Fine mapping of quantitative trait loci for improved fruit characteristics from *Lycopersicon chmielewskii* chromosome 1

A. Frary, S. Doganlar, A. Frampton, T. Fulton, J. Uhlig, H. Yates, and S. Tanksley

Abstract: The near-isogenic line (NIL) TA1150 contains a 56-cM introgression from *Lycopersicon chmielewskii* chromosome 1 and has several interesting phenotypic characteristics including fruit with orange color, high levels of soluble solids, thick pericarp, small stem scars, and good firmness. A set of overlapping recombinant lines (subNILs) was developed and field tested to fine map the quantitative trait loci (QTL) controlling these traits. The results indicated that the solids, pericarp thickness, and firmness QTL are distinct from the color locus. Several of the QTL mapped in this study, including the soluble-solids QTL, probably correspond to QTL mapped in other wild species of tomato. However, analysis of a set of TA523 subNILs containing complementary introgressions from *Lycopesicon hirsutum* chromosome 1 suggests that this wild species may contain a different locus for improved soluble solids. Thus, it might be possible to combine the *L. chmielewskii* and *L. hirsutum* alleles for these loci in a single line with the potential for extremely highly soluble solids. The TA1150 subNIL TA1688 contains the smallest introgression of the solids locus (approximately 19 cM), as well as the pericarp thickness and firmness QTL, with a yield that was equivalent to two of the three control lines. Isolation of recombinant subNILs from TA1688 should break the linkage between orange color and high solids and provide a small introgressed segment for marker-assisted breeding and genetic improvement of processing tomato.

Key words: tomato, QTL, soluble solids, Brix, colour.

Résumé : La lignée quasi-isogénique (NIL) TA1150 contient un segment de 56 cM introgressé à partir du chromosome 1 du *Lycopersicon chmielewskii* et elle présente de nombreuses caractéristiques phénotypiques intéressantes dont des fruits orange, une forte teneur en solides solubles, un péricarpe épais, de petites cicatrices axillaires et une bonne fermeté. Des lignées recombinantes chevauchantes (subNIL) ont été développées et évaluées au champ pour réaliser une cartographie fine des locus à caractère quantitatif (QTL) contrôlant ces caractéristiques. Les résultats indiquent que les QTL pour les solides, l'épaisseur du péricarpe et la fermeté sont différents de ceux contrôlant la couleur. Plusieurs des QTL qui ont été localisés au cours de cette étude, dont les QTL pour les solides solubles, correspondent vraisemblablement à des QTL identifiés chez d'autres espèces de tomate sauvages. Cependant, une analyse de subNIL du TA523 qui comprend des introgressions supplémentaires du chromosome 1 du *Lycopesicon hirsutum* suggère que cette espèce pourrait contenir un locus différent pour ce qui est de la teneur en solides solubles. Ainsi, il pourrait s'avérer possible de combiner les allèles du *L. chmielewskii* et du *L. hirsutum* pour ces deux locus chez une seule lignée, laquelle présenterait potentiellement une très forte teneur en solides solubles. La subNIL TA1688 de la lignée TA1150 contient la plus petite introgression du locus des solides solubles (environ 19 cM) de même que les QTL pour l'épaisseur du péricarpe et la fermeté, tout cela avec un rendement égal à celui de deux des trois témoins. Le développement de lignées recombinantes subNIL à partir de TA1688 devrait permettre de briser l'association entre la couleur orange des fruits et la forte teneur en solides. Cela fournirait également un petit segment introgressé pour des fins de sélection assistée de marqueurs et pour l'amélioration génétique de la tomate de conserve.

Mots clés : tomate, QTL, solides solubles, Brix, couleur.

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Introduction

Wild germplasm is a tremendous source of novel genetic variability for the improvement of crop species (Tanksley and McCouch 1997). However, the use of such unadapted material to improve a cultivar for one or more traits can be difficult because of linkage drag, the transfer of linked, undesirable loci with the gene(s) of interest. Linkage drag can be especially problematic when loci from multiple sources are pyramided into an elite line. When too much unadapted DNA is introgressed into a cultivar, important agronomic traits such as yield can degrade to unacceptable levels. An obvious way of reducing linkage drag is to minimize the amount of unadapted genome introgressed into an elite line. The advent of molecular mapping techniques has made it possible to map quantitative trait loci (QTL) to specific chromosomal regions (Tanksley 1993) and to target breeding efforts using near-isogenic lines (NILs). Thus, the use of molecular markers and NILs can significantly reduce the amount of wild germplasm introduced into a line. In many cases, however, even an NIL may still contain both beneficial and undesirable traits from the wild species. Therefore, it is often necessary to fine map QTL within an NIL to further reduce linkage drag and develop lines with smaller introgressions (subNILs) that are even more useful for breeding. In addition, such mapping may allow one to determine if the favorable effects of the introgression are indeed caused by linkage drag (i.e., separate, linked loci) or by the pleiotropic effects of a single locus.

TA1150 is a tomato NIL that contains a chromosome 1 introgression (56 cM) from the wild species *Lycopersion chmielewskii* in the *Lycopersicon esculentum* cv. E6203 background. This introgression has been reported to cause a significant increase in soluble-solids content (Paterson et al. 1990). Soluble-solids content is of paramount importance for processing tomatoes, because lines with higher sugar content require less energy input (i.e., less cooking) during the concentration process. In addition to high Brix, TA1150 has orange fruit that are slightly pear shaped, firmer, and have a thicker pericarp and smaller stem scar than 'E6203'. Fruits with thick pericarps are desirable for processing because they yield more product when diced. Fruits with smaller stem scars release from the stem better during harvest, are easier to peel, and result in a more attractive whole or diced product. For effective use in processing tomato improvement, the favorable traits contained within the introgression (high solids, firmness, thick pericarp, and small stem scar) must be uncoupled from orange fruit color. Therefore, the objectives of this study were to determine the number of loci controlling each trait within the introgression, to map these QTL using a set of overlapping recombinant subNILs, and to determine whether the phenotypic effects exhibited by TA1150 are caused by linked loci or by a single locus with pleiotropic effects. In addition, QTL for some of the same traits were mapped using a set of overlapping recombinant TA523 subNILs containing chromosome 1 introgressions from a different wild species (Monforte and Tanksley 2000). TA523 contains a 40-cM introgression of the distal portion of chromosome 1 from *Lycopersicon hirsutum* and reportedly has high soluble-solids content, enhanced fruit color, and reduced yield (Bernacchi et al. 1998*a*). Comparison of the locations of the QTL identified in these two complementary sets of subNILs suggested whether the similar phenotypic effects of the *L. chmielewskii* and *L. hirsutum* introgressions were the result of allelism or distinct QTL.

Materials and methods

Development of subNILs

For development of the TA1150 subNILs, a total of 380 F_2 plants were derived from a cross between the *L. esculentum* processing inbred 'E6203' (TA209) and the *L. chmielewskii* NIL TA1150 (Paterson et al. 1990). DNA was extracted from the plants, digested with *Eco*RI, separated by electrophoresis through 1% w/v agarose, and Southern blotted as described by Bernatzky and Tanksley (1986). RFLP marker probes were selected from the high-density tomato map (Tanksley et al. 1992). Recombinant plants were identified by hybridization of the Southern blots with TG19 and TG27, the RFLP markers that flanked the TA1150 introgression (Fig. 1). Putative recombinant plants were selfed to generate F_3 lines. The F_3 plants were screened with TG19, TG27, 12 additional RFLP markers (CT267, TG237, CT163, TG245, TG375, TG17, TG157, TG369, TG267, TG158, TG258, TG269), and 2 PCR assays (TG83 and TG142) in the interval to determine the exact location of their recombination break points and to select homozygous individuals. PCR primers were as follows: TG 83 forward, 5′-TTGCACATGACGAAAGAAGC-3′; TG83 reverse, 5′-TGGCTCTAATCCTTGGATGG-3′; TG142 forward, 5′-ATGAAAGGAGCCGATCACAC-3′; TG142 reverse, 5′-AGGAGGCCCAACTCTCACTT-3′. The 100-μL amplification reaction mixtures contained approximately 100 ng genomic DNA, 10 mM Tris–HCl (pH 8.0), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% w/v gelatin, 200 μM of each dNTP, and 1 U *Taq* DNA polymerase. PCR amplification was performed in a Perkin Elmer Thermocycler using the following profile: 35 cycles of 94°C for 1 min, 50°C for 1 min, and 72°C for 2 min, followed by 72°C for 5 min, and then held at 4°C. The 600-bp product of the TG83 primers was digested with *Taq*I and the 900-bp product of the TG142 primers was digested with *Eco*RI. After digestion, the TG83 assay gave diagnostic fragments of 400 bp for TA209 and 450 bp for TA1150. Similarly, the TG142 assay gave fragments of 500 and 600 bp for TA209 and TA1150, respectively.

A total of 39 independent subNILs were isolated in this way. A molecular linkage map for the bottom of chromosome 1 was generated from the TA1150 F_2 population using the Mapmaker 3.0 program (Lander et al. 1987). The Kosambi mapping function (Kosambi 1944) was used to convert recombination frequencies to map distances in centimorgans (cM). The development of the TA523 subNILs is described in Monforte and Tanksley (2000). Eleven subNILs from that work were field tested with the TA1150 subNILs.

Field evaluations

Field evaluations for the TA1150 subNILs were conducted at two locations: Woodland, Calif., and Ithaca, N.Y. In Woodland, a subset of 28 homozygous TA1150 subNILs, the 2 controls (TA209 and TA1150), and 2 commercial stan-

dards (BOS3155 and H8892) were transplanted to the field on 12 April 2000 in plots of 10 plants each with 3 replications of each plot at a single location. In Ithaca, a complete random block experimental design was used for the 39 subNILs. Thus, a single plant for each homozygous subNIL was randomly assigned a position within each block and 10 blocks of the subNILs were transplanted to the field on 1 June 2000. At least 20 plants for each of the controls were also included within the blocks. Trait evaluations were done in late July in Woodland and in early October in Ithaca. Because one section of the field in Ithaca had poor growing conditions, some plants were excluded from the experiment. However, in most cases, data could be collected from eight or nine replicates. The 11 TA523 subNILs were grown only in Woodland using the same experimental design that was used for the evaluation of the TA1150 subNILs.

Trait evaluations

The 33 and 15 traits measured in Woodland and Ithaca, respectively, are listed in Table 1. Details of trait evaluations are given below.

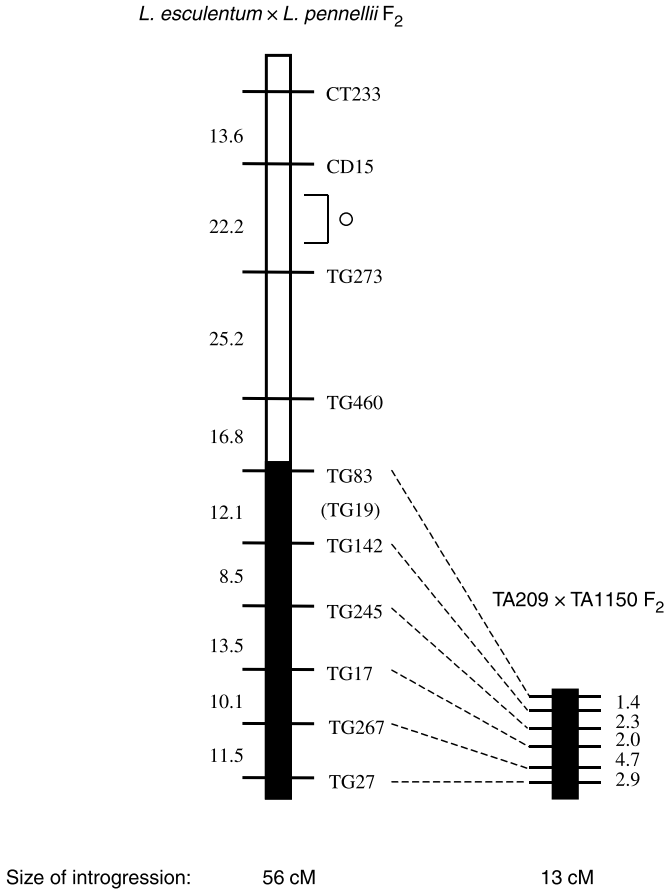
Fruit color

Fruit color was evaluated in several ways. External (ECol) and internal (ICol) fruit color was determined visually on 10 ripe fruit from each plot (Woodland) or plant (Ithaca) using a scale from 1 to 5. These color measurements took into account both the hue and intensity of fruit color as follows: 1, light or medium orange; 2, dark orange or light red; 3, medium red; 4, dark red; and 5, very intense dark red. Internal orange (IOrange) fruit color assessed only whether or not there was any orange coloration in the fruit interior and was also rated using a scale from 1 to 5 with 1 being no orange and 5 being completely orange. The lycopene content (ppm) was measured on a fresh weight basis for both a small subset (FrLyco) and a larger sample (50–75 fruit; Lyco) of ripe fruit. Chromaticity (Chroma), redness (A/B), and hue (Hue) were measured for deaerated, deseeded, and deskinned tomato pulp using a Minolta CM-508d Spectrophotometer using specular component excluded, 10° observer, and D65 illuminant. The colorimetric notation was according to Hunter Lab.

Fruit quality

All fruit quality traits were evaluated for 10 ripe fruit per plot/plant. Fruit weight (FW) was determined by weighing (in grams) the 10 fruit for each line. Firmness (Firm) was evaluated by hand squeezing the fruit (1, soft; 5, very firm). Puffiness (Puff) was the amount of intralocular air space in transversely cut fruit (1, no air space not puffy; 5, very puffy). Stem-scar size (Scar) was based on a visual examination of the fruit (1, small scar; 5, large scar). Pericarp thickness (Wall) was evaluated for transversely cut fruit at the midpoint of the locules and measured in millimeters in the Woodland population and on a scale from 1 to 5 (1, thin; 5, thick pericarp) in the Ithaca population. Fruit cracking (Crack) was also rated on a scale from 1 (no cracks) to 5 (many cracks), as was the amount of white vascular veins (Veins) visible in ripe, transversely cut fruit (1, no venation; 5, much venation).

Fig. 1. Comparison of chromosome 1 linkage maps for the *L. esculentum* × *L. pennellii* (Tanksley et al. 1992) and TA209 × TA1150 F_2 populations. The shaded region shows the extent of the introgression in TA1150. The position of the centromere is also indicated.



Fruit shape

Several different fruit-shape parameters were measured. In Woodland, overall fruit shape (Shape) was determined visually using a scale from 1 to 5 (1, round; 3, blocky; 5, very elongated). Pear shape (Pear) was also evaluated using a scale from 1 (symmetric fruit) to 5 (pear-shaped fruit). In Ithaca, twp fruit per plant were cut transversely so that fruit length (FLeng), fruit width (FWid) at the widest point, and fruit length to the widest point could be measured (in cm). These measurements were then used to calculate the fruit-shape (ShInd = FLeng/FWid) and -eccentricity (EccInd = length to widest point/FLeng) indices. A shape index of 1 indicated that the fruit were round, whereas a shape index greater than 1 indicated that the fruit were more elongated. An eccentricity index greater than 0.5 indicated that the fruit were pear shaped.

Processing quality

Soluble-solids content (°Brix) of the fruit was measured with a refractometer as described in Tanksley et al. (1996). Higher °Brix values indicated increased sugar content. Several components of fruit acids content were considered: titratable acidity (TA), estimated total organic acids (TOA), fruit pH (pH), and the ratio of sugars to acid (S/A). For TA

Table 1. Means and standard errors of quantitative traits for the control (TA209) and the TA1150 NIL.

		Woodland, Calif.		Ithaca, N.Y.	
Category	Trait[a]	TA209	TA1150	TA209	TA1150
Fruit Color	ECol	3.1±0.1	2.2±0.2***	3.5±0.1	2.0±0.0***
	ICol	3.6±0.2	1.0±0.0***	3.3±0.1	1.0±0.0***
	IOrange	1.0±0.0	4.2±0.3***	nd	nd
	FrLyco (ppm)	125.7±2.8	61.5±5.4***	nd	nd
	Lyco (ppm)	91.5±4.7	67.0±3.1*	nd	nd
	Chroma	ns	ns	nd	nd
	A/B	2.4±0.1	1.6±0.0***	nd	nd
	Hue	32.6±0.1	22.3±0.5***	nd	nd
Fruit Quality	FW (g)	ns	ns	ns	ns
	Firm	ns	ns	3.1±0.1	4.0±0.1***
	Puff	2.2±0.2	1.3±0.2*	ns	ns
	Scar	3.0±0.0	2.0±0.0***	3.3±0.1	2.8±0.1**
	Wall	ns	ns	2.5±0.2	4.1±0.1***
	Crack	ns	ns	nd	nd
	Vein	ns	ns	nd	nd
Fruit Shape	Shape	ns	ns	nd	nd
	ShInd	nd	nd	ns	ns
	Pear	ns	ns	nd	nd
	EccInd	nd	nd	ns	ns
	Fleng (cm)	nd	nd	5.6±0.1	5.8±0.1*
	Fwid (cm)	nd	nd	ns	ns
Processing Quality	Brix (°Brix)	4.7±0.1	5.4±0.1***	5.8±0.1	7.0±0.1***
	TA	4.5±0.4	6.0±0.3*	nd	nd
	TOA	24.1±2.0	32.1±1.9*	nd	nd
	pH	ns	ns	nd	nd
	S/A	ns	ns	nd	nd
	JBost	16.0±0.4	12.8±0.8**	nd	nd
	PBost	ns	ns	nd	nd
	Ostwald	ns	ns	nd	nd
Agronomic Quality	Tyld (lbs)	ns	ns	ns	ns
	Ryld (lbs)	ns	ns	nd	nd
	%Red	94.3±2.0	79.9±2.8**	nd	nd
	B*RY	ns	ns	nd	nd
	B*TY	ns	ns	ns	ns
	PW (lbs)	nd	nd	ns	ns
	HortAcc	3.1±0.2	2.2±0.3*	nd	nd
	1stRed (d)	32.5±1.8	42.3±2.7*	nd	nd
	Stem (%)	19.5±2.6	30.8±2.5*	nd	nd

Note: Asterisks indicate a significant difference from the control: *, $P = 0.05$; **, $P = 0.01$; ***, $P = 0.001$; ns, not significant; nd, trait was not measured at that location.

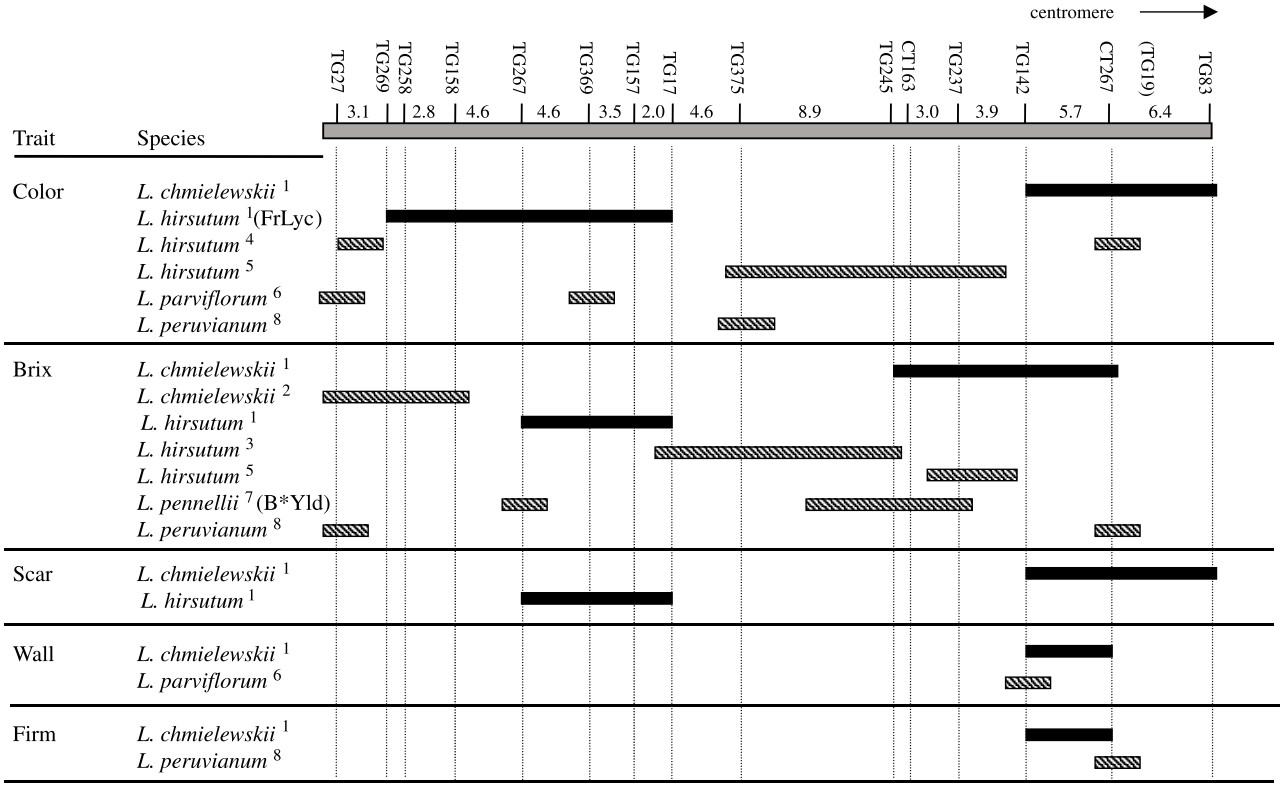
[a]For definitions of abbreviations, see Materials and methods.

and TOA, a higher value indicated that the fruit were more acidic. Processed product was used to measure juice viscosity, juice Bostwick (JBost), and the Ostwald (Ost) or serum viscosity according to a previously published protocol (Anonymous 1977). For JBost, a lower value indicated a greater viscosity (i.e., juice that is less runny), whereas, for Ost, a higher value indicated less separation of water from the product. Paste Bostwick (PBost) was calculated from JBost and °Brix.

Agronomic quality

Total and red-fruit yields (TYld and RYld, respectively) were measured in pounds. Total fruit yield included both ripe and unripe (green) fruit, whereas red-fruit yield was determined using only fruit that had started to ripen. These measurements were then used to calculate several derived parameters: percent of the total yield that was ripe or starting to ripen (%Red), Brix × red yield (Brix*RY) and Brix × total yield (Brix*TY). After harvesting all fruit, the soil was shaken from each uprooted plant and individual fresh plant weights (PW) were measured in pounds. Overall horticultural acceptability (HortAcc) of a line was determined by a visual evaluation of the appearance of each plot in Woodland (1, poor; 5, excellent horticultural attributes). Ripening time (1stRed) was assessed by counting the number of days from 1 June 2000 to the first ripe fruit. Stem retention (Stem) of the fruit was evaluated as the percent of fruit that retained their stems after harvest.

Fig. 2. Locations of the QTL identified in this (black bars) and other (hatched bars) studies. Positioning of bars is approximate for the AB-QTL studies. (1) This work. (2) Paterson et al. (1990) (comparison of NILs). (3) Bernacchi et al. (1998*a*) (comparison of NILs). (4) Bernacchi et al. (1998*b*) (AB-QTL). (5) Monforte and Tanksley (2000) (comparison of subNILs). (6) Fulton et al. (2000) (AB-QTL). (7) Eshed and Zamir (1995) (comparison of NILs). (8) Fulton et al. (1997) (AB-QTL).



Data analysis

Trait means for the controls and the homozygous TA1150 and TA523 subNILs were compared with *t* tests at $P \leq 0.05$ using the StatView software package for Macintosh (SAS Institute Inc., Cary, N.C.). The genomic region included in a subNIL was considered to be harboring a QTL for that trait when the mean for a particular trait showed a highly significant difference between that subNIL and the TA209 control. Confirmation was provided when this difference was consistent for the same line in both locations and for other lines containing similar introgressions. Only QTL that were confirmed in this way are included in the summary figure (Fig. 2). When several subNILs showed the same effect, the QTL was localized to the smallest chromosomal region shared by all of those subNILs.

Results and discussion

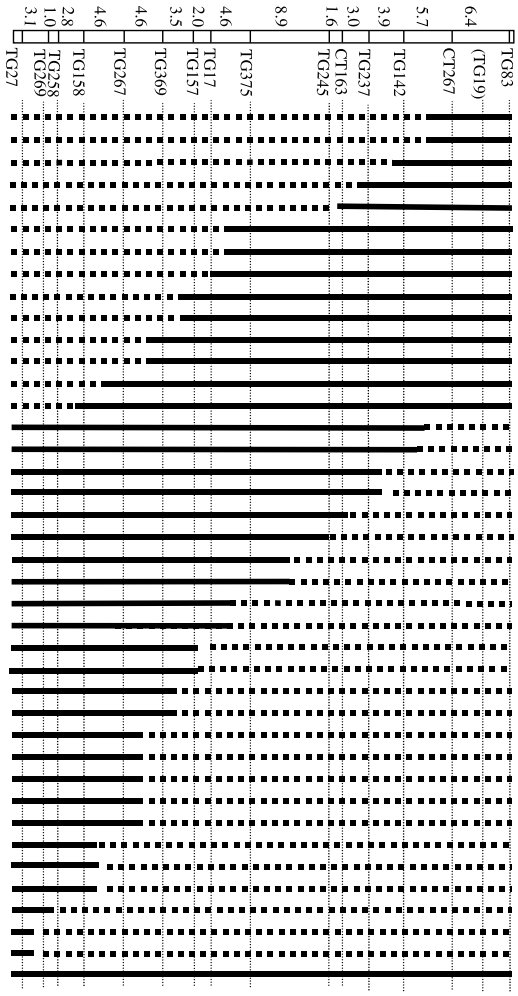
Performance of TA1150

Table 1 summarizes the means and standard errors of the quantitative traits evaluated for the control (TA209) and for the TA1150 NIL. Means are only shown if the two lines were significantly different at $P \leq 0.05$. In both Woodland and Ithaca, TA1150 had fruit that were significantly less red (more orange) than TA209. In addition, the TA1150 fruit tended to be firmer, less puffy and have a smaller stem scar and a thicker pericarp than the inbred control. However, with the exception of stem-scar size, these differences were not consistent over both locations. For most of the fruit shape traits and many of the processing quality traits, there were no significant differences between TA1150 and TA209. However, soluble-solids content was significantly higher in TA1150 than TA209 in both locations. TA1150 also had higher titratable acidity and organic acids and lower juice Bostwick than the control. Although TA1150 had higher soluble solids than TA209, no significant effects were seen on total or red yield or on their derived characters (Brix*RY and Brix*TY). In contrast, for other agronomic quality traits, including percent red fruit, horticultural acceptability, fruit ripening time, and stem retention, the processing inbred control was significantly better than the *L. chmielewskii* NIL. Based on these results, the region encompassed by the TA1150 introgression is expected to contain QTL for several traits including external and internal fruit color, firmness, pericarp thickness, stem-scar size, and soluble-solids content.

SubNIL development

The TA1150 NIL contains a 56-cM introgression from *L. chmielewskii* that includes nearly half of chromosome 1 (Fig. 1). To fine map the different QTL contained within the introgression and determine which are controlled by linked loci as opposed to a single locus with pleiotropic effects, a series of TA1150 subNILs was created. A total of 62 recombinants were identified in the TA1150 × TA209 F_2 population of 380 individuals. By selfing these recombinants

Fig. 3. Graphical genotypes and *t* test results for mean comparisons between TA1150 subNILs and TA209 from Woodland, Calif., and Ithaca, N.Y. Results for four traits are shown: internal fruit color (ICol), soluble-solids content (Brix), stem-scar size (Scar), and pericarp thickness (Wall). Asterisks indicate a significant difference from the control: *, $P = 0.05$; **, $P = 0.01$; ***, $P = 0.001$; ****, $P = 0.0001$; ns, not significant at $P > 0.05$; nd, line was not tested at that location. Letter in parentheses following significance level indicates which parental allele was associated with an increase in the trait: E, *L. esculentum*; C, *L. chmielewskii*.



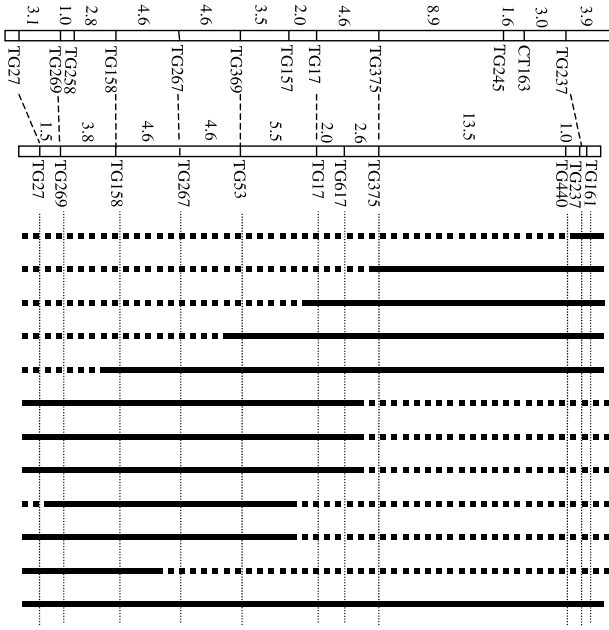
	#reps	ICol CA/NY	Brix CA/NY	Scar CA/NY	Wall NY
TA1756	3/9	*/* (E)	ns/ns	*/ns (E)	ns
TA1700	3/9	***/**** (E)	ns/ns	*/ns (E)	ns
TA1796	0/9	nd/**** (E)	nd/** (C)	nd/** (E)	*** (C)
TA1707	3/8	***/**** (E)	ns/ns	*/ns (E)	*** (C)
TA1688	3/10	****/**** (E)	***/**** (C)	***/*** (E)	*** (C)
TA1704	0/8	nd/**** (E)	nd/**** (C)	nd/*** (E)	**** (C)
TA1784	0/3	nd/** (E)	nd/*** (C)	nd/ns	* (C)
TA1744	0/9	nd/**** (E)	nd/*** (C)	nd/** (E)	*** (C)
TA1684	3/8	****/**** (E)	***/**** (C)	****/**** (E)	** (C)
TA1705	0/8	nd/**** (E)	nd/*** (C)	nd/** (E)	**** (C)
TA1692	3/10	*/**** (E)	*/* (C)	***/**** (E)	*** (C)
TA1787	0/8	nd/**** (E)	nd/** (C)	nd/**** (E)	**** (C)
TA1794	3/7	****/**** (E)	***/*** (C)	****/**** (E)	*** (C)
TA1752	0/9	nd/ns	nd/ns	nd/* (C)	ns
TA1691	2/8	ns/ns	ns/ns	ns/ns	ns
TA1694	0/8	nd/* (E)	nd/** (C)	nd/* (E)	ns
TA1726	3/9	ns/ns	*/* (C)	*/ns (E)	ns
TA1746	3/9	*/* (E)	ns/ns	***/ns (E)	ns
TA1733	3/8	ns/ns	ns/** (C)	ns/ns	ns
TA1789	0/9	nd/* (E)	nd/ns	nd/ns	ns
TA1748	3/9	**/** (E)	ns/* (E)	***/ns (E)	ns
TA1751	0/9	nd/**** (E)	nd/ns	nd/ns	ns
TA1712	3/9	*/*** (E)	ns/ns	ns/* (C)	ns
TA1761	3/9	ns/ns	ns/ns	ns/ns	ns
TA1709	3/8	ns/ns	ns/ns	***/ns (E)	* (E)
TA1777	0/6	nd/ns	nd/ns	nd/* (C)	ns
TA1722	3/7	*/ns (E)	*/ns (C)	****/* (E/C)	ns
TA1768	3/8	**/ns (E)	ns/ns	ns/**** (C)	* (C)
TA1717	3/8	*/ns (E)	ns/ns	ns/* (C)	ns
TA1739	3/10	ns/* (E)	ns/ns	*/** (E/C)	ns
TA1762	3/9	ns/ns	*/* (C)	ns/* (C)	* (C)
TA1766	3/9	ns/ns	ns/ns	ns/** (C)	ns
TA1793	3/9	ns/ns	*/ns (C)	ns/** (C)	ns
TA1718	3/8	ns/ns	ns/ns	ns/* (C)	* (C)
TA1729	3/9	ns/ns	ns/ns	ns/**** (C)	ns
TA1759	3/9	**/*** (E)	ns/ns	*/ns (E)	ns
TA1706	3/9	ns/** (E)	ns/ns	****/ns (E)	ns
TA1725	3/8	ns/** (E)	ns/ns	ns/ns	ns
TA1740	3/9	ns/* (E)	ns/ns	ns/ns	ns
TA1150					

and screening the resulting F_3 individuals, 39 independent, homozygous subNILs were generated. When categorized by recombination point (as delimited by the two flanking markers), these subNILs fell into 22 different classes. Using the TA1150 × TA209 F_2 population, a linkage map was constructed for the bottom of chromosome 1 (Fig. 1). Compared with an *L. esculentum* × *L. pennellii* F_2 population (Tanksley et al. 1992), the NIL population exhibited a 76% reduction in recombination. Suppression of recombination was also observed in the population from which TA1150 was derived (Paterson et al. 1990), as well as in advanced generations of other interspecific populations (Ganal and Tanksley 1991; Grandillo et al. 1996; Monforte and Tanksley 2000). Reduced recombination in such populations has been attributed to sequence divergence between the species, which results in preferential recombination within homozygous regions of a chromosome (i.e., the regions that do not contain an introgression) (Paterson et al. 1990).

Fine mapping of QTL within TA1150

Graphical genotypes for the 39 TA1150 subNILs are shown in Fig. 3. Discussions about map distances are based on the tomato high-density map (Tanksley et al. 1992). Many of the subNILs for the top third of the introgression (TG83 to TG245) had fruit that were significantly more orange, higher in soluble solids, and had smaller scars than TA209 both in Woodland and Ithaca (Fig. 3). The same introgression lines also had fruit that were firmer (data not shown) and had thicker pericarps than the control; however, this effect of the *L. chmielewskii* segment was only significant in the Ithaca population (Fig. 3). By comparisons between the different subNILs, it was possible to fine map each QTL within the TA1150 introgression region. TA1752 presented anomalous results for all traits, therefore the data for this line was not included for the fine-mapping analysis. Because even the subNILs with the shortest introgression from the region (TA1700, TA1756) had significantly reduced fruit color compared with TA209, the ICol QTL was localized to the 12.1-cM interval between markers TG83 and TG142 (Figs. 2 and 3). The ECol and FrLyco QTL also mapped to the same location (data not shown). Because these same subNILs (TA1700, TA1756) did not exhibit in-

Fig. 4. Comparison of TA1150 and TA523 introgressions, graphical genotypes, and *t* test results for mean comparisons between TA523 subNILs and TA209 from Woodland, Calif. Results for three traits are shown: lycopene content (FrLyco), soluble-solids content (Brix), and stem-scar size (Scar). Asterisks indicate a significant difference from the control: *, $P = 0.05$; **, $P = 0.01$; ***, $P = 0.001$; ****, $P = 0.0001$; ns, not significant at $P > 0.05$. Letter in parentheses following significance level indicates which parental allele was associated with an increase in the trait: E, *L. esculentum*; H, *L. hirsutum*.



	#reps	FrLyco	Brix	Scar
TA1218	3	ns	ns	ns
TA1226	3	* (H)	ns	ns
TA1227	3	ns	ns	ns
TA1223	3	ns	* (H)	* (E)
TA1224	3	ns	* (H)	* (E)
TA1220	3	** (E)	** (H)	* (E)
TA1228	3	** (E)	* (H)	ns
TA1229	3	** (E)	ns	* (E)
TA1225	3	** (E)	* (H)	* (E)
TA1219	3	ns	ns	**** (E)
TA1230	3	ns	ns	ns
TA523				

creased soluble-solids content, the Brix QTL was delimited to the 14.2-cM region of the chromosome between CT267 and TG245 (Figs. 2 and 3). Based on the Woodland data, the Scar QTL mapped to the same location as the color QTL, between TG83 and TG142 (Figs. 2 and 3). The Wall and Firm QTL, which were identified only in Ithaca, were localized to the same region of the chromosome, the 5.7-cM interval between CT267 and TG142 (Figs. 2 and 3).

Fine mapping of QTL within TA523

Figure 4 shows a comparison of the TA523 and TA1150 introgressions and the graphical genotypes for the 11 TA523 subNILs. Of the five traits for which QTL were mapped using the *L. chmielewskii* subNILs, only fruit color (FrLyco), soluble solids, and stem-scar size loci could be mapped using the *L. hirsutum* lines. The TA523 subNILs containing the middle portion of the introgression from the wild species (e.g., TA1220) had significantly lower levels of lycopene, higher Brix, and smaller stem scars than individuals that contained either end of the introgression (Fig. 4). Given these results, the FrLyco QTL was localized to the 21.6-cM interval between TG17 and TG269, whereas the Brix and Scar QTL colocalized to the 10.1-cM interval between TG17 and TG267 (Figs. 3 and 4).

In a previous examination of these subNILs (Monforte and Tanksley 2000), an external-color QTL was localized to the top portion of the introgression (Fig. 3). It was not expected that the lycopene locus identified in the current study would overlap with this other QTL because the two have opposite effects. The *L. hirsutum* allele for the ECol QTL is associated with increased red color, whereas that of the FrLyco QTL is associated with decreased lycopene. The Brix locus identified by Monforte and Tanksley (2000) also mapped to the top part of the introgression (TG237) and did not coincide with the Brix QTL detected in the present study (Fig. 2). This discrepancy might be due to environmental variability, because the two studies were performed at two different locations, New York (Monforte and Tanksley 2000) and California (present work), during two different years. Alternatively, there may indeed be two soluble solids loci within the *L. hirsutum* introgression.

Comparison of TA1150 QTL with QTL mapped in other populations

Several of the QTL identified in the TA1150 subNILs have also been mapped in other populations (Fig. 2). The fruit color QTL located between TG83 and TG142 was identified by Bernacchi et al. (1998*b*) in an *L. hirsutum* advanced backcross QTL (AB-QTL) study. However, the *L. hirsutum* allele of the QTL increased color, whereas the *L. chmielewskii* allele decreased fruit redness. The TA523 subNILs were not expected to contain this locus because it is located outside of the TA523 introgression. It is also important to note that none of the known carotenoid biosynthetic pathway genes that give rise to orange or reduced red fruit color have been mapped to chromosome 1.

The Brix QTL detected between CT267 and TG245 in the TA1150 subNILs coincided with the chromosome 1 soluble-solids QTL identified in a previous evaluation of the TA523 *L. hirsutum* subNILs (Monforte and Tanksley 2000) and in an *L. peruvianum* AB-QTL population (Fulton et al. 1997). The Brix QTL also colocalized with a Brix*yield QTL identified in *L. pennellii* (Eshed and Zamir 1995). Interestingly, Paterson et al. (1990), using introgression lines from *L. chmielewskii*, fine mapped a soluble-solids QTL to the bottom of chromosome 1 (TG158 to TG27), which was not identified in the current study and did not detect the QTL located between CT267 and TG245. In addition, the

position of the Brix QTL mapped in the TA523 subNILs in the present study did not coincide with the *L. chmielewskii* locus or either of the previously mapped *L. hirsutum* QTL (Monforte and Tanksley 2000; Bernacchi et al. 1998*a*). These results suggest that this region of chromosome 1 may contain more than one locus controlling soluble-solids content. The gene encoding a large subunit of ADPglucose pyrophosphorylase, an enzyme that is essential for starch accumulation in the fruit and is more active in tomato lines with high soluble solids content, has been mapped to the lower portion of chromosome 1 (Schaffer et al. 2000) and is an obvious candidate for one of the Brix QTL in this region.

The Scar QTL identified in this study did not correspond to any previously published stem-scar size locus and did not colocalize with the QTL identified in the *L. hirsutum* subNILs. In contrast, the Wall and Firm QTL that mapped to the interval between CT267 and TG142 coincided with a pericarp-thickness locus identified in a *L. parviflorum* AB-QTL study (Fulton et al. 2000) and a firmness locus found in a similar *L. peruvianum* study (Fulton et al. 1997). Like the *L. chmielewskii* allele, the *L. parviflorum* allele for the Wall QTL increased pericarp thickness. In contrast, the *L. peruvianum* allele for the Firm QTL reduced firmness, whereas the *L. chmielewskii* allele improved the trait.

Linkage versus pleiotropy in the TA1150 subNILs

Substitution mapping using the TA1150 subNILs allowed a determination of whether some of the traits contained within the introgression are controlled by linked loci or by the pleiotropic effects of a single locus. Although all of the QTL mapped to overlapping regions of the chromosome, some subNILs showed breakage of the linkage between certain traits. For example, subNILs TA1756 and TA1700 both had orange fruit color indicating that they contained the *L. chmielewskii* allele for the color QTL; however, neither line had a Brix level that was significantly different from the control (Fig. 3). Thus, the QTL controlling fruit color and soluble-solids content could be distinguished as two separate, linked loci. Similarly, the color QTL was found to be distinct from the pericarp thickness and firmness locus and (or) loci. The color and stem-scar size QTL could not be resolved nor could the pericarp thickness, firmness, and soluble-solids loci be distinguished. Because of the different results in Woodland and Ithaca, it was also impossible to differentiate the Brix and Scar QTL. The colocalization of QTL for different traits suggests that some or all of these traits, especially those that are physiologically related, may be controlled by single loci with pleiotropic effects. For example, the gene that controls pericarp thickness might also affect fruit firmness because fruit with thicker walls might feel firmer than those with less pericarp and more placenta. Finer mapping or cloning of the loci in the region will be necessary to determine whether the traits are the effects of pleiotropy or closely linked loci.

Implications for breeding

The primary hurdle preventing the use of TA1150 to improve soluble-solids content of tomato was the linkage between high solids and orange fruit color. In this study, subNILs that affect only fruit color were identified, which indicates that the two traits are controlled by different loci. Thus, it will be possible to break the linkage between high Brix and poor color and to retain only the portion of the *L. chmielewskii* introgression that increases solids by creating subNILs for line TA1688. TA1688 is an obvious candidate for this work because it contained the smallest introgression, which showed a highly significant increase in Brix (as compared with TA209) in both locations, i.e., 1.0 and 0.8°Brix increases in Woodland and Ithaca, respectively. Moreover, although the difference was not statistically significant for BOS3155, the line outperformed both of the industry standards in Woodland (5.7°Brix versus 5.3 and 4.9 for BOS3155 and H8892, respectively).

In addition to improved soluble solids, TA1688 had red and total yields that were comparable with TA209 and BOS3155 (data not shown), indicating that there was no severe yield penalty for the increase in soluble-solids content. The pericarp-thickness and firmness QTL within TA1150 may also be useful for processing tomato improvement because the locus and (or) loci for these traits could be distinguished from the color QTL. Because the Wall and Firm QTL could not be distinguished from the Brix QTL, it is possible that use of the Brix QTL will also improve these other characteristics. Analysis of subNILs for TA1688 should clarify whether or not these three traits are controlled by the same locus. If, as suggested by the current results for the TA523 subNILs and the work of Bernacchi et al. (1998*a*), the *L. hirsutum* introgression does indeed contain one or more soluble-solids QTL that are distinct from the locus identified in *L. chmielewskii*, it should be possible to use a high-Brix, red-fruited, TA1688 subNIL and TA1220 to combine the *L. chmielewskii* and *L. hirsutum* alleles for these different QTL into a single line with the potential for extremely high solids.

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References

Anonymous. 1977. Tomato products bulletin 27-L. 5th ed. National Canner's Association, Washington, D.C.

Bernacchi, D., Beck-Bunn, T., Emmatty, D., Eshed, Y., Inai, S., Lopez, J., Petiard, V., Sayama, H., Uhlig, J., Zamir, D., Tanksley, S.D. 1998*a*. Advanced backcross QTL analysis of tomato. II. Evaluation of near-isogenic lines carrying single-donor introgressions for desirable QTL-alleles derived from *L. hirsutum* and *L. pimpinellifolium*. Theor. Appl. Genet. **97**: 170–180.

Bernacchi, D., Beck-Bunn, T., Eshed, Y., Lopez, J., Petiard, V., Uhlig, J., Zamir, D., and Tanksley, S.D. 1998*b*. Advanced backcross QTL analysis in tomato. I. Identification of QTLs for traits of agronomic importance from *L. hirsutum*. Theor. Appl. Genet. **97**: 381–397.

Bernatzky, R., and Tanksley, S.D. 1986. Majority of random cDNA clones correspond to single loci in the tomato genome. Mol. Gen. Genet. **203**: 8–14.

Eshed, Y., and Zamir, D. 1995. An introgression-line population of *Lycopersicon pennellii* in the cultivated tomato enables the identification and fine mapping of yield-associated QTLs. Genetics, **141**: 1147–1162.

Fulton, T.M., Beck-Bunn, T., Emmatty, D., Eshed, Y., Lopez, J., Petiard, V., Uhlig, J., Zamir, D., and Tanksley, S.D. 1997. QTL analysis of an advanced backcross of *Lycopersicon peruvianum* to the cultivated tomato and comparisons with QTLs found in other wild species. Theor. Appl. Genet. **95**: 881–894.

Fulton, T.M., Grandillo, S., Beck-Bunn, T., Fridman, E., Frampton, A., Lopez, J., Petiard, V., Uhlig, J., Zamir, D., and Tanksley, S.D. 2000. Advanced backcross QTL analysis of a *Lycopersicon esculentum* × *L. parviflorum* cross. Theor. Appl. Genet. **100**: 1025–1042.

Ganal, M.W., and Tanksley, S.D. 1991. Recombination around the *Tm2a* and *Mi* resistance genes in different crosses of *Lycopersicon peruvianum*. Theor. Appl. Genet. **92**: 101–108.

Grandillo, S., Ku, H-M., and Tanksley, S.D. 1996. Characterization of *fs8.1*, a major QTL influencing fruit shape in tomato. Mol. Breed. **2**: 251–260.

Kosambi, D.D. 1944. The estimation of map distances from recombination values. Ann. Eugen. **12**: 172–175.

Lander, E.S., Green, P., Abrahamson, J., Barlow, A., Daly, M.J., Lincoln, S.E., and Newburg, L. 1987. MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics, **1**: 174–181.

Monforte, A.J., and Tanksley, S.D. 2000. Fine mapping of a quantitative trait locus (QTL) from *Lycopersicon hirsutum* chromosome 1 affecting fruit characteristics and agronomic traits: breaking linkage among QTLs affecting different traits and dissection of heterosis for yield. Theor. Appl. Genet. **100**: 471–479.

Paterson, A.H., DeVerna, J.W., Lanini, S., and Tanksley, S.D. 1990. Fine mapping of quantitative trait loci using selected overlapping recombinant chromosomes in a interspecies cross of tomato. Genetics, **124**: 735–742.

Schaffer, A.A., Levin, I., Oguz, I., Petreikov, M., Cincarevsky, F., Yeselson, Y., Shen, S., Gilboa, N., and Bar, M. 2000. ADPglucose pyrophosphorylase activity and starch accumulation in immature tomato fruit: the effect of a *Lycopersicon hirsutum*-derived introgression encoding for the large subunit. Plant Sci. **152**: 135–144.

Tanksley, S.D. 1993. Mapping polygenes. Annu. Rev. Genet. **27**: 205–233.

Tanksley, S.D., and McCouch, S.R. 1997. Seed banks and molecular maps: unlocking genetic potential from the wild. Science (Washington, D.C.), **277**: 1063–1066.

Tanksley, S.D., Ganal, M.W., Prince, J.P., deVicente, M.C., Bonierbale, M.W., Broun, P., Fulton, T.M., Giovannoni, J.J., Grandillo, S., Martin, G.B., Messeguer, R., Miller, J.C., Paterson, A.H., Pineda, O., Roder, M.S., Wing, R.A., Wu, W., and Young, N.D. 1992. High density molecular linkage maps of the tomato and potato genomes. Genetics, **132**: 1141–1160.

Tanksley, S.D., Grandillo, S., Fulton, T.M., Zamir, D., Eshed, Y., Petiard, V., Lopez, J., and Beck-Bunn, T. 1996. Advanced backcross QTL analysis in a cross between an elite processing line of tomato and its wild relative *L. pimpinellifolium*. Theor. Appl. Genet. **92**: 213–224.