Theor Appl Genet (2007) 115:429–442
DOI 10.1007/s00122-007-0578-1

ORIGINAL PAPER

Both additivity and epistasis control the genetic variation for fruit quality traits in tomato

Mathilde Causse · Jamila Chaïb · Laurent Lecomte · Michel Buret · Frédéric Hospital

Received: 16 December 2006 / Accepted: 14 May 2007 / Published online: 15 June 2007

Abstract The effect of a gene involved in the variation of a quantitative trait may change due to epistatic interactions with the overall genetic background or with other genes through digenic interactions. The classical populations used to map quantitative trait loci (QTL) are poorly efficient to detect epistasis. To assess the importance of epistasis in the genetic control of fruit quality traits, we compared 13 tomato lines having the same genetic background except for one to five chromosome fragments introgressed from a distant line. Six traits were assessed: fruit soluble solid content, sugar content and titratable acidity, fruit weight, locule number and fruit firmness. Except for firmness, a large part of the variation of the six traits was under additive control, but interactions between QTL leading to epistasis effects were common. In the lines cumulating several QTL regions, all the significant epistatic interactions had a sign opposite to the additive effects, suggesting less than additive epistasis. Finally the re-examination of the segregating population initially used to map the QTL confirmed the extent of epistasis, which frequently involved a region where main effect QTL have been detected in this progeny or in other studies.

Communicated by Q. Zhang.

M. Causse (✉) · J. Chaïb · L. Lecomte
INRA, UR1052, Unité de Génétique et Amélioration des Fruits et Légumes, Domaine Saint-Maurice, 84143 Montfavet, France
e-mail: Mathilde.Causse@avignon.inra.fr

M. Buret
INRA, UMR408 Sécurité et Qualité des Produits d'Origine Végétale, Domaine Saint-Paul, 84914 Avignon, France

F. Hospital
INRA, UMR de Génétique Végétale, Ferme du Moulon, 91190 Gif-sur-Yvette, France

Present Address:
F. Hospital
Laboratoire de Physique Théorique et Modèles Statistiques, Université Paris Sud, UMR8626, Bâtiment 100, Orsay Cedex 91405, France

Introduction

Epistasis—the interaction between genes at different loci—has two related but distinct definitions depending on the way it is revealed (Phillips 1998). In 1909, Bateson used the term epistasis to describe the masking effect of an allele at one locus upon an allele at another locus. This definition, first used in Mendelian segregation studies was further used by biologists when interactions among proteins were detected. In quantitative genetics, the term epistasis has been used in a different sense. In 1918, Fisher defined epistasis as the deviation from additivity of the contributions of several loci to a quantitative phenotype. Epistasis may exert important effect on the dynamics of evolving populations (Cheverud and Routman 1996; Elena and Lenski 2001). In the evolutionary history of species, complementary epistatic interactions due to the isolation of subspecies explain some epistatic interactions (Fenster et al. 1997) for instance for female sterility in rice (Kubo and Yoshimura 2005) or seed yield in bean (Johnson and Gepts 2002). Epistasis is also supposed to be the main factor responsible for overdominance and heterosis in rice (Yu et al. 1997; Li et al. 2001; Xing et al. 2002, Hua et al. 2003; Mei et al. 2003), maize (Doebley et al. 1995) and *Arabidopsis thaliana* (Syed and Chen 2005). Its presence may have important consequences on the success of detection, introgression and characterization of the genes

controlling quantitative traits (Carlborg and Haley 2004). Indeed as additive and epistatic effects are partially confounded, analysing only single locus effects can lead to detect a quantitative trait locus (QTL) that has no actual main effect but interact epistatically with another (Purcell and Sham 2004). This could result in a restricted genetic gain from marker-assisted selection (Liu et al. 2003) as well as some difficulties when trying to isolate the QTL. Ignoring the epistatic interactions also leads to underestimate genetic variance and to overestimate individual QTL effects (Carlborg and Haley 2004). Any departure from a specific linear model describing the relationships between alleles at different genetic loci may be attributed to epistatic interactions (Wade et al. 2001). Scaling the phenotypic trait may be important, as in some cases, a log transformation of the data reduces the departure from the additive model (Cordell et al. 2001).

The studies aiming at dissecting QTL with molecular markers contribute to bridge the gap between the two definitions of epistasis. However, due to the number of statistical tests performed to detect epistatic interactions, significance thresholds must be very stringent and thus the power to detect interactions between QTL is low. Consequently, the number of significant epistatic interactions is usually close to that expected by chance (Tanksley 1993). Nevertheless, several cases of epistatic interactions have been detected in plants, for flowering time (Li et al. 2001; Monna et al. 2002), inflorescence development (Ungerer et al. 2002), plant development (Doebley et al. 1995) or metabolic traits (McMullen et al. 2001; Mahmood et al. 2003; Zhao et al. 2005). Several cases of epistasis were also detected for disease resistance (Visker et al. 2003; Thabuis et al. 2004; Zhao and Meng 2003; Calenge et al. 2005; Clarke et al. 2001; Ahmadi et al. 2001; Coaker and Francis 2004). In tomato, significant interactions were detected for fruit shape (Van Der Knaap et al. 2002), locule number (Lippman and Tanksley 2001), fruit colour (Kabelka et al. 2004), soluble solid content (Monforte et al. 2001), fructose to glucose ratio (Levin et al. 2004), fruit firmness and aroma production (Saliba-Colombani et al. 2001; Causse et al. 2002).

Several methods have been proposed to search simultaneously for multiple QTL (Kao et al. 1999; Wang et al. 1999). An alternative approach consists in restricting the search to the portions of the genome carrying main effect QTL (Lark et al. 1995). Because in statistical genetics epistasis is a by-product of the additive model, its effect is rarely significant, except when specific designs are used (Kearsey et al. 2003). Indeed, interactions with the genetic background could be revealed by multiple crosses between related inbred lines (Charcosset et al. 1995; Jannink and Jansen 2001; Cockerman and Zeng 1996) or by transferring the same mutation or QTL in several genetic backgrounds (Elena and Lenski 2001; Lecomte et al. 2004). Introgression lines are also particularly adapted to reveal epistatic interactions. By crossing near-isogenic lines carrying different QTL, Eshed and Zamir (1996) showed that the addition of favourable alleles for fruit weight provided less progress than expected. Crosses between lines carrying interacting QTL were also used to confirm epistatic effects detected for bacterial canker resistance in tomato (Coaker and Francis 2004). At the molecular level, many epistasis examples can be found in the analysis of mutations controlling developmental or physiological traits such as flowering time (Caicedo et al. 2004) or defence mechanisms (Li et al. 2004).

In this study, we assessed the amount of epistasis for six traits involved in tomato development and tomato fruit quality. These traits are known to have different genetic architectures: fruit weight is controlled by many QTL (Grandillo et al. 1999), locule number is linked to organogenesis and probably controlled by a few loci with both additive and epistatic effects (Lippman and Tanksley 2001), fruit firmness results from several biological processes (Seymour et al. 2002), soluble solid content is controlled by many QTL and has been extensively studied in processing tomato (Fulton et al. 2002), contrarily to sugar content and titratable acidity, both linked to the primary metabolism in fruit. Several QTL controlling these traits were previously mapped in a recombinant inbred line population (hereafter CL-RIL, Saliba-Colombani et al. 2001). Very few epistatic interactions were detected at the whole genome level using a stringent threshold. A marker-assisted backcross scheme was then performed in order to introgress five major regions carrying QTL for quality traits into a recipient line (Lecomte et al. 2004). During the marker-assisted backcross, BC3S2 lines carrying favorable alleles at one to five QTL regions were produced (Lecomte et al. 2004). The comparison of lines carrying only one introgressed region (hereafter named QTL-NIL, for near isogenic lines) with the recipient line allowed additive QTL effects to be assessed. Interactions among QTL were assessed by comparing the values of the lines cumulating several introgressed regions (hereafter named QTL-CIL, for cumulating introgressed lines) to their expected values based on additive QTL effects estimated in QTL-NIL. As significant epistatic effects were detected for all the traits, epistatic interactions in the CL-RIL population were then re-examined in the light of these results. Putative epistatic loci were compared with the QTL detected for the same traits in other QTL mapping experiments, showing that a majority of the interacting loci in the present study actually corresponded to additive QTL detected in other studies.

Materials and methods

Plant material

In a previous study (Saliba-Colombani et al. 2001), QTL analysis was performed using a population (named CL-RIL) of 144 recombinant inbred lines developed from a cross between Cervil (a cherry tomato, *Solanum lycopersicum*, formerly *Lycopersicon esculentum*, var. *cerasiforme*, with 7 g fruits, a good taste and a high aroma intensity) and Levovil (a *S. lycopersicum* line, with 125 g fruits and a common taste). Several QTL controlling the variation of organoleptic quality traits were detected (Causse et al. 2002). The favorable alleles for fruit quality were conferred by Cervil in all cases. Two to five QTL were detected in the CL-RIL population for the six traits of interest (Table 1). Five regions (called 1, 2, 4, 9a and 9b, according to their chromosome location) were then chosen to be introgressed into Levovil. The five chromosome regions selected to be introgressed covered the major QTL intervals detected in the CL-RIL. The regions to transfer were chosen not only on the basis of QTL described in Table 1, but also on the basis of the QTL for sensory traits and volatile aroma content (Causse et al. 2002). For instance the segment 9b was retained because it carried a QTL controlling pharmaceutical aroma. No effect is thus expected in this region for the traits studied here. A single RIL with Cervil alleles for the five regions was used as donor parent in the introgression program. The implementation of the selection scheme is described by Lecomte et al. (2004). The five regions were controlled by one to three markers during marker-assisted introgression (Fig. 1). After three marker-assisted backcrosses, one BC3 plant heterozygous at the five regions of interest was selected and selfed. In two generations of selection and selfing, BC3S2 lines with homozygous Cervil genotypes at one, three, four or five regions were derived. Lines having a single introgressed region were called QTL-NIL (Van Berloo et al. 2001), those cumulating three to five introgressed regions were called QTL-CIL (Cumulating Isogenic Lines). The lines are named Q_i, i corresponding to the introgressed fragment(s) carried by the line. Markers covering the whole genome were used to characterize the genotypes of each line and to assess the remaining percentage of donor genome, as described in Lecomte et al. (2004). In the end, the introgressed segments were often larger than the QTL region because of linkage drag (Lecomte et al. 2004).

Phenotypic evaluations

Three trials were performed in heated glasshouses in Southern France. The first trial consisted in the 144 RIL as described in Saliba-Colombani et al. (2001). Red fruits of each plot were harvested twice a week during six weeks. The QTL-NIL and QTL-CIL were evaluated in two trials in greenhouses in the South East of France, one performed from February to June 2002, the other from February to June 2003. The recipient line Levovil, Cervil and 13 BC3S2 lines were grown, each represented by a plot of six plants. Fruits were harvested in bulk on the six plants of each plot twice a week during 6 weeks. Each week, seven fruits were sampled per plot. It was preferred to bulk fruits harvested each week on several plants rather than several weeks of harvest per plant in order to maximize the environmental variability, as previous experiments showed that variability among weeks of harvest is higher than among plants of the same genotype (MC unpublished data). A total of 42 fruits per plot were evaluated for the three physical and the three chemical traits. Fruit-by-fruit evaluations were performed for fruit weight (FW) and firmness (FIR). Fruit firmness was evaluated with a penetrometer in CL-RIL and with a Durofel (a probe was applied at two points on the fruit equator, the moves of the probe was recorded and the average of the two measurements was used as an indicator of fruit firmness) in introgressed lines. Then, fruits were cut to determine the locule number (LCN) and then frozen (–30°C). Chemical analyses were performed on fruit powder derived from blending a bulk of seven fruits with liquid nitrogen. Soluble solids content (SSC), sugar content (SUC) and titratable acidity (TA) were evaluated as described in Saliba-Colombani et al. (2001).

Statistical analyses

BC3S2 lines having one to five introgressed regions were analyzed using the SAS software (SAS Institute 1988). Mean values over the two trials were estimated for each line after testing the genotype and year effects by ANOVA. The difference between each line mean and the recipient line mean was tested by a Dunnett's test. Let Q and Q_i be the average values of the recipient line and of the QTL-NIL carrying region i, respectively. QTL effect was estimated from the values of the lines having a single introgressed region, as additive effect a_i of region i:

$$a_i = (Q_i - Q)/2 \quad (1)$$

and multiplicative effect of the region i:

$$m_i = Q_i/Q. \quad (2)$$

According to these individual effects, the expected effect for a line having several introgressed regions i, j and k was predicted based on a linear additive model:

Table 1 Characteristics of the QTL detected by interval mapping in the CL-RIL population derived from the cross between a cherry tomato (Cervil) and a large fruited line (Levovil) for six fruit quality traits

Trait	Chromosome[a]	Position on reference map[b]	Markers[c]	R^2	Additive effect	LOD (SIM)	Introgressed region
Fruit weight (h^2 = 0.75)	2 (1)	56	CT103	13.3	–8.9	3.37	Q_2
	2 (2)	110	TG492	40.9	–12.4	13.99	Q_2
	3	83	CT85	20.2	–9.1	5.39	–
	11	84	CT65	13.9	–8.9	4.45	–
	12	30	CT120B	12.4	–8.5	3.69	–
Firmness[d] (h^2 = 0.63)	4	48	TG287	31.9	6,745	9.78	Q_4
	9	39	CT32 (SIM only)	9.8	–4,936	3.20	Q_{9a}
Locule number (h^2 = 0.92)	1	75	TG116	7.0	–0.39	2.46	Q_1
	2	71	TG484	49.0	–1.08	15.40	Q_2
	12	30	TG367	8.0	–0.44	2.65	–
Soluble solid content (h^2 = 0.58)	2 (1)	56	CT103	26.5	0.94	8.26	Q_2
	2 (2)	78	AE4-0.9C	31.9	1.01	10.26	Q_2
	9	39	CT32 (CIM only)	7.3	0.49	2.14 NS	Q_{9a}
Sugar content (h^2 = 0.61)	2 (1)	56	CT103	22.5	0.53	7.62	Q_2
	2 (2)	78	AE4-0.9C	29.0	0.59	9.19	Q_2
	10	62	TG1 (CIM only)	9.5	0.34	2.94	–
	11	74	TG36	12.5	0.39	3.42	–
Titratable acidity (h^2 = 0.81)	1	100	TG77	14.9	1.00	4.89	Q_1
	2 (2)	110	TG492	14.3	1.01	4.50	Q_2
	3	71	H42M47.112L	16.6	1.05	4.70	–
	9	39	CT32	11.5	0.85	3.76	Q_{9a}
	12	30	CT120B	9.7	0.80	2.79	–

Negative additive effect indicates that the alleles from the Levovil parent increase the trait value. Heritability (h^2) measured in the CL-RIL (according to Saliba-Colombani et al. 2001; unpublished data for locule number). A LOD threshold of 2.36 was used, corresponding to a 0.10 genome-wise error. The effects detected in regions introgressed by marker assisted backcross (noted Q_1, Q_2, Q_4, Q_{9a} and Q_{9b}) are indicated

[a] The 2 regions carrying QTL on chromosome 2 are indicated with (1) and (2)

[b] The marker position is indicated as extrapolated on the tomato reference map (Tanksley et al. 1992)

[c] The QTL detected only by Simple Interval mapping (SIM only) or only by Composite Interval Mapping (CIM only) are indicated

[d] A penetrometer was used to measure firmness in CL-RIL whereas it was measured with a Durofel in QTL-NIL and QTL-CIL

$$A_{ijk} = a_i + a_j + a_k$$

and based on a multiplicative model:

$$M_{ijk} = m_i \times m_j \times m_k.$$

Observed effects of QTL in QTL-CIL were compared to predicted effect through the comparison of the residual sum of squares of both models by a F-test.

Epistatic effects e_i were estimated for each region i by the differences:

$$\begin{aligned} e_1 &= Q_{1/4/9a/9b} - Q_{4/9a/9b} - 2a_1 \\ e_2 &= Q_{1/2/4/9a/9b} - Q_{1/4/9a/9b} - 2a_2 \\ e_4 &= Q_{1/4/9a/9b} - Q_{1/9a/9b} - 2a_4 \\ e_{9a} &= Q_{1/4/9a/9b} - Q_{1/4/9b} - 2a_{9a}. \end{aligned}$$

These epistasis effects are thus the mixture of interactions between region i and the three or four other regions. Significance of e_i was tested by comparing with a t-test the two contrasts Q_i-Q and Q_{ijkl}-Q_{jkl} which should be equal in absence of epistasis.

In the CL-RIL population, epistasis was tested by 2-way ANOVAs among all the 129 markers covering the genome at a significance threshold of $P < 0.001$. In RIL, the epistatic interaction was assessed by an index adapted from Keightley (1996):

$$I_K = \frac{|m_{CC} - m_{LL}|}{[m_{CL} + m_{LC} - 2 \times \min(m_{LL}, m_{CC})]},$$
$$\text{if } (m_{CC} + m_{LL}) > (m_{CL} + m_{LL}),$$

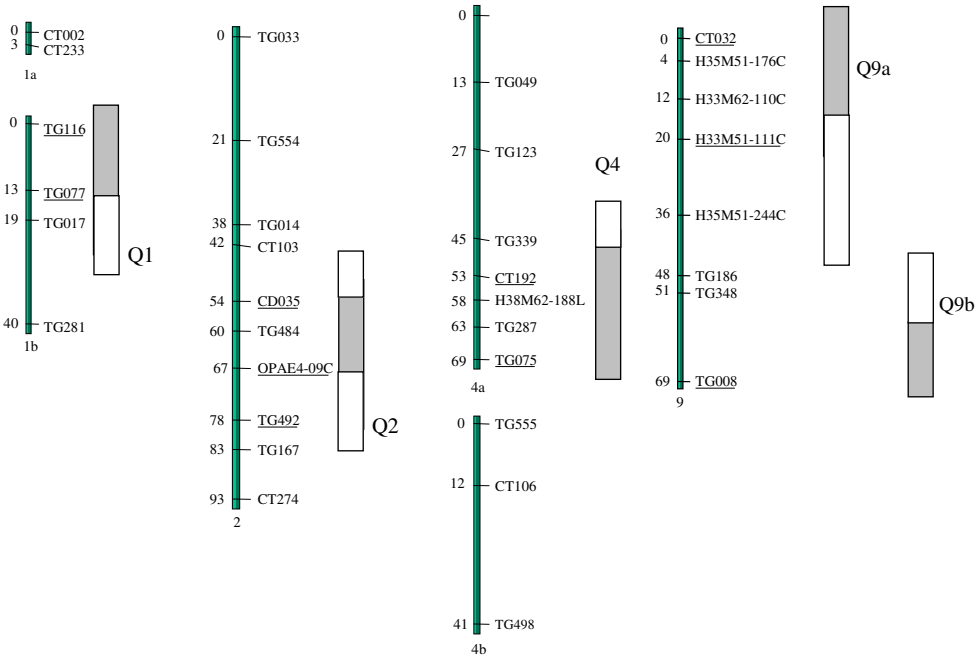

Fig. 1 Genetic map showing the 5 regions of interest introgressed in each QTL-NIL (named Q_i) on chromosome 1, 2, 4 and 9. The molecular markers used to control these regions during the marker assisted backcrosses are underlined (according to Lecomte et al. 2004). *Grey boxes* correspond to the regions chosen to be introgressed, *white boxes* indicate the regions which were introgressed by hitch-hiking with the QTL regions in the corresponding QTL-NILs

$$I_K = \frac{|m_{CL} - m_{LC}|}{[m_{CC} + m_{LL} - 2 \times \min(m_{CL}, m_{LC})]}, \quad \text{if } (m_{CL} + m_{LC}) > (m_{CC} + m_{LL}),$$

where m_{XY} is the average value of the plants with a genotype homozygous X at the first locus and Y at the second.

Results

Genotype and phenotype of the introgressed lines

The 13 introgressed lines carried one to five chromosome segments from the cherry tomato genome. Eight lines cumulating three, four or five regions were characterized, and each region was thus introgressed into four or five different lines. During marker-assisted backcross process, markers were used to control the genetic background and selection was performed to limit undesired introgression. Table 2 shows the percentage of Cervil genome remaining in each introgressed line, based on more than 100 markers spread over the genome. Over the whole genome, the QTL-NIL contained 4 to 14 % Cervil genome, and the QTL-CIL contained 12–30% Cervil genome. QTL-CIL carried Cervil alleles on a few chromosome fragments apart from the QTL regions. Six lines had a segment of about 27 cM at the top of chromosome 3, seven lines had 15–27 cM at the bottom of chromosome 4, two lines had a fragment of about 10 cM on chromosome 6 and 5 lines had a fragment at the top of chromosome 12 covering about 10 cM. We verified that all these regions did not carry any QTL (Saliba-Colombani et al. 2001). Residual Cervil alleles were found close to QTL regions in only two QTL-NIL (5 cM above CT32 on chromosome 9 in Q_4 and 30 cM from the top of chromosome 4a in Q_{9a}). As these segments did not correspond to any QTL region, we did not take them into account. In the introgression lines, the correlation between the percentage of Cervil genome and the phenotypic value was significant for fruit weight, soluble solid content and sugar content due to the high effect of the QTL controlling these traits. Indeed, when the fragments carrying the QTL were not taken into account, the correlation remained only significant for soluble solid content, confirming the low impact of the residual introgressions outside these regions.

Differences between means of lines over the two years were significant for all the traits, except for locule number, but the year × genotype interaction was only significant for sugar content and acidity and in both cases the genotype effect was much more significant than the interaction (data not shown). The comparison of the QTL-NIL to the recipient line allowed QTL effect in a homogenous genetic background to be assessed (Table 2). We considered only one effect on chromosome 2, as it was impossible to separate the two QTL on this chromosome in the QTL-NIL. Three regions (on chromosomes 1, 2, and 9a) showed significant effects for more than one trait, revealing either pleiotropic effects or linkage of several QTL in the same region. These colocations were in accordance with the correlations observed between soluble solid and sugar content ($r = 0.78$ in the CL-RIL and 0.90 in the introgressed lines) or acidity ($r = 0.59$ in the CL-RIL and 0.80

Table 2 Genotypic and phenotypic characteristics of the near isogenic lines (QTL-NIL) and lines cumulating three to five chromosome regions (QTL-CIL) introgressed from the cherry tomato line (Cervil) into Levovil genetic background

	Cervil (%)	FW	LCN	FIR	SSC	SUC	TA
Donor line mean (Cervil)	100	6.76	2.00	58.33	11.68	5.91	8.16
Recipient line mean (Levovil)	0	124.1	4.27	60.97	5.73	2.77	4.61
QTL-NIL							
Q_1	11	79.5*	3.75 NS	56.24 NS	7.03*	3.41*	5.22*
Q_2	7	57.1*	2.04*	53.58*	6.98*	3.40*	5.91 *
Q_4	8	128.1 NS	4.75 NS	56.74 NS	5.90 NS	3.06 NS	4.82 NS
Q_{9a}	14	94.7*	4.03 NS	57.65 NS	6.78*	3.29*	5.24 *
Q_{9b}	4	112.0 NS	4.40 NS	58.92 NS	5.54 NS	2.69 NS	4.74 NS
QTL-CIL							
$Q_{1/2/9b}$	12	57.7*	2.21*	55.90 NS	7.27*	3.61*	5.90*
$Q_{1/4/9b}$	12	97.8*	3.72 NS	60.61 NS	5.67 NS	3.07 NS	4.89 NS
$Q_{1/9a/9b}$	15	87.4*	3.24*	54.76 NS	6.52*	3.35*	5.44*
$Q_{2/4/9b}$	17	47.6*	2.29*	62.57 NS	6.78*	3.49*	4.87 NS
$Q_{2/9a/9b}$	16	64.0*	2.40*	51.42*	7.98*	3.79*	6.02*
$Q_{4/9a/9b}$	15	82.5*	3.99 NS	62.64 NS	6.46*	3.44*	4.77 NS
$Q_{1/4/9a/9b}$	20	68.3*	3.46*	62.45 NS	6.54*	3.74*	5.19*
$Q_{1/2/4/9a/9b}$	30	29.7*	2.14*	60.04 NS	8.55*	4.57*	5.81*

The percentage of Cervil genome (% Cervil) was assessed based on the genotypes of 91 markers spread over the genome. The means of the QTL-NIL and the QTL-CIL over two trials were compared to the mean of the recipient line for *FW* fruit weight, *LCN* locule number, *FIR* firmness, *SSC* soluble solid content, *SUC* sugar content, *TA* titratable acidity. The regions for which a QTL was detected in the CL-RIL population are underlined

* Significant difference from the recipient line, in Dunnett's test ($P < 0.05$), *NS* non significant

in introgressed lines), as well as between fruit weight and locule number ($r = 0.91$ in introgressed lines). Firmness was not correlated to any other trait except to acidity in the introgressed lines ($r = –0.71$). All the eight QTL-CIL had fruits much smaller than the recipient line, the line with five QTL fragments having the smallest fruits. Locule number of six lines was significantly lower than the recipient line, and only one line ($Q_{2/9a/9b}$) was different for firmness. For sugar content and soluble solid content, seven lines were different from the recipient line and five lines differed in acidity from the recipient line.

Additive and epistatic effects of the QTL

The average values of lines cumulating three, four or five introgressed fragments were compared to the predicted values based on the additive effects of each region assessed from the QTL-NIL (Fig. 2). Then, an overall epistatic effect (e_i) was assessed for each chromosome segment (except 9b) by subtracting from the line with four or five segments the mean of the line with the same fragments except the i fragment and the substitution allelic effect ($2a_i$) (Table 3). The epistatic effects thus corresponded to a mixture of epistatic interactions, between the region of interest on chromosome 1 and chromosomes 4 and 9, between the region of interest on chromosome 2 and chromosomes 1, 4 and 9, between the region of interest on chromosome 4 and chromosomes 1 and 9, and between the region of interest on chromosome 9a and chromosomes 1 and 4, for e_1, e_2, e_4 and e_{9a}, respectively. The region carrying the bottom part of chromosome 9 (region 9b) was present in every QTL-CIL and could not be individually studied, but it did not show any effect on the traits studied here.

For fruit weight, the Cervil alleles had a negative effect for all the three significant QTL on chromosomes 1, 2 and 9a, with a major effect on chromosome 2. A significant reduction of fruit weight was observed when several donor regions were introgressed. The QTL-CIL showed a significant reduction of 26–94 g in fruit mass compared to the recipient line. Expected values were lower than observed values, except in the two QTL-CIL $Q_{2/4/9b}$ and $Q_{4/9a/9b}$ (Fig. 2), indicating less than additive interactions. All the combinations involving the region Q_1 had much lower effects than expected, as if the additive QTL effect of this region had been overestimated in the QTL-NIL. This was confirmed by the estimation of epistatic effect which was very large for Q_1. Epistatic effects appeared as high or higher than additive effects. The sign of all epistatic effects was opposite to that of additive effect, showing that epistasis was reducing the additive effect of the QTL when

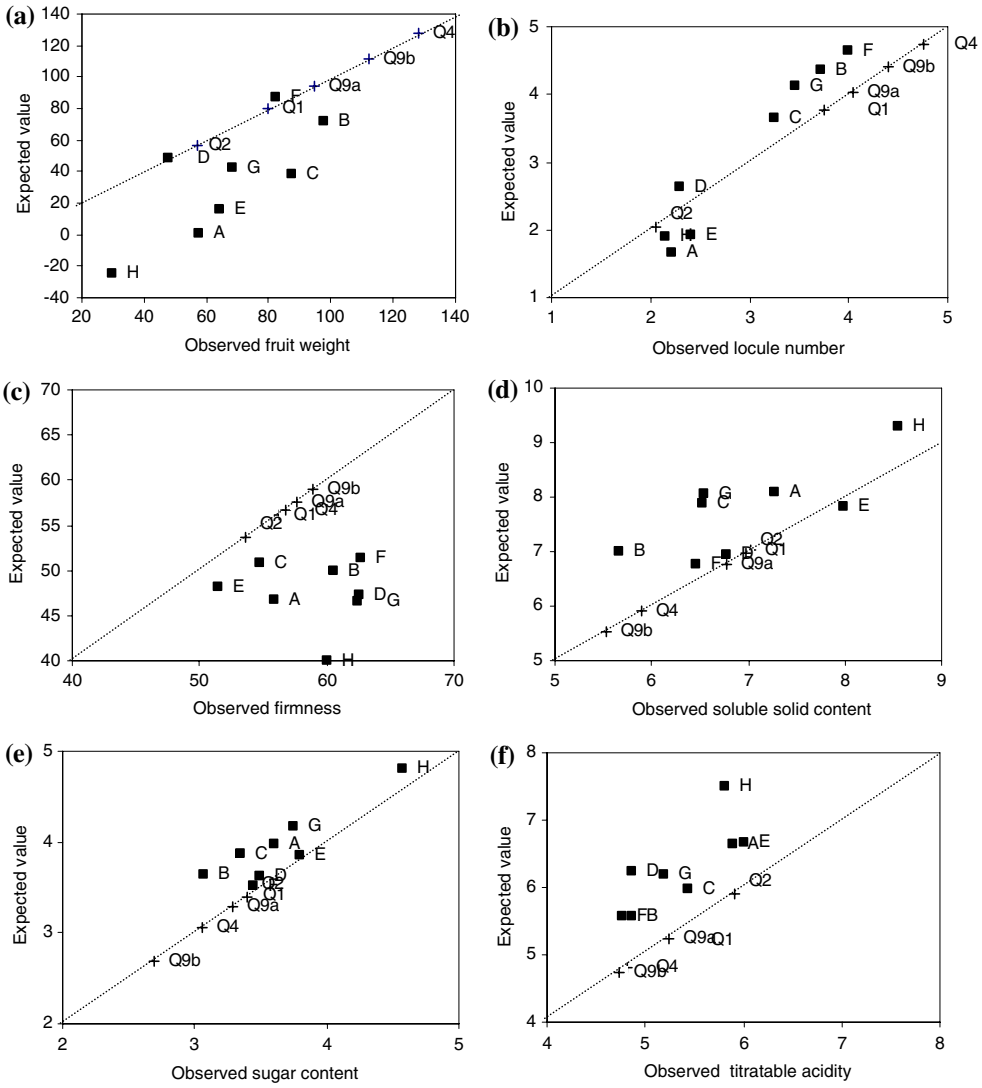

Fig. 2 Relation between expected value of QTL-CIL based on the additive model (*y*-axis) and observed values (*x*-axis) for fruit weight (**a**), locule number (**b**), firmness (**c**), soluble solid content (**d**), sugar content (**e**) and titratable acidity (**f**). The value of the lines with only one introgressed region are indicated on the *datched line* corresponding to $y = x$. The lines cumulating several regions are coded: $Q_{1/2/9b}$ (*A*), $Q_{1/4/9b}$ (*B*), $Q_{1/9a/9b}$ (*C*), $Q_{2/4/9b}$ (*D*), $Q_{2/9a/9b}$ (*E*), $Q_{4/9a/9b}$ (*F*), $Q_{1/4/9a/9b}$ (*G*), $Q_{1/2/4/9a/9b}$ (*H*)

Table 3 Additive (a_i) and epistatic (e_i) effect of each region *i* for for fruit weight (FW), locule number (LCN), firmness (FIR), soluble solid content (SSC), sugar content (SUC) and titratable acidity (TA)

	FW		LCN		FIR		SSC		SUC		TA	
Fragment	a_i	e_i	a_i	e_i	a_i	e_i	a_i	e_i	a_i	e_i	a_i	e_i
Q_1	–22.29*	30.4*	–0.26 NS	–0.01 NS	–2.37 NS	4.54*	0.65*	–1.22*	0.32*	–0.34*	0.31*	–0.19 NS
Q_2	–33.52*	28.4*	–1.12*	0.91*	–3.69*	4.98*	0.62*	0.76*	0.32*	0.20 NS	0.65*	–0.68*
Q_4	2.00 NS	–23.1*	0.24 NS	–0.26 NS	–2.12 NS	11.92*	0.09 NS	–0.15 NS	0.15 NS	0.10 NS	0.10 NS	–0.46*
Q_{9a}	–14.68*	–0.1 NS	–0.12 NS	–0.02 NS	–1.66 NS	5.16*	0.52*	–0.18 NS	0.26*	0.15 NS	0.32*	–0.33*
$r(a_I - e_i)$	–0.93*		–0.99*		0.27 NS		–0.08 NS		0.34 NS		–0.58 NS	

$r(a_i - e_i)$: correlations between additive and epistatic effect

* Significant effect ($P < 0.05$), *NS* non significant

The additive effect a_i was estimated by the difference between the average of the QTL NIL carrying fragment *i* and the recipient line. The epistatic effect was deduced from the the comparison of QTL CIL and QTL NIL values as detailed in ''Materials and methods''

several QTL were combined. Individual effects of Q_1 and Q_{9a} QTL were not detected in the CL-RIL population probably because of the masking effect of Q_2.

Only Q_2 showed a significant additive effect on locule number. The observed and predicted values were relatively close, three QTL-CIL exhibiting observed values higher

than expected, contrarily to the five others. The QTL-CIL cumulating the fragments on chromosome 1 and 9a showed a significant effect which was masked when added to the fragments on chromosome 2, as locule number was the same in $Q_{1/2/9b}$ combination, in $Q_{2/9a/9b}$ combination and in the line carrying the five introgressed regions. This was confirmed by the estimation of epistasis effects: e_2 was highly significant with a sign opposite to the a_2 additive effect.

Firmness exhibited the strongest epistatic effects. For this trait, the additive effect detected in CL-RIL on chromosome 4 and 9a were not significant in QTL-NIL, whereas an additive effect was detected for Q_2, the Cervil alleles reducing firmness. The expected means of QTL-CIL were all lower than the observed values, and the correlation between predicted and observed values was not significant. The epistatic effects were significant for the four regions. As all the QTL-CIL containing the Q_4 fragment were not different in average from the recipient line, the epistatic effect for this fragment was highly significant.

For soluble solid content, sugar content and titratable acidity, significant additive effects were detected for fragments Q_1, Q_2, Q_{9a}, the Cervil alleles always increasing the trait value. Expected values of the QTL-CIL were all equal or higher than observed values, suggesting less than additive interactions. For soluble solid content, it seemed that the presence of Q_4 in a combination, although not significant at the QTL-NIL level, masked the Q_1 effect. The fragment Q_2 also masked the effect of the other fragments except when all the five fragments were included together in the genotype, leading to an interaction e_2 positive as a_2. The epistatic effects e_1 and e_2 were very high for soluble solid content, while for sugar content, only e_1 was significant. For titratable acidity, the epistatic interactions e_2, e_4 and e_{9a} were significant, the presence of Q_2 masking the effect of Q_1 and Q_{9a}. Q_4 also masked the expression of Q_2 in $Q_{2/4/9b}$ and the effect of Q_{9a} in $Q_{4/9a/9b}$, but not in $Q_{1/4/9a}$ nor in the line with the five fragments.

Prediction with a multiplicative model

A multiplicative model was also tested to predict the cumulative effects of the four regions. Such model could be more realistic in the case of additive-by-additive epistasis (Wade et al. 2001). For fruit weight and locule number, both additive and multiplicative models gave similar results. For fruit firmness, the correlation between predicted and observed values was very low and non significant whatever the model. For soluble solid content, sugar content and titratable acidity, the departure from the observed value was higher with the multiplicative model than with the additive one (data not shown).

A re-examination of epistatic interactions in the segregating population

The screening for epistatic interactions in the CL-RIL population was first performed at a $P < 10^{-4}$ threshold (Saliba-Colombani et al. 2001) in order to avoid false positive effects (0.5 significant test per trait expected by chance if we assume the independence of every tests). At this threshold, 6 significant interactions were detected for the traits studied here. They concerned locule number (between chromosomes 8 and 10), firmness (between regions carrying Q_1 and Q_{9a} and among two distant loci of chromosome 6), soluble solid content (among two distant loci of chromosome 6 and between chromosomes 11 and 12), and sugar content (between chromosomes 8 and 10). Taking into account the amount of putative epistatic interactions detected in QTL-CIL, we then re-analysed these data with a less stringent threshold ($P < 10^{-3}$) and in regard to the epistatic effects detected with the QTL-CIL. When all the markers were considered, the number of significant tests was higher than expected by chance only for firmness and sugar content. When we screened for interactions between at least one marker within one of the five regions introgressed and one outside, the number of significant tests exceeded the number expected by chance for firmness, sugar content and acidity. The number of interactions among two of the five regions was low, but interactions were detected for firmness, soluble solid content, and sugar content (Table 4). Among the 39 significant interactions, some involved two or three closely linked markers. In these cases, only the pair of markers with the most significant interaction was taken into account, reducing to 30 the number of putative interactions. Several significant interactions involved loci on chromosome 2, 3, 4 and 6. The epistatic interaction was assessed by an index I_K adapted from Keightley (1996), which ranges from 0 to 1 for antagonistic (or duplicate) epistatic interactions and is higher than 1 for synergistic or complementary epistatic interactions. Among the 30 epistatic interactions 19 had an I_k lower than 1. The Fig. 3 illustrates a few cases of epistasis: (i) ''complementary'', when the two parental genotypes had close averages, opposite to the two recombinant ones (Fig. 3d, f), (ii) ''duplicate'' when three classes had the same average and only one was different (Fig. 3a, c), or (iii) ''intermediate'' when only two genotypes (CC and CL or LL and LC) differed, not the two other ones (Fig. 3b, e, g, h).

Table 4 Significant digenic interactions detected in the CL-RIL population at $P < 10^{-3}$ for fruit weight (FW), locule number (LCN), firmness (FIR), soluble solid content (SSC), sugar content (SUC) and titratable acidity (TA)

Trait	Chr1	Position[a]	Marker 1	Chr2	Position[a]	Marker 2	Probability	R^2	mCC	mLC	mCL	mLL	I_k
FW	Q_2	62	H35M47l	6	50	H38M47g	4.88E-04	0.10	22.03	27.18	20.64	42.01	5.31
LCN	1a	0	CT233	Q_2	73	H35M47l	8.66E-04	0.07	2.39	2.44	3.91	3.15	2.23
	3	10	TG131a	4b	124	TG498	1.96E-04	0.11	3.15	2.74	2.75	3.37	0.27
	8	**38**	**CT287a**	**10**	**62**	**TG001**	**5.55E-06**	**0.15**	**2.86**	**3.65**	**3.06**	**2.61**	0.91
FIR	Q_{1b}	100	TG077	4a	2	CT063a	1.91E-04	0.10	38,613	45,830	39,910	38,895	2.56
	Q_{1b}	133	TG281	Q_{4a}	48	TG287	5.49E-04	0.08	40,863	45,155	38,545	35,800	15.48
	Q_{1b}	**103**	**CT259**	**Q_{9a}**	**55**	**TG079**	**2.13E-05**	**0.12**	**39,655**	**39,514**	**38,601**	**47,473**	6.54
	Q_2	56	CT103	Q_{4a}	50	TG075	3.56E-04	0.07	42,188	44,032	39,171	34,168	2.45
	Q_2	62	H35M47l	Q_{4a}	31	TG339	6.20E-04	0.2	39,742	44,245	38,660	33,397	1.34
	Q_2	71	TG484	8	36	CT245	4.85E-04	0.09	39,988	42,099	42,225	37,028	0.02
	3	3	TG040	7	56	TG639	1.71E-04	0.10	38,068	42,004	42,126	37,998	0.02
	3	76	H35M48d	4a	21	TG123	1.70E-04	0.16	44,674	39,207	37,278	40,245	1.11
	6	42	TG365	8	38	CT287a	6.53E-04	0.10	41,424	37,345	38,499	42,635	0.17
	6	**51**	**TG357**	**6**	**94**	**TG314**	**9.19E-05**	**0.11**	**38,754**	**43,722**	**43,348**	**39,222**	0.04
SSC	Q_2	110	TG492	Q_2	123	CT274	5.67E-04	0.07	7.58	6.23	7.13	6.93	1.30
	3	76	H35M48d	12	14	TG068	7.35E-04	0.13	7.23	7.57	7.83	6.83	0.24
	6	**42**	**TG365**	**6**	**94**	**TG314**	**7.58E-05**	**0.13**	**7.35**	**6.85**	**6.61**	**7.41**	0.05
	11	**83**	**H33M51b**	**12**	**33**	**GC092**	**2.81E-05**	**0.22**	**7.91**	**6.88**	**6.82**	**7.42**	0.43
SUC	Q_2	110	TG492	Q_2	123	CT274	6.22E-04	0.07	4.32	3.53	3.94	3.89	1.39
	Q_2	105	H33M49i	12	35	H33M50p	2.44E-04	0.14	4.13	4.13	4.48	3.63	0.70
	3	103	TG152	Q_{9a}	60	H35M51s	1.38E-04	0.17	3.68	4.34	4.20	3.92	0.17
	3	120	TG267	12	17	H33M61a	5.70E-04	0.17	3.61	4.44	3.99	3.88	0.92
	4b	124	TG498	6	30	H33M47l	4.14E-04	0.17	3.91	4.30	4.27	3.70	0.03
	5	47	CT091a	Q_{9a}	43	H35M51s	3.03E-04	0.14	3.76	4.45	4.11	3.91	0.62
	6	5	GC094	6	18	GC099	7.00E-04	0.10	3.89	4.38	4.28	3.84	0.12
	8	**27**	**H33M49g**	**10**	**62**	**TG001**	**7.26E-05**	**0.20**	**4.3**	**3.80**	**3.70**	**4.25**	0.05
	11	83	H33M51b	12	33	GC092	1.34E-04	0.18	4.44	3.70	3.91	4.10	0.58
TA	3	3	TG040	8	41	CT069	5.08E-04	0.09	7.34	8.07	8.46	7.57	0.32
	3	83	CT085	Q_{4a}	31	TG339	2.19E-04	0.13	8.01	7.72	9.22	6.93	3.00
	Q_{4a}	45	H38M62f	6	30	H33M47l	2.57E-04	0.16	8.18	7.59	6.97	8.58	0.22

The average value of each genotypic class (CC, CL, LC and LL) is indicated. The magnitude of epistasis is estimated by an Index I_k (see ''Materials and methods'')

Significant effects at the $P < 10^{-4}$ threshold are in bold

[a] The marker position is indicated as extrapolated on the high-density map (Tanksley et al. 1992)

For fruit weight, a significant interaction was detected between the fragment Q_2 and a marker on chromosome 6. This interaction corresponded to complementary epistasis, where only the genotype combining recipient alleles at both loci increased fruit weight. For locule number, three significant interactions involved six chromosome regions. Firmness showed the largest number of significant tests (ten interactions involving ten chromosome regions). Four interactions involved two QTL regions and two others involved one QTL region. Some regions on chromosome 1, 2, 4 and 8 were involved in two or three different interactions. For soluble solid content, four significant interactions were detected, one between two loci of Q_2, one between two loci on chromosome 6, the others involving the chromosomes 3, 11 and 12. For sugar content, four interactions among nine involved at least one QTL region (Q_2 or Q_{9a}). For acidity, three interactions were significant, two involving Q_4. For chemical traits, most of the interactions were of the duplicate type.

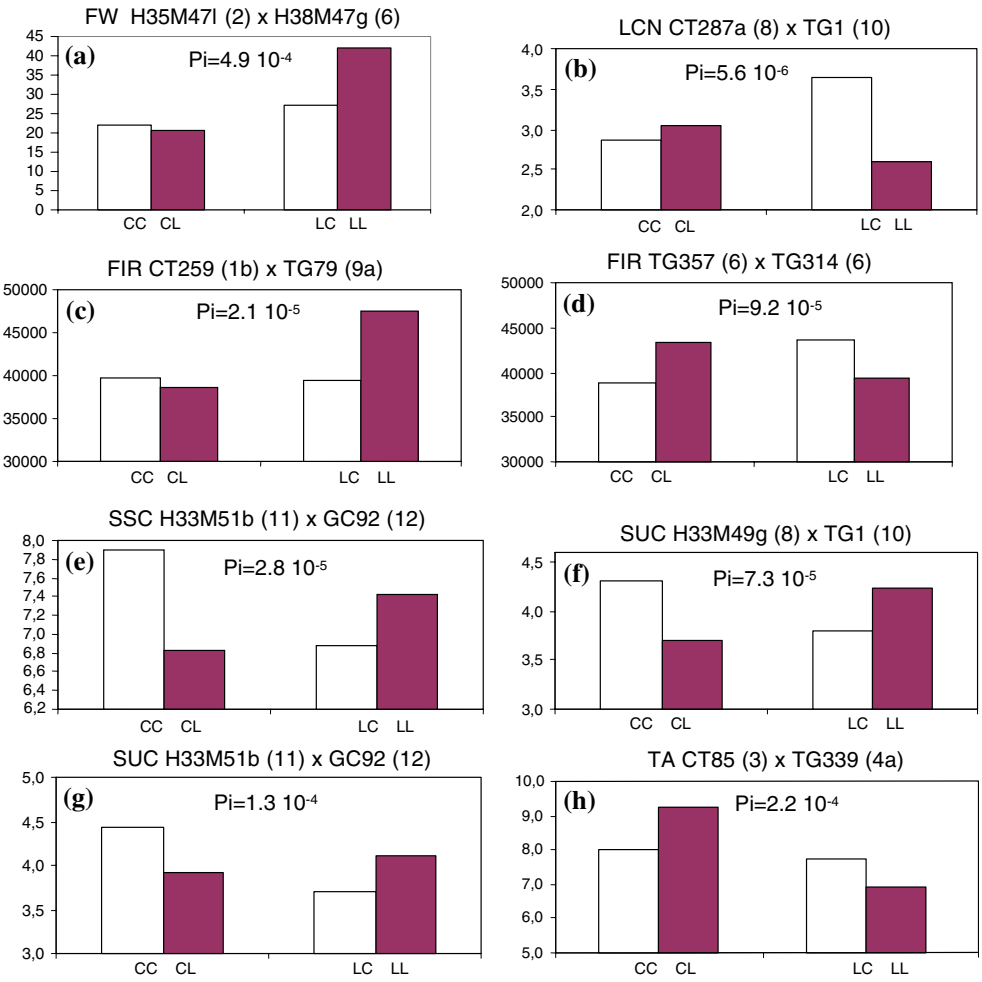

Fig. 3 Examples of epistatic interactions detected in CL-RIL population between pairs of markers. The probability (*Pi*) of the interaction is indicated. Average values of the four genotypic classes (*CC*, *CL*, *LC*, *LL*) according to the genotypes at each marker are shown for (**a**) fruit weight, interaction between H35M47l and H38M47g, (**b**) locule number, interaction between CT287a and TG1, (**c**) firmness, interaction between CT259 and TG79, (**d**) firmness, interaction between TG357 and TG314, (**e**) soluble solid content, interaction between H33M51b and GC92, (**f**) sugar content, interaction between H33M49g and TG1, (**g**) sugar content, interaction between H33M51b and GC 92, (**h**) titratable acidity, interaction between CT85 and TG339. Chromosome number is indicated after each marker

Discussion

Interest of QTL-NIL and QTL-CIL for revealing epistasis

Our results showed that cumulating QTL may not always provide the trait values expected based on additive effects. The interest to physically generate the genetic decomposition of interactions by limiting the total genotypic variation to just a few sites has already been underlined either with point mutations in *Drosophila* (Clark and Wang 1997) or with introgressed regions in tomato (Eshed and Zamir 1996). Introgression lines allow one to attribute all the observed variation only to the difference in the introgressed region and they facilitate fine mapping experiment (Eshed and Zamir 1996). The development of QTL-NIL also provides valuable material for marker-assisted selection, permitting a more detailed evaluation of the effect of a given QTL in a new genetic composition (Van Berloo et al. 2001). Due to epistasis, selection of lines carrying several QTL combinations may also be interesting, as the best genotype may not always be the line having the highest number of QTL introgressed (Robert et al. 2001; Yousef and Juvik 2002).

Importance of non additive interactions in the genetic control of fruit quality traits

Except for firmness, a large part of the variation of the six fruit quality traits was under additive control, but interactions leading to less than additive effects were common. This study revealed that epistasis may control a significant part of the genetic variation for quantitative traits. A previous experiment, where the same five QTL were simultaneously transferred into three genetic backgrounds and then crossed in a half diallel design, also resulted in the evidence of QTL by genetic background interactions for most of the quality traits (Lecomte et al. 2004). Nevertheless, it was not yet possible to assess epistasis at the QTL level. In the lines cumulating several QTL regions, most of the significant epistatic effects (e_i) had a sign opposite to the additive effects (a_i), suggesting less than

additive epistasis. Eshed and Zamir (1996) found that 28% of the epistatic interactions were significant and generally independent of the scale, for yield-related traits. Most of the interactions they found were also less than additive. Furthermore they found that the combinations of three QTL exhibited even more important epistatic effect than combinations of two. The additive and epistatic effects were negatively correlated for fruit weight, locule number and acidity, the strongest additive effects having thus the most masking effects on the other regions. These traits were also those exhibiting the highest heritability in CL-RIL (Table 1).

Epistasis was detected more frequently in introgression lines than in the CL-RIL, confirming that epistasis detection in a RIL population is powerless. In CL-RIL, additive and epistatic effects are partially confounded, as the analysis can in fact detect as a single QTL a region that has no main effect but interacts epistatically with another one (Purcell and Sham 2004). Testing only single point effects may thus reveal statistical additive effects that actually correspond to physiological epistasis: indeed, in case of duplicate epistatic interactions, two apparently additive QTL could be detected, whereas in case of complementary epistatic interactions, no main effect should be detected. The major limit in testing two-point interactions in RIL is due to the population size, but otherwise, RIL population, with only four homozygous genotypic classes is the best situation among segregating populations to test additive × additive interactions.

In CL-RIL, epistatic interactions frequently involved regions where additive effects were already detected. Limiting the analysis to these regions reduced the number of tests performed and allowed us to use a less stringent threshold, but some important interactions could be missed, as for instance between chromosome 8 and 10, for locule number and sugar content, or between markers of chromosome 6 for firmness and soluble solid content. Some of the interactions were detected between linked markers on chromosome 6 and 2. Such interactions between linked loci have already been detected in several studies (Visker et al. 2003; Steinmetz et al. 2002) and their evidence is particularly clear in fine mapping experiments (Kroymann and Mitchell-Olds 2005; Monforte and Tanksley 2000).

Origin of epistatic interactions

The intensity of epistatic interactions varied according to the traits and several causes of epistasis could be proposed. In order to understand the consistency of QTL effects when they are simultaneously introgressed with other QTL, two prediction models were used to estimate the value of lines having several introgressed regions, a linear additive model and a multiplicative model. The multiplicative model fitted slightly better only for fruit weight. For the other traits, none of the models gave good predictions using QTL-NIL data, and when expected values were estimated from the additive effects assessed in the CL-RIL, the prediction did not provide better results (data not shown). The presence of interactions among QTL revealed that loci epistatically control the same developmental process. It is thus important to dissect the processes underlying complex traits either through the analysis of metabolic pathway (McMullen et al. 2001) or by ecophysiological modeling (Quilot et al. 2004), and search for QTL of the individual processes. This should increase the efficiency of the choice of QTL for gene pyramiding as more epistatic interactions are expected between QTL corresponding to the same process. Some masking effect may also result from QTL acting successively on the same pathway. Whole genome scan and systems biology approaches reveal the importance of epistatic interactions and may further conciliate the two definitions of epistasis as shown in *Drosophila* (Anholt et al. 2003) or yeast (Segre et al. 2005).

Genetic control of tomato quality traits

Expression of genetic interactions may depend on the genetic backgrounds. For instance, in tomato, introgressions of a QTL region of chromosome 4 from three wild species showed differences in the magnitude of main effects and interactions. QTL locations were conserved across species, but they exerted additive or epistatic effect according to their origin (Monforte et al. 2001). We thus compared the results presented here with those obtained in the literature with other progenies (mostly advanced backcross and introgression lines), in order to compare the location of epistatic interactions with the locations of main effect QTL. Two QTL were considered to be putatively the same locus if they mapped to the same 20 cM region of the high density tomato map (Tanksley et al. 1992). Thus many QTL detected in this study (with additive or epistatic effect) corresponded to main effect QTL in other studies on tomato, confirming that QTL are consistent over species, but that some QTL may be detected as additive in one cross and epistatic in another one. Particularly many QTL detected in advanced backcross progenies exhibited apparent additive effect, as interaction can not be tested in such progeny unless crosses between advanced backcrosses are studied.

For example, a set of at least 28 QTL controlling *fruit weight* variation has been identified in a synthesis of 15 studies on tomato (Grandillo et al. 1999). In the CL-RIL population, five QTL were mapped and two other (on Q_1 and Q_{9a}) were detected in QTL-CIL. Fruit weight QTL have already been found in all these regions, in at least two other progenies (Grandillo et al. 1999), except on

chromosome 12. In CL-RIL, a duplicate epistatic interaction was shown between Q_2 and a region of chromosome 6 where a QTL was detected by Grandillo et al. (1999).

For *locule number*, at least eight QTL were previously detected in four progenies (Lippman and Tanksley 2001; Van Der Knaap and Tanksley 2003; Barrero and Tanksley 2004), five of which being detected in at least two progenies. Interactions between QTL were detected only in certain progenies, and three new putative epistatic interactions involving five other fragments were detected in the CL-RIL.

Many biological processes control fruit *firmness*: the change of color during ripening, the ethylene synthesis partly responsible of cell wall loosening, cell adhesion and osmotic pressure modifications (Seymour et al. 2002). Although a few major mutations are known to influence fruit ripening and firmness (Giovannoni 2001), firmness has a low heritability and is generally quantitatively inherited. A comparison of previously mapped QTL for firmness in progenies of *S. pimpinellifolium* (Tanksley et al. 1996), *S. peruvianum* (Fulton et al. 1997), *S. neorickii* (Fulton et al. 2000), *S. habrochaites* (Bernacchi et al. 1998) and *S. pennellii* (M. C. unpublished data) revealed 28 QTL in 18 regions. Firmness had the lowest heritability and the lowest number of QTL detected in CL-RIL. QTL on chromosome 4 and 9 were already detected twice in the same regions in studies of advanced backcross progenies involving wild species. For this trait the epistatic interactions appeared much more important than additive effects in both CL-RIL and QTL-CIL. In QTL-CIL the four regions exhibited significant epistatic interactions and ten interactions were significant in the CL-RIL, among which four involved Q_4 or Q_{9a}, three Q_2 and one Q_1, highlighting the consistency of results in QTL-CIL and CL-RIL. Overall, 13 of the 20 loci involved in interactions corresponded to main effect QTL detected in another progeny.

Soluble solid content is mainly determined by the content in sugars and acids. Many studies have focused on the identification of QTL controlling this trait, and more than 20 QTL were mapped (Fulton et al. 2002; Eshed and Zamir 1995; Causse et al. 2004). The two additive QTL detected in the CL-RIL on chromosome 2 and 9 corresponded to regions where several QTL were already detected, as well as the epistatic loci. The region Q_1 detected in QTL-CIL also corresponded to a QTL for soluble solid content in Fulton et al. (2002). Thus for this trait, no new QTL location was shown.

QTL for *sugars* have been mapped in at least 35 regions (Fulton et al. 2002; Causse et al. 2004). All the QTL regions detected with CL-RIL and QTL-NIL have already been detected with other populations. In the CL-RIL a complex network of interactions was shown. A total of 9 interactions involved 14 regions, among which 8 corresponded to QTL regions in other studies.

Acidity relies on the content of citric and malic acids, for which at least 29 QTL have been detected (Fulton et al. 2002; Causse et al. 2004). All the additive QTL detected in CL-RIL or QTL-NIL have already been detected in another progeny. Four of the 6 loci involved in interactions corresponded to QTL in other progenies. Thus, more than 30 QTL could be involved in the control of acid content.

In conclusion, we showed that most of the chromosome regions where loci control genetic variation for fruit quality traits had already been identified in one of about ten studies published. Several QTL may lay in each of these regions and only high resolution mapping experiments can resolve the number of underlying genes. The actual effect of these QTL on trait variation is strongly dependent on the genetic background and the influence of epistasis needs to be reconsidered in order to increase efficiency of marker-assisted selection and QTL characterization. The future progresses in tomato genome sequencing (Mueller et al. 2005) will provide new tools to be combined with quantitative genetics for this purpose.

Acknowledgments We are grateful to anonymous reviewers for their helpful comments. Many thanks to A. Gautier for genotypic and phenotypic works, to the greenhouse experimental team for plant cultivation, to A. M. Cossalter for taking care of the plants and for her help during the phenotypic evaluations, to P. Duffé for plant genotyping, to R. Matthieu for chemical evaluations and to C. Dillmann for SAS programs. Thanks to Vilmorin for providing lines and the trial in Ledenon. Laurent Lecomte was partly supported by the Conseil Régional Provence-Alpes-Côte d'Azur (France). The experiments comply with the current French laws.

References

Ahmadi N, Albar L, Pressoir G, Pinel A, Fargette D, Ghesquiere A (2001) Genetic basis and mapping of the resistance to Rice yellow mottle virus. III. Analysis of QTL efficiency in introgressed progenies confirmed the hypothesis of complementary epistasis between two resistance QTLs. Theor Appl Genet 103:1084–1092

Anholt RRH, Dilda CL, Chang S, Fanara JJ, Kulkarni NH, Ganguly I, Rollmann SM, Kamdar KP, Mackay TFC (2003) The genetic architecture of odor-guided behavior in *Drosophila*: epistasis and the transcriptome. Nature Genet 35:180–184

Barrero LS, Tanksley SD (2004) Evaluating the genetic basis of multiple-locule fruit in a broad cross section of tomato cultivars. Theor Appl Genet 109:669–679

Bernacchi D, Beck-Bunn T, Eshed Y, Lopez J, Petiard V, Uhlig J, Zamir D, Tanksley SD (1998) Advanced backross QTL analysis in tomato. I. Identification of QTL for traits of agronomic importance from *Lycopersicon hirsutum*. Theor Appl Genet 97:381–397

Caicedo AL, Stinchcombe JR, Olsen KM, Schmitt J, Purugganan MD (2004) Epistatic interaction between *Arabidopsis* FRI and FLC flowering time genes generates a latitudinal cline in a life history trait. Proc Natl Acad Sci USA 101:15670–15675

Calenge F, Drouet D, Denance C, Van De Weg WE, Brisset M-N, Paulin JL, Durel CE (2005) Identification of a major QTL together with several minor additive or epistatic QTL for resistance to fire blight in apple in two related progenies. Theor Appl Genet 111:128–135

Carlborg O, Haley CS (2004) Epistasis: too often neglected in complex trait studies? Nat Rev Genet 5:618–624

Causse M, Saliba-Colombani V, Lecomte L, Duffé P, Rousselle P, Buret M (2002) QTL analysis of fruit quality in fresh market tomato: a few chromosome regions control the variation of sensory and instrumental traits. J Exp Bot 53:2089–2098

Causse M, Duffe P, Gomez MC, Buret M, Damidaux R, Zamir D, Gur A, Chevalier C, Lemaire-Chamley M, Rothan C (2004) A genetic map of candidate genes and QTL involved in tomato fruit size and composition. J Exp Bot 55:1671–85

Charcosset A, Causse M, Moreau M, Gallais A (1995) Investigation into the effect of genetic background on QTL expression using three connected maize recombinant inbred lines (RIL) populations. In: van Ooijen JW (ed) Biometrics in plant breeding: applications of molecular markers, pp 83–89

Cheverud JM, Routman EJ (1996) Epistasis as a source of increased additive genetic variance at population bottlenecks. Evolution 50:1042–1051

Clark AG, Wang L (1997) Epistasis in measured genotypes: Drosophila P-element insertions. Genetics 147:157–163

Clarke JD, Aarts N, Feys BJ, Dong XN, Parker JE (2001) Constitutive disease resistance requires EDS1 in the *Arabidopsis* mutants cpr1 and cpr6 and is partially EDS1-dependent in cpr5. Plant J 26:409–420

Coaker GL, Francis DM (2004) Mapping, genetic effects, and epistatic interaction of two bacterial canker resistance QTLs from *Lycopersicon hirsutum*. Theor Appl Genet 108:1047–1055

Cockerham CC, Zeng ZB (1996) Design III with marker loci. Genetics 143:1437–1456

Cordell HJ, Todd JA, Hill NJ, Lord CJ, Lyons PA, Peterson LB, Wicker LS, Clayton DG (2001) Statistical modeling of interlocus interactions in a complex disease: rejection of the multiplicative model of epistasis in type 1 diabetes. Genetics 158:357–367

Doebley J, Stec A, Gustus C (1995) Teosinte Branched 1 and the origin of maize—evidence for epistasis and the evolution of dominance. Genetics 141:333–346

Elena SF, Lenski RE (2001) Epistasis between new mutations and genetic background and a test of genetic canalization. Evolution 55:1746–1752

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

Eshed Y, Zamir D (1996) Less-than-additive epistatic interactions of quantitative trait loci in tomato. Genetics 143:1807–1817

Fenster C, GallowayLF, Chao L (1997) Epistasis and its consequences for the evolution of natural populations. Trends Ecol Evol 12:282–286

Fisher RA (1918) The correlation between relatives on the supposition of Mendelian inheritance. Trans R Soc Edinb 52:399–433

Fulton TM, BeckBunn T, Emmatty D, Eshed Y, Lopez J, Petiard V, Uhlig J, Zamir D, Tanksley SD (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 TM, Grandillo S, Beck-Bunn T, Fridman E, Frampton A, Lopez J, Petiard V, Uhlig J, Zamir D, Tanksley SD (2000) Advanced backcross QTL analysis of a *Lycopersicon esculentum x Lycopersicon parviflorum cross*. Theor Appl Genet 100:1025–1042

Fulton TM, Bucheli P, Voirol E, Lopez J, Petiard V, Tanksley SD (2002) Quantitative trait loci (QTL) affecting sugars, organic acids and other biochemical properties possibly contributing to flavor, identified in four advanced backcross populations of tomato. Euphytica 127:163–177

Giovannoni J (2001) Molecular biology of fruit maturation and ripening. Ann Rev Plant Phys Plant Mol Biol 52:725–749

Grandillo S, Ku HM, Tanksley SD (1999) Identifying the loci responsible for natural variation in fruit size and shape in tomato. Theor Appl Genet 99:978–987

Hua JP, Xing YZ, Wei WR Xu C, Sun X, Yu S, Zhang Q (2003) Single-locus heterotic effects and dominance by dominance interactions can adequately explain the genetic basis of heterosis in an elite rice hybrid. Proc Natl Acad Sci USA 100:2574-2579

Jannink JL, Jansen R (2001) Mapping epistatic quantitative trait loci with one-dimensional genome searches. Genetics 157:445–454

Johnson WC, Gepts P (2002) The Role of epistasis in controlling seed yield and other agronomic traits in an Andean x Mesoamerican cross of common bean (*Phaseolus vulgaris* L.). Euphytica 125:69–79

Kabelka E, Yang WC, Francis DM (2004) Improved tomato fruit color within an inbred backcross line derived from *Lycopersicon esculentum* and *L. hirsutum* involves the interaction of loci. J Amer Soc Hort Sci 129:250–257

Kao CH, Zeng ZB, Teasdale RD (1999) Multiple interval mapping for quantitative trait loci. Genetics 152:1203–1216

Kearsey M.J, Pooni HS Syed NH (2003) Genetics of quantitative traits in *Arabidopsis thaliana*. Heredity 91:456–464

Keightley PD (1996) Metabolic models of selection response. J Theor Biol 182:311–316

Kroymann J, Mitchell-Olds T (2005) Epistasis and balanced polymorphism influencing complex trait variation. Nature 435:95–98

Kubo T, Yoshimura A (2005) Epistasis underlying female sterility detected in hybrid breakdown in a Japonica-Indica cross of rice (*Oryza sativa* L.). Theor Appl Genet 110:346–355

Lark KG, Chase K, Adler F, Mansur LM, Orf JH (1995) Interactions between quantitative trait loci in soybean in which trait variation at one locus is conditional upon a specific allele at another. Proc Natl Acad Sci USA 92:4656–4660

Lecomte L, Duffé P, Buret M, Servin B, Hospital F, Causse M (2004) Marker-assisted introgression of five QTLs controlling fruit quality traits into three tomato lines revealed interactions between QTLs and genetic background. Theor Appl Genet 109:658–668

Levin I, Lalazar A, Bar M, Schaffer AA (2004) Non GMO fruit factories strategies for modulating metabolic pathways in the tomato fruit. Ind Crops Prod 20:29–36

Li ZK, Luo LJ, Mei HW, Wang DL, Shu QY, Tabien R, Zhong DB, Ying CS, Stansel JW, Khush GS, Paterson AH (2001) Overdominant epistatic loci are the primary genetic basis of inbreeding depression and heterosis in rice. I. Biomass and grain yield. Genetics 158:1737–1753

Li J, Brader G, Palva ET (2004) The WRKY70 transcription factor: a node of convergence for jasmonate-mediated and salicylate-mediated signals in plant defense. Plant Cell 16:319–331

Lippman Z, Tanksley SD (2001) 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. Genetics 158:413–422

Liu PY, Zhu J, Lou XY, Lu Y (2003) A method for marker-assisted selection based on QTLs with epistatic effects. Genetica 119:75–86

Mahmood T, Ekuere U, Yeh F, Good AG, Stringam GR (2003) RFLP linkage analysis and mapping genes controlling the fatty acid profile of *Brassica juncea* using reciprocal DH populations. Theor Appl Genet 107:283–290

McMullen MD, Snook M, Lee EA, Byrne PF, Kross H, Musket TA, Houchins K, Coe EH (2001) The biological basis of epistasis between quantitative trait loci for flavone and 3-deoxyanthocyanin synthesis in maize (*Zea mays* L.) Genome 44:667–676

Mei HW, Luo LJ, Ying CS, Wang YP, Yu XQ, Guo LB, Paterson AH, Li ZK (2003) Gene actions of QTLs affecting several agronomic traits resolved in a recombinant inbred rice population and two testcross populations. Theor Appl Genet 107:89–101

Monforte AJ, Tanksley SD (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

Monforte AJ, Friedman E, Zamir D, Tanksley SD (2001) Comparison of a set of allelic QTL-NILs for chromosome 4 of tomato: deductions about natural variation and implications for germplasm utilization. Theor Appl Genet 102:572–590

Monna L, Lin HX, Kojima S, Sasaki T, Yano M (2002) Genetic dissection of a genomic region for a quantitative trait locus, Hd3, into two loci, Hd3a and Hd3b, controlling heading date in rice. Theor Appl Genet 104:772–778

Mueller LA, Tanksley SD, Giovannoni JJ, van Eck J, Stack S, Choi D, Kim BD, Chen MS, Cheng ZK, Li CY, Ling HQ, Xue YB, Seymour G, Bishop G, Bryan G, Sharma R, Khurana J, Tyagi A, Chattopadhyay D, Singh NK, Stiekema W, Lindhout P, Jesse T, Lankhorst RK, Bouzayen M, Shibata D, Tabata S, Granell A, Botella MA, Giullano G, Frusciante L, Causse M, Zamir D (2005) The tomato sequencing project, the first cornerstone of the International Solanaceae Project (SOL). Compar. Funct. Genomics 6:153–158

Phillips PC (1998) The language of gene interaction. Genetics 149:1167–1171

Purcell S, Sham PC (2004) Epistasis in quantitative trait locus linkage analysis: interaction or main effect? Behav Genet 34:143–152

Quilot B, Génard M, Kervella J, Lescourret F (2004) Analysis of genotypic variations in flesh total sugar content via a modelling approach applied to peach fruit. Theor Appl Genet 109:440–449

Robert VJM, West MAL, Inai S, Caines A, Arntzen L, Smith JK, St Clair DA (2001) Marker-assisted introgression of blackmold resistance QTL alleles from wild *Lycopersicon cheesmanii* to cultivated tomato (*L. esculentum*) and evaluation of QTL phenotypic effects. Mol Breed 8:217–233

Saliba-Colombani V, Causse M, Langlois D, Philouze J, Buret M (2001) Genetic analysis of organoleptic quality in fresh market tomato. 1. Mapping QTLs for physical and chemical traits. Theor Appl Genet 102:259–272

SAS Institute (1988) SAS users guide: statistics (SAS Institute, ed). Cary, North Carolina

Segre D, DeLuna A, Church GM, Kishony R (2005) Modular epistasis in yeast metabolism. Nat Genet 37:77–83

Seymour G, Manning K, Eriksson EM, Popovich AH, King GJ (2002) Genetic identification and genomic organization of factors affecting fruit texture. J Exp Bot 53:2065–2071

Steinmetz LM, Sinha H, Richards DR, Spiegelman JI, Oefner PJ, McCusker JH, Davis RW (2002) Dissecting the architecture of a quantitative trait locus in yeast. Nature 416:326–330

Syed NH, Chen ZJ (2005) Molecular marker genotypes, heterozygosity and genetic interactions explain heterosis in *Arabidopsis thaliana*. Heredity 94:295–304

Tanksley SD (1993) Mapping polygenes. Annu Rev Genet 27:205–233

Tanksley SD, Ganal MW, Prince JP, de Vicente MC, Bonierbale MW, Broun P, Fulton TM, Giovannoni JJ, Grandillo S, Martin GB, Messeguer R, Miller JC, Miller L, Paterson AH, Pineda O, Roder MS, Wing RA, Wu W, Young ND (1992) High density molecular linkage maps of the tomato and potato genomes. Genetics 132:1141–1160

Tanksley SD, Grandillo S, Fulton TM, Zamir D, Eshed Y, Tanksley SD, Grandillo S, Fulton TM, Zamir D, Eshed Y, Petiard V, Lopez J, 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

Thabuis A, Palloix A, Servin B, Daubèze AM, Signoret P, Hospital F, Lefebvre V (2004) Marker-assisted introgression of 4 *Phytophthora capsici* resistance QTL alleles into a bell pepper line: validation of additive and epistatic effects. Mol Breed 14:9–20

Ungerer MC, Halldorsdottir SS, Modliszewski JL, Mackay TFC, Purugganan MD (2002) Quantitative trait loci for inflorescence development in *Arabidopsis thaliana*. Genetics 160:1133–1151

van Berloo R, Aalbers H, Werkman A, Niks RE (2001) Resistance QTL confirmed through development of QTL-NILs for barley leaf rust resistance. Mol Breed 8:187–195

van der Knaap E, Tanksley SD (2003) The making of a bell pepper-shaped tomato fruit: identification of loci controlling fruit morphology in Yellow Stuffer tomato. Theor Appl Genet 107:139–147

van der Knaap E, Lippman ZB, Tanksley SD (2002) Extremely elongated tomato fruit controlled by four quantitative trait loci with epistatic interactions. Theor Appl Genet 104:241–247

Visker MHPW, Keizer LCP, Van Eck HJ, Jacobsen E, Colon LT, Struik PC (2003) Can the QTL for late blight resistance on potato chromosome 5 be attributed to foliage maturity type? Theor Appl Genet 106:317–325

Wade MJ, Winther RG, Agrawal AF, Goodnight CJ (2001) Alternative definitions of epistasis: dependence and interaction. Trends Ecol Evol 16:498–504

Wang DL, Zhu J, Li ZK, Paterson AH (1999) Mapping QTLs with epistatic effects and QTL x environment interactions by mixed linear model approaches. Theor Appl Genet 99:1255–1264

Yousef GG, Juvik JA (2002) Enhancement of seedling emergence in sweet corn by marker-assisted backcrossing of beneficial QTL. Crop Sci 42:96–104

Yu SB, Li JX, Xu CG, Tan YF, Gao YJ, Li XH, Zhang Q, Saghai Maroof MA (1997) Importance of epistasis as the genetic basis of heterosis in an elite rice hybrid. Proc Natl Acad Sci USA 94:9226–9231

Xing YZ, Tan YF, Hua JP, Sun XL, Xu CG, Zhang Q (2002) Characterization of the main effects, epistatic effects and their environmental interactions of QTLs on the genetic basis of yield traits in rice. Theor Appl Genet 105:248–257

Zhao JW, Meng JL (2003) Genetic analysis of loci associated with partial resistance to Sclerotinia sclerotiorum in rapeseed (*Brassica napus L.*). Theor Appl Genet 106(4):759–764

Zhao JY, Becker HC, Zhang DQ, Zhang YF, Ecke W (2005) Oil content in a European x Chinese rapeseed population: QTL with additive and epistatic effects and their genotype-environment interactions. Crop Sci 45:51–59