yellow pear and the round-fruited introgression line (IL2–5) which carried the distal portion of chromosome 2 from the *Lycopersicon pennellii* genome. Based on its map position, we propose that the locus detected on chromosome 2 is the same as a locus referred to as *ovate* in the early tomato literature (Linstrom 1926, 1927). The fruit-shape index (length/diameter) and neck constriction were highly correlated in both populations suggesting that *ovate* exerts control over both traits or that the genes for these traits are tightly linked on chromosome 2. Using two-way ANOVA test, the minor QTL on chromosome 10 showed no significant interaction with the *ovate* locus on chromosome 2 with respect to the fruit-shape index. For *ovate* round fruit was dominant to elongated fruit in the *L. pimpinellifolium* populations, but additive in the IL2–5 population. Thus far, no genes controlling fruit shape have been cloned. The molecular mapping of the *ovate* locus may ultimately lead to its isolation via map-based cloning.

Key words Molecular markers · Mapping · Quntitative trait loci · Fruit development · Map-based cloning

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Introduction

In contrast to wild tomato species, which typically bear small, round fruit adapted for seed production and dispersal, cultivated tomato varieties bear fruit with extreme variation in both size and shape. While probably not adaptive in the wild, these unusual fruit types have been selected and propagated by humans, either for their utility or for their sheer novelty. One can now find tomato varieties bearing long slender fruit resembling those of Japanese eggplants, for example, an heirloom variety “Sausage” (Anonymous 1999), or fruit nearly identical in appearance to bell peppers (Frary et al. 1998).

One of the most striking fruit types is that of the heirloom variety, Yellow Pear, which bears small, oval-shaped fruit with a distinct constriction at the stem end (Fig. 1). This variety is not widely grown anymore, but is still used for pickling, canning and making preserves, and was one of the favorite varieties of U.S. President Thomas Jefferson (DuBose 1985).

In the early part of this century it was proposed that a single recessive gene is responsible for pear-shaped tomato fruit (Hendrick and Booth 1907; Price and Drinkard 1908). The locus controlling pear shape was called *pr* (Hedrick and Booth 1907) but was later renamed *o* (*ovate*) after it was shown to cosegregate with the locus conditioning oblate-oval fruit shape (MacArthur 1925; Lindstrom 1926, 1927). *ovate* was placed on the linkage group corresponding to chromosome 2 by virtue of its linkage to the *dwarf* locus. In addition, it was demonstrated that early removal of the corollas from pear-shaped tomatoes results in oval-shaped fruit and thus pear shape may be a pleiotropic effect of constricting corollas (Yeager 1937). More recently, in the study of Warnock (1990a), an unlinked gene, constricting corolla (*cc*), was proposed to control pear shape. In the presence of *ovate*, *cc* was suggested to be associated with a change from an oval to a pear-shaped fruit (Warnock 1990b). It was also suggested that variation in the severity of the constriction of the fruit within the same plant can be explained by fruit size, temperature and moisture conditions (Warnock 1990c).

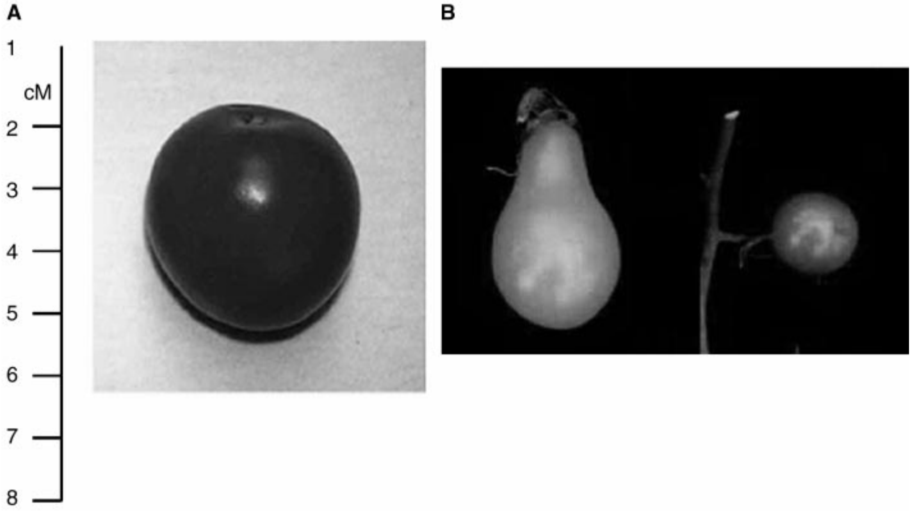


Fig. 1 Fruit of: **A** *L. esculentum* IL2–5; **B** *L. esculentum* cv Yellow Pear TA503 (left), and *L. pimpinellifolium* LA1589 (right)

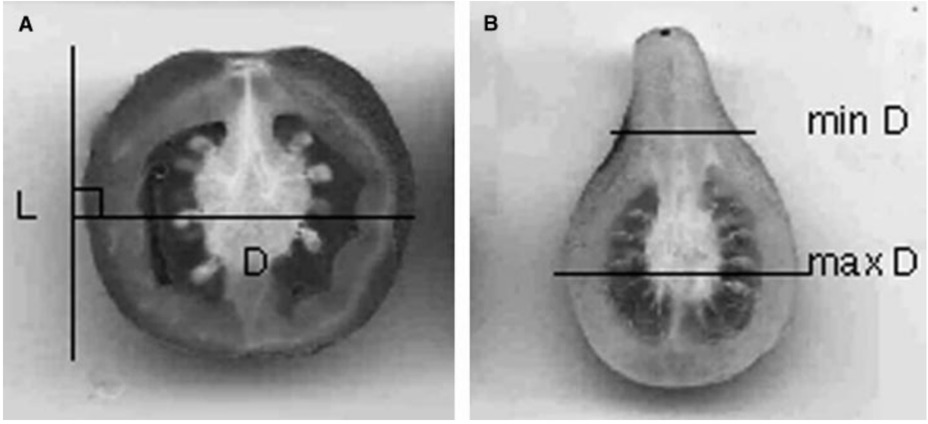


Fig. 2. Measurement of **A** fruit-shape index=L/D and B neck-constriction index=minD/maxD. *L*=the length of the fruit; *D*=the equatorial diameter; *minD*=the minimal diameter; *maxD*=the maximal diameter

The goals of the present study were to determine the genetic basis by which the Yellow Pear variety produces elongated fruit with a distinctive neck constriction and to locate the loci responsible for these characteristics on the tomato high-density molecular map as a prerequisite for map-based cloning. Thus far, few genes controlling fruit shape in plants have been identified and none have been cloned. Isolation of such genes should provide new insights into fruit developmental biology and new opportunities for engineering fruit crops.

Materials and methods

Population development

L. pimpinellifolium population: *L. esculentum* cv Yellow Pear, TA503 (hereafter referred to as YP) was crossed to the small red-fruited, wild species *L. pimpinellifolium* LA1589 (hereafter referred to as PM) (Fig. 1). The resulting hybrid was selfed to produce an F_2 population. A total of 82 F_2 plants were transplanted to the greenhouse in Ithaca, N.Y., for phenotypic evaluation and DNA extraction for molecular mapping.

L. pennellii population: YP was crossed to the round-fruited introgression line IL2–5 which carries the distal portion of chromosome 2 from the *L. pennellii* genome in an otherwise *L. esculentum* background (Eshed and Zamir 1994) (Fig. 1). Sixty F_2 plants (hereafter referred to as PN), were transplanted to the field in Ithaca, N.Y., for phenotypic evaluation and molecular mapping.

Phenotypic analysis

A minimum of ten ripe fruit per plant were evaluated for fruit length and diameter. The fruit-shape index (L/D) was calculated as the ratio of fruit length to diameter. In addition, the constriction of fruit (hereafter referred to as the ”neck-constriction index”) was measured by the ratio of minimal diameter (minD) to maximal diameter (maxD) (Fig. 2). All the measurements were taken with a hand-held caliper.

Genotypic analysis

For the PM population, parental DNA was digested with six restriction enzymes (*Hin*dIII, *Dra*I, *Eco*RV, *Xba*I, *Eco*RI, and *Sca*I) and subjected to Southern-blot analysis as described by Bernatzky and Tanksley (1986). A total of 20 markers (cDNA and genomic) on chromosome 2 and 80 markers on the other 11 chromosomes that showed polymorphism between YP and LA1589 (Tanksley et al. 1992; Grandillo and Tanksley 1996) were used to perform QTL analysis on the 82 F_2 plants in this population.

The 60 PN F_2 plants were subjected to the same procedures except that the restriction enzymes *Bst*NI, *Eco*RI, and *Eco*RV and *Dra*I were used (surveyed on YP and IL2–5) and 15 informative RFLP markers from chromosome 2 were probed.

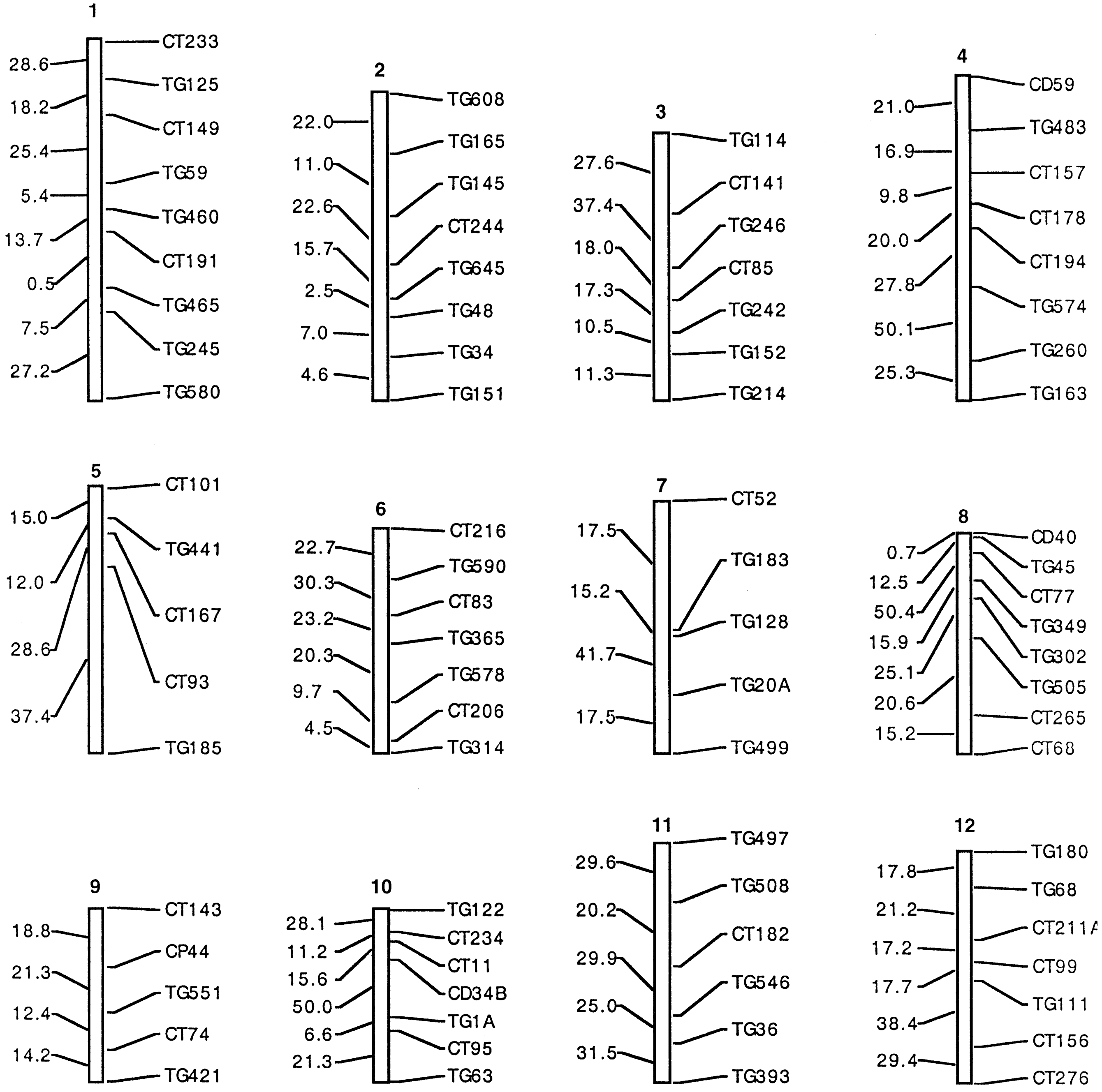


Fig. 3 Linkage map derived from the PM population. The *numbers* listed on the left side of each chromosome indicate the map distances (cM) between markers

Statistical analysis

Linkage analysis of chromosome 2 markers on the 82 PM plants and 60 PN plants was performed using the software package MAPMAKER V2.0 (Lander et al. 1987). Markers and their corresponding distances (cM) were included within the framework map only if the LOD value for the ripple was >3. The Kosambi mapping function was used to convert recombination frequencies to map distances in cM (Kosambi 1944).

The genome-wide QTL analysis was performed by both a single-point linear model and interval analysis using QGENE (Nelson 1997). The R-square explained by *ovate* was obtained by the single-point linear model from QGENE. For fruit-shape index, two-way interactions were tested for each significant QTL via two-way ANOVA using QGENE.

Results and discussion

QTL analysis

A total of 82 polymorphic markers covering the 12 chromosomes of tomato were mapped on 82 PM F_2 plants (Fig. 3) and tested for association with fruit shape. The results from QTL interval mapping (Fig. 4) were consistent with those from a single-point linear model (Table 1). In summary, the fruit-shape index (L/D) was found to be controlled by two loci; a major locus near TG645 on

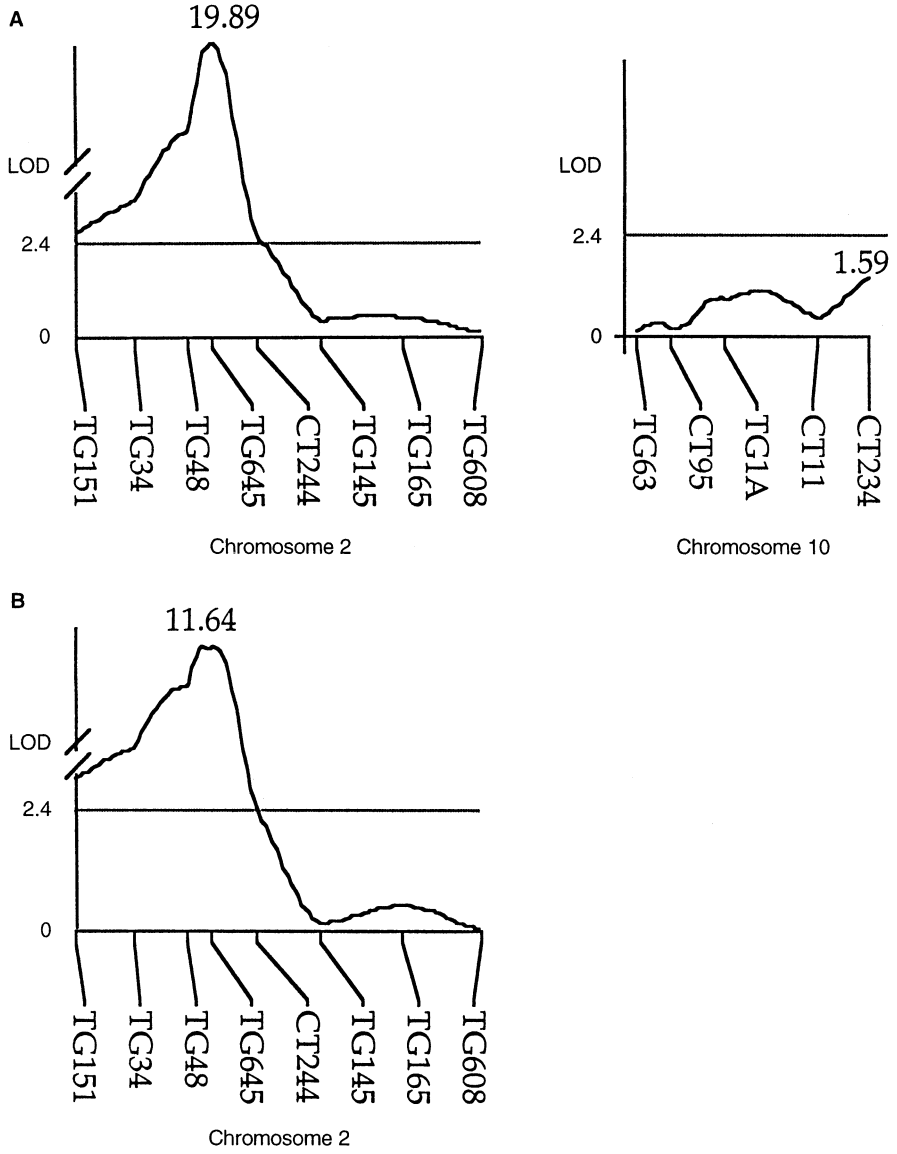


Fig. 4 The significant loci detected by interval mapping for **A** fruit-shape index **B** neck-constriction index in the PM population

chromosome 2 (*P*<0.0001) and a locus of lesser significance (*P*=0.1004) near CT234 on the top of chromosome 10. For both loci, the YP alleles caused fruit to be more elongated (L/D>1.00). The loci on chromosomes 2 and 10 explained 67% and 17% of the variance for L/D, respectively. However, only the locus on chromosome 2 showed a significant association with the constriction at the stem end of the fruit, explaining 48% of the phenotypic variation for this character. The YP allele for the locus on chromosome 2 showed largely recessive gene action compared with the PM allele for both L/D (D/A=–0.91) and the neck constriction (D/A=–0.83). In addition, the YP allele for the locus on chromosomne 10 also showed recessive gene action for L/D (D/A=–1.14).

Two-way ANOVA analysis revealed no significant interaction between TG645 (chromosome 2) and CT234 (chromosome 10) in the control of L/D, which suggests that these loci act independently in controlling fruit-shape development. Grandillo et al. (1996) reported a locus on chromosome 8 (*fs8.1*) controlling round versus blocky fruit-shape in crosses between processing tomato and wild tomato species such as *L. pimpinellifolium* However, *fs8.1* was not detected in the current study, most likely because the *L. pimpinellifolium* and YP parents both carry round-fruited alleles, hence the locus did not segregate in this population.

The QTL detected on chromosome 2 most likely corresponds to the *ovate* locus

The phenotypic effect, chromosomal localization and gene action of the QTL detected on chromosome 2 are consistent with a locus referred to as *ovate* in the early tomato literature (Hederick and Booth 1907; MacArthur 1925; Lindstrom 1926, 1927). The finding that the fruit-shape index and neck constriction both map to the same locus on chromosome 2 is also consistent with these ear-

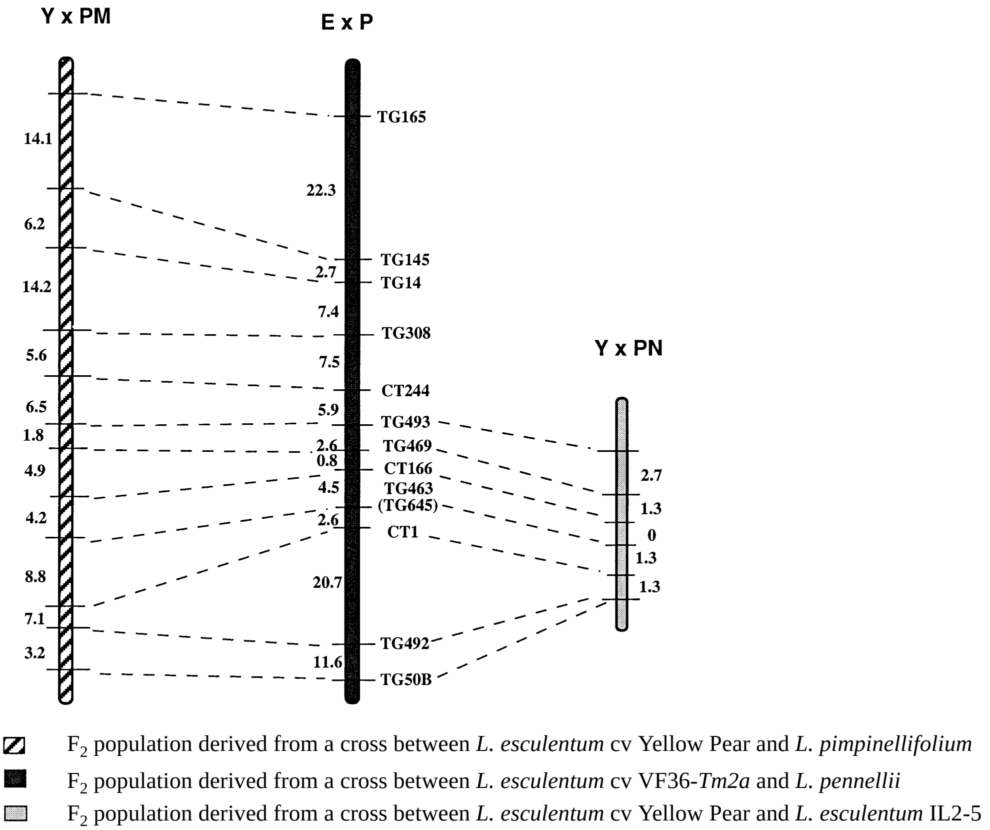
Table 1 Comparisons of fruit-shape index (A) and neck-constriction index (B) between different genotypes in PM and PN populations

Marker[a]	Population[b]	% of R-square	*P*	YY	N	PM/PM or PN/PN	N	Y/PM or Y/PN	N	D/A
(A) Fruit-shape index										
TG645	PM	67.2	<0.0001	1.32	22	1	11	1.02	49	–0.9
CT234	PM	16.8	0.004	1.2	16	1.06	20	1.05	27	–1.1
TG645	PN	46.3	<0.0001	1.35	12	1	16	1.14	32	–0.1
(B) Neck-constriction index										
TG645	Pm	47.5	<0.0001	0.75	22	0.88	11	0.88	49	–0.8
CT234	Pm	10.1	0.0406	0.8	16	0.85	20	0.87	27	–2
TG645	PN	54.3	<0.0001	0.41	12	0.78	16	0.61	32	–0.1

[a] The chromosome marker which showed most significant associations with fruit-shape and neck-constriction index

[b] PM=F_2 population derived from a cross between PM and YP; PN=F_2 population derived from a cross between PN and YP D/A=degree of dominance of the YP allele

Fig. 5 Comparisons of interval distances of chromosome 2 markers in the PM (Y×PM) and PN (Y×PN) populations and the tomato high-density map (E×P) (Tanksley et al. 1992)



▨ F_2 population derived from a cross between *L. esculentum* cv Yellow Pear and *L. pimpinellifolium*

■ F_2 population derived from a cross between *L. esculentum* cv VF36-*Tm2a* and *L. pennellii*

□ F_2 population derived from a cross between *L. esculentum* cv Yellow Pear and *L. esculentum* IL2-5

ly studies of *ovate*. An attempt to locate the stocks used in the early studies was unsuccessful, hence allelism testing was not possible. However, observing the rule of parsimony, we propose that the chromosome-2 locus detected in the current study is the same as *ovate* and hereafter we refer to this locus by that name.

Independent verification of the *ovate* locus in the PN population

In order to verify the position of *ovate* on the molecular linkage map, an F_2 population was developed from a cross between YP and IL2–5. IL2–5 contains only the lower portion of chromosome 2 (including the *ovate*-containing region) from the wild species *L. pennellii*, while the rest of the genome is from the modern *esculentum* variety M82 (Eshed and Zamir 1994, Fig. 2). *L. pennellii* and *L. esculentum* differ for alleles at most marker loci, hence molecular probes in the *ovate* region, not polymorphic in the PM F_2, could be examined. Also, the total phenotypic variation for fruit and plant characteristics was reduced in this F_2 since the remainder of the genome in IL2–5 is from *L. esculentum*, as is YP.

The linear order of the RFLP markers of chromosome 2 in the PN population (15 markers) was consistent with both the PM map (20 markers) and the previously published high-density linkage map (Tanksley et al. 1992,

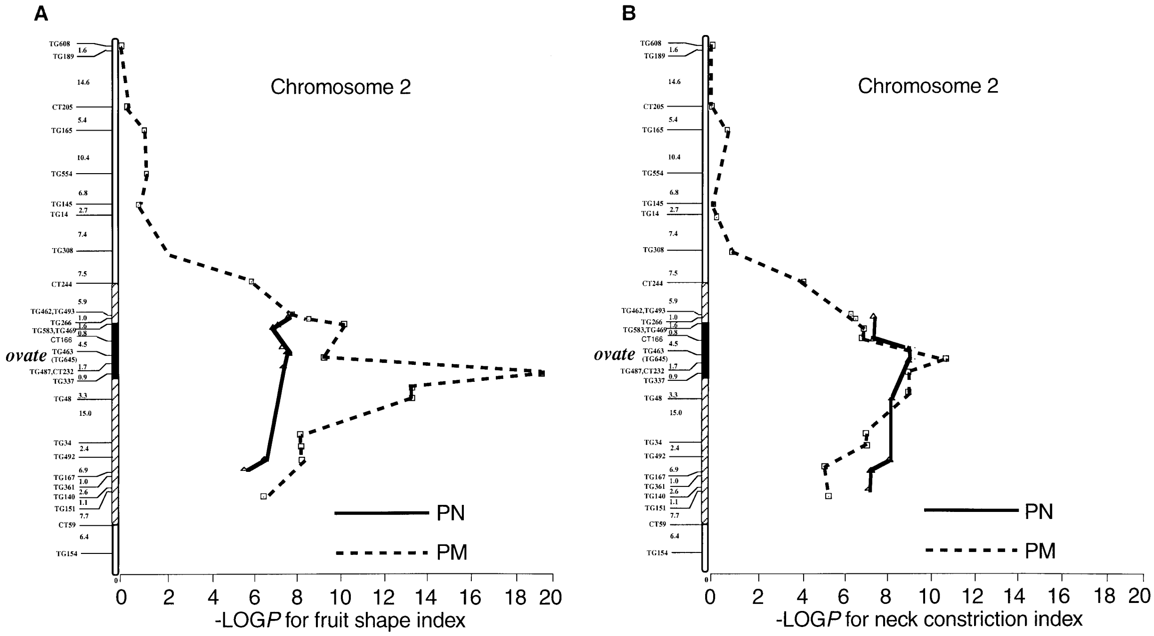


Fig. 6 The association between **A** fruit-shape index, **B** neck-constriction index and tomato chromosome 2 markers in the PN population (*solid line*) and PM population (*dashed line*). The *P* value of each marker was obtained from a single-point linear model using the program QGENE (Nelson 1997). Map distances are based on the previously published tomato high-density linkage map (Tanksley et al. 1992). The *hatched box* corresponds to the *L. pennellii* region of chromosome 2 contained in the introgression line (IL2–5). The *black filled area* indicates the likely position of *ovate*

Fig. 5). However, there were some examples where the map distances between markers differed significantly. For example, the interval between CT1 and TG492 was 20.7 cM in the high-density map but only 7.1 cM and 1.3 cM in the PM and PN maps, respectively. The total recombination in the PN segment (from TG493 to TG50B) was 6.6 cM, much less than in the PM and the high-density map populations, 30 cM and 42.8 cM (Fig. 5). This reduced recombination is most likely due to the fact that the PN population involved an intogressed segment of DNA on chromosome 2, whereas the rest of the chromosome was composed of DNA from the cultivated tomato. Previous studies have shown that recombination typically is reduced when less than an entire introgressed chromosome is involved in pairing with a chromosome from the cultivated tomato (Paterson et al. 1990). This may be due to crossing-over occurring preferentially in the non-introgressed portion of the chromosome (Paterson et al. 1990).

A locus with a major effect on the fruit-shape index (L/D) and the neck-constriction index (minD/maxD) was localized to the same chromosome 2 region in the PN and PM populations. In both populations, TG645 showed the most significant association with both traits (Fig. 6). The amount of phenotypic variation explained by *ovate* in the PM population was 67.2% for L/D, versus 46.2% for PN, with 47.5% for the pear-shaped index in the PM, versus 54.3% for PN. The *ovate* allele from YP in the PM cross showed recessive gene action compared with the PM allele; however, in the PN population the *ovate* allele behaved in an additive manner (D/A=0.06) for both L/D and the neck-constriction index (Table 1). This suggests that the *ovate* alleles in YP, PN and PM are all distinct, despite the fact that both PN and PM alleles condition round fruit without a constriction. Interestingly, homozygous YP gave on average a more elongated fruit-shape index (2.44) in the PN population than in the PM population (1.30) which may be due to the fact that *fs8.1* also segregates in the PN population, but not in PM (Grandillo et al. 1996). Moreover, plants with much more extremely elongated pear-shaped fruit were found in the PN population which suggests an interaction with the genetic background.

Map position and gene action consistent with a single gene controlling both fruit length and fruit constriction

In both the PM and PN populations, genetic control of the fruit length and the constriction at the stem end of fruit mapped to the same locus suggesting either a single gene with pleiotropic effects or two tightly linked genes, each affecting a separate character. The fact that the gene action was similar for both traits in each population is more consistent with the pleiotrophy hypothesis, since correlation in gene action would not be predicted by linked, but discrete loci (Table 1).

Implications and future work

Nearly all wild tomato species have small round fruit. In contrast, cultivated tomatoes display a wide range of sizes and shapes. The current study shows that both red-fruited (*L. pimpinellifolium*) and green-fruited (*L. pen-*

nellii) tomato species contain the wild-type *ovate* allele conditioning rounded fruit without a neck constriction. One possible explanation is that elongated- and pear-shaped fruit in modern cultivars, such as Yellow/Red Pear and Yellow/Red Plum, are a result of a mutation in the *ovate* gene that might have been selected during the domestication and breeding process. A similar macromutation involving the domestication of crop varieties from their wild ancestors has been described for tomato fruit size (Alpert et al. 1995). The eventual molecular cloning of the *ovate* locus may open the door to a better understanding of the molecular basis of fruit development and the process of domestication of fruit-bearing plants.

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