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Mapping of QTLs for glandular trichome densities and *Trialeurodes vaporariorum* (greenhouse whitefly) resistance in an F_2 from *Lycopersicon esculentum* × *Lycopersicon hirsutum* f. *glabratum*

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An F_2 of an interspecific cross between cultivated tomato (*Lycopersicon esculentum* cv. Moneymaker) and *L. hirsutum* f. *glabratum* was used to generate an RFLP linkage map. Distortion of single locus segregation (1:2:1) was observed for a number of markers from different chromosomes, always with a prevalence for *L. hirsutum* f. *glabratum* alleles. To identify quantitative trait loci (QTLs) for greenhouse whitefly (*Trialeurodes vaporariorum*) resistance in this F_2 population, life history components of the greenhouse whitefly population were evaluated. Two QTLs affecting oviposition rate mapped to chromosome 1 (*Tv-1*) and 12 (*Tv-2*). F_3 lines homozygous for either the *L. esculentum* allele or the *L. hirsutum* f. *glabratum* allele at one or both loci confirmed the effects of *Tv-1* and *Tv-2*. The F_2 population was also evaluated for segregation of type IV and type VI glandular trichome densities. Two QTLs affecting trichome type IV density (*TriIV-1* and *TriIV-2*) and one affecting type VI trichome density (*TriVI-1*) mapped to chromosomes 5, 9 and 1, respectively. These results do not support the hypothesis that the density of type IV trichomes is involved in whitefly resistance.

Keywords: insect resistance, *Lycopersicon esculentum*, *L. hirsutum* f. *glabratum*, *Trialeurodes vaporariorum*, QTL mapping, segregation distortion.

Introduction

The greenhouse whitefly (*Trialeurodes vaporariorum* Westw.) is an endemic pest in greenhouse tomato (*Lycopersicon esculentum*) cultivation in northern Europe. As all tomato cultivars are susceptible (Gentile *et al.*, 1968; De Ponti *et al.*, 1975) and because considerable costs are involved in controlling the pest chemically or biologically, there is an urgent need for resistant cultivars. Unfortunately, breeding for whitefly resistance is hampered by the apparent quantitative inheritance of the resistance, the variation in the whitefly population and large environmental variation of population growth (De Ponti *et al.*, 1975; Bas *et al.*, 1992). Hence, large plant populations are required for resistance tests.

A high level of resistance has been found in the wild species *L. hirsutum* f. *glabratum* (De Ponti *et al.*, 1975; Bas *et al.*, 1992) and *L. pennellii* (Gentile *et al.*, 1968). Tests for resistance using clip-on cages (Berlinger & De Ponti, 1981; Romanow *et al.*, 1991) have revealed large differences between *L. hirsutum* f. *glabratum* and *L. esculentum* with respect to the life history components oviposition rate (OR), adult survival (AS) and preadult survival (PS) (Bas *et al.*, 1992).

An association between insect resistance and the presence and density of type IV and type VI glandular trichomes in *L. hirsutum* f. *glabratum* and *L. pennellii* has been reported by several authors (Snyder & Carter, 1984; Fery & Kennedy, 1987; Goffreda *et al.*, 1988, 1990a,b; Weston *et al.*, 1989). Trichome type IV is present in *L. pennellii* and *L. hirsutum* f. *glabratum* but absent in *L. esculentum*.

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Trichome type VI is present in all *Lycopersicon* species (Luckwill, 1943) but more abundant in *L. hirsutum* f. *glabratum* than in *L. esculentum* (Fery & Kennedy, 1987). In F_2 progenies from *L. esculentum* × *L. hirsutum* f. *hirsutum* an association was observed between resistance to the two-spotted spider mite (*Tetranychus urticae* Koch) and type IV and type VI trichome densities (Carter & Snyder, 1985). Also the exudates from glandular trichomes of wild tomato species may be involved in insect resistance. Goffreda *et al.* (1988) reported that glucose esters in the exudate of type IV trichomes in *L. pennellii* were involved in mediating resistance to the potato aphid (*Macrosiphum euphorbiae* Thomas). Williams *et al.* (1980) showed that 2-tridecanone in type VI trichome glands of *L. hirsutum* f. *glabratum* had an insecticide activity to *Manduca sexta* L., *Heliothis zea* (Boddie) and *Aphis gossypii* Glover. Moreover, 2-tridecanone is far more abundant in *L. hirsutum* f. *glabratum* than in *L. esculentum* (Williams *et al.*, 1980; Zamir *et al.*, 1984). Fery & Kennedy (1983, 1987) observed significant effects of both type VI trichome density and 2-tridecanone concentration on resistance to *Manduca sexta* in segregating progenies from *L. esculentum* × *L. hirsutum* f. *glabratum* but no association was found between these two characters.

The genetics of these characters related to insect resistance appears to be complex. For example, Zamir *et al.* (1984) reported association of the level of 2-tridecanone in *L. hirsutum* f. *glabratum* with five marker loci on at least four different chromosomes. Nienhuis *et al.* (1987) found association of 2-tridecanone level with marker loci on three linkage groups and of type VI trichome density with one of these marker loci. Lemke & Mutschler (1984) suggested that the presence of type IV trichomes in *L. pennellii* is controlled by two dominant unlinked genes whereas type IV trichome density is conditioned by more than two genes.

Despite these studies, the mechanism and inheritance of whitefly resistance still remains obscure. Nowadays, inheritance of quantitative traits is successfully investigated by using molecular markers such as restriction fragment length polymorphisms (RFLPs). Quantitative trait loci (QTLs) for insect-resistance-related characters have been identified in several crops like tomato (Nienhuis *et al.*, 1987), potato (Bonierbale *et al.*, 1992, 1994) and maize (Schön *et al.*, 1993).

To map QTLs involved in whitefly resistance and trichome densities, we have used a set of RFLP markers with known map positions (Tanksley *et al.*, 1992). Here we present the identification of two major QTLs affecting whitefly oviposition rate and three genomic regions associated with trichome densities. Marker-aided selection of F_3 lines confirmed the presence of the QTLs for whitefly oviposition rate.

Materials and methods

Plant material

A cross was made between *L. esculentum* cv. Moneymaker and *L. hirsutum* f. *glabratum* using one plant of Moneymaker as the pistil parent and one plant of a resistant accession of *L. hirsutum* f. *glabratum* (CGN1.1561) as the pollen parent. This accession is maintained by selfing at the Centre of Genetic Resources (part of CPRO-DLO). The F_1 was selfed and an F_2 population of 288 plants was obtained from two F_1 plants. All F_2 plants were selfed by hand-pollination to generate F_3 seeds.

Greenhouse whitefly

A stock culture of whiteflies was reared on susceptible 'Moneymaker' plants. Tomato plants were inoculated as described by Bas *et al.* (1992) and life history components of the greenhouse whitefly were calculated per cage according to Van Giessen *et al.* (1995):

oviposition rate $(\mathrm{OR}) = 2e/\{d(m+n)\}\ \mathrm{day}^{-1}$,
adult survival $(\mathrm{AS}) = (m/n)^{1/d}\ \mathrm{day}^{-1}$,
preadult survival $(\mathrm{PS}) = p/e$,

where e = number of eggs, d = number of days (= 3) between redistribution and removal of the adult whiteflies, m = number of surviving adults after 3 days, n = number of adults used for inoculation (= 5) and p = number of pupae after 5 weeks.

If there were fewer than five eggs or if the leaves deteriorated before the flies emerged, the estimate for PS was excluded from the analysis. If more than half of the inoculated whiteflies died, OR could not be determined accurately and, as a consequence, they were excluded. To produce a normally distributed error term, an arcsin transformation was used for AS and PS.

Clip-on cage tests

In two neighbouring greenhouse compartments at 20/15°C, 12 *L. esculentum* cv. Moneymaker, 12 *L. hirsutum* f. *glabratum*, 12 F_1 and 288 F_2 plants were grown. Plants were pruned regularly and were lowered so as to keep the height at 2 m above the soil.

Each compartment contained six rows of 27 plants each: one plant of each of the parents, one F_1 and 24 F_2 plants. One block consisted of two rows. In the analysis individual plant observations for OR, AS and PS were corrected for block effects. The block effect was calculated as the difference between the block mean of the 48 F_2 plants in a block and the overall mean of the F_2. Considering the large number of F_2 plants in each block (48) we assumed that differences between blocks resulted from environmental rather than genetic factors.

Fourteen weeks after sowing, whiteflies were collected at random from a stock culture. For adaptation, whitefly females were released in clip-on cages attached to each plant. After 4 days, flies were collected from each plant and 20 survivals were evenly redistributed in four clip-on cages attached to the abaxial side of two young leaves of the same plant. Three days later the surviving whiteflies were counted and all flies removed. Four to 5 weeks later, after the next generation of whiteflies had emerged, the numbers of eggs and empty pupae were counted.

The densities of Type IV and type VI trichomes were evaluated by taking three leaf disks from one leaflet of each plant and counting the trichomes on an area of 2.5 (for type IV) and 4.9 mm^2 (for type VI) on the abaxial side of the leaf. The leaves were the same age and from the same plants as were used in the resistance test. The densities were calculated per plant as the average number of trichomes per mm^2.

Heritabilities for the life history components and trichome densities were estimated on a single plant basis using the values of the parents and the F_1 to estimate environmental variance:

$$\hat{h}^2 = \frac{\hat{\sigma}^2_g}{\hat{\sigma}^2_g + \hat{\sigma}^2_{eb} + \frac{1}{k}\hat{\sigma}^2_{ew}}$$

where $\hat{h}^2$ is the broad sense heritability, $\hat{\sigma}^2_g$ is the estimated genetic variance, $\hat{\sigma}^2_{eb}$ is the estimated environmental variance between plants, $\hat{\sigma}^2_{ew}$ is the estimated environmental variance within plants and k is the number of observations per plant ($k = 4$ for whitefly life history components; $k = 3$ for trichome densities).

To verify the presence of QTLs detected in the F_2, a clip-on cage test was used to determine whitefly resistance in a marker-based selection of F_3 lines. Twelve or 24 plants of 14 F_3 lines were grown under similar conditions as in the F_2 trial. The two compartments each consisted of six rows. One plant of each parent and the F_1 were grown together with one or two plants of each of the F_3 lines in every row. The plants were randomized within a row. One block consisted of two rows. Plant cultivation and the determination of the whitefly life history components were the same as in the F_2 clip-on cage test.

F_3 compartment test

Ten F_3 lines and controls (parents and F_1) were grown separately in isolated greenhouse compartments under commercial tomato production conditions at 20/15°C. Each compartment consisted of 20 plants. Fifteen female whiteflies were released near the bases of every plant. After 94 days, empty pupae present on the three top leaflets of each leaf were counted.

To adjust for differences in leaf architecture, the relative leaf area of these leaflets was estimated by dividing the area of three top leaflets of one leaf by the total area of this leaf. For the F_3 genotypes this was carried out for all plants separately, for the parents and the F_1 for one plant only. On the basis of these relative leaf areas, total numbers of empty pupae per plant were estimated.

RFLP analysis

A set of tomato genomic DNA clones was obtained from Dr S.D. Tanksley (Cornell University, New York, USA). Ninety-four clones were screened for polymorphisms between the parents, using the restriction enzymes *Eco*RI, *Taq*I and *Xba*I (essentially as described by Van der Beek *et al.*, 1992). Sixty-four of the clones were used as RFLP markers for the evaluation of 278 of the 288 F_2 plants. No DNA could be isolated from the remaining 10 F_2 plants.

QTL mapping

Marker genotypes were determined for 278 F_2 plants and a linkage map was constructed using JoinMap with Kosambi mapping function (Stam, 1993; version 1.1). For mapping QTLs involved in whitefly resistance, the interval mapping method of Lander & Botstein (1989) was used, which implies normality for the quantitative trait. For trichome densities, where normality could not be assumed, the nonparametric rank sum test of Kruskal–Wallis (Lehmann, 1975) was performed for each marker individually (Van Ooijen *et al.*, 1993). In this test the plants are ranked according to their trait value and the mean ranks of the marker genotype classes are compared. Large differences between the mean

ranks of marker genotype classes indicate an association between the marker and the trait. Both QTL mapping methods were performed with the computer program MAPQTL (developed by J. W. van Ooijen at CPRO-DLO).

Results

Segregation of the marker loci

From the 94 clones screened, 78 (83 per cent) generated an RFLP between the parents with at least one of the three restriction enzymes used. Sixty-four markers were used for evaluating the 278 F_2 plants. The segregation of 16 markers (25 per cent) deviated significantly ($P<0.001$) from the expected 1:2:1 ratio, with the *L. hirsutum* f. *glabratum* allele always more abundant than the *L. esculentum* allele, as was also observed for most of the remaining markers. Distortion was most extreme on chromosome 7, where for some markers the frequency of *L. hirsutum* f. *glabratum* homozygotes exceeded the frequency of heterozygotes while hardly any *L. esculentum* homozygotes occurred.

Linkage map of L. esculentum × L. hirsutum *f.* glabratum

Using a LOD threshold of 3 all markers were assigned to 10 linkage groups, nine of which corresponded to nine known tomato chromosomes. Because of the strong unidirectional skewed segregation of the markers of chromosomes 7, 9 and 10, these markers were grouped in one linkage group and could only be assigned to their respective chromosomes on applying a LOD score threshold of 7. Thus, a linkage map could be constructed that corresponded to the 12 chromosomes of tomato.

From the 64 markers used in the present study, 62 had previously been mapped in an *L. esculentum* × *L. pennellii*(*esc* × *pen*) F_2 population (Tanksley *et al.*, 1992). Two markers absent in the *esc* × *pen* map, TG190 and TG138, mapped to chromosome 7 and chromosome 10, respectively. Three markers (TG145, TG151 from chromosome 2 and TG264 from chromosome 4) were unambiguously mapped to different chromosomes: 4, 6 and 3, respectively. The order of the remaining markers was identical to the order of the markers on the *esc* × *pen* map, thus corroborating the high congruency of genetic maps within the *Lycopersicon* genus (Tanksley *et al.*, 1992; Van Ooijen *et al.*, 1994).

QTL mapping

Means, standard errors of the means and heritability estimates were determined for whitefly life history components and trichome densities in the F_2 of *L. esculentum* × *L. hirsutum* f. *glabratum* (Table 1). The whitefly life history components PS, AS and OR were considered as measures of plant resistance. The variance of AS within the F_2 was smaller than within the parents and the F_1. As a consequence, the heritability estimate was negative. The heritability estimates for PS and OR were 0.85 and 0.65, respectively. Correlations between life history components and trichome densities were small.

Significant LOD scores were found for OR on chromosomes 1 (TG142) and 12 (TG296; Fig. 1). Nonsignificant high LOD scores were found on chromosomes 3 (TG264), 8 (TG346) and 10 (TG241) but these putative QTLs need further investigation.

Of the total variance for OR in the F_2, 6.4 per cent was explained by the QTL on chromosome 1 and 8.0 per cent by the QTL on chromosome 12. We propose to name these QTLs *Tv-1* (chromosome 1) and *Tv-2* (chromosome 12). For both loci the *L. hirsutum* f. *glabratum* allele increased resistance and was partially dominant over the *L. esculentum* allele.

Table 1 Means and standard errors of means of life history components of the greenhouse whitefly and type IV and type VI trichome densities

Gen.	OR	AS	PS	Density IV	Density VI
P_1	4.2±0.2 (12)	0.96±0.01 (12)	0.87±0.03 (9)	0 (10)	1.1±0.2 (10)
P_2	1.7±0.2 (11)	0.91±0.01 (11)	0.05±0.04 (4)	7.6±1.1 (12)	3.2±0.3 (12)
F_1	2.5±0.1 (12)	0.96±0.01 (12)	0.90±0.01 (12)	0.4±0.2 (12)	3.5±0.4 (12)
F_2	3.1±0.1 (274)	0.95±0.00 (276)	0.76±0.01 (254)	2.5±0.3 (283)	3.6±0.1 (283)
	$\hat{h}^2 = 0.65$	$\hat{h}^2 = 0$	$\hat{h}^2 = 0.85$	$\hat{h}^2 = 0.87$	$\hat{h}^2 = 0.64$

Plant numbers are indicated in parentheses. Gen.: generation; P_1: *Lycopersicon esculentum* cv. Moneymaker; P_2: *Lycopersicon hirsutum* f. *glabratum*; OR: oviposition rate; AS: adult survival; PS: preadult survival; Density IV: density of type IV trichomes; Density VI: density of type VI trichomes; $\hat{h}^2$: broad sense heritability.

The estimated single locus additive and dominance effects were similar (0.35 and −0.20 for *Tv-1* and 0.37 and −0.20 for *Tv-2*, respectively). The combined effects of the two loci were additive although epistatic effects were also observed (Table 2). However, the small plant numbers for some of the genotype classes did not allow reliable conclusions to be drawn about epistatic effects. Using linear regression of OR with both markers as explanatory variables, the additive and dominance effects of the two loci were estimated to account for 13 per cent of the total phenotypic variation in the F_2. Given the heritability estimate of 0.65, this corresponds to an estimated 20 per cent of the genetic variance. The difference in OR between the means of the individuals homozygous for the *L. esculentum* allele and those homozygous for the *L. hirsutum* f. *glabratum* allele at both marker loci was 4.1 − 2.7 = 1.4 (Table 2), which is 56 per cent of the difference between the parental means (4.2 and 1.7 for *L. esculentum* and *L. hirsutum* f. *glabratum*, respectively).

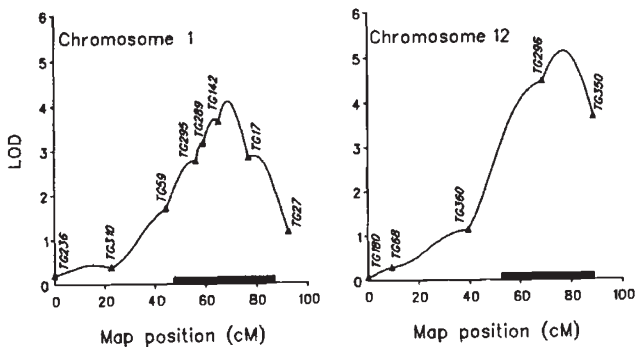

Fig. 1 LOD-score plots of chromosome 1 and chromosome 12 for putative quantitative trait loci affecting oviposition rate of the greenhouse whitefly (*Trialeurodes vaporariorum*). The bar at the bottom of each plot indicates the 2-LOD support interval for each of the two loci. The markers (▲) indicate RFLP marker positions.

No QTLs were detected for the whitefly life history components AS and PS. As for type IV trichome density, the Kruskal–Wallis test statistic (K^*) was significant ($P<0.001$) for three markers on each of the chromosomes 5 (TG441, TG379 and TG32) and 9 (TG254, TG223 and TG186), indicating QTLs on these chromosomes. For chromosome 5, the highest value for the test statistic was found at the TG379 marker locus ($K^* = 42.1$; $P<0.0001$) and for chromosome 9 at TG223 ($K^* = 24.9$; $P<0.0001$). The mean type IV trichome density of F_2 plants homozygous for the *L. hirsutum* f. *glabratum* allele at both loci was similar to the wild parent value (8.0 and 7.6, respectively) and almost twice as high as the overall mean of the F_2 plants which were homozygous for the *L. hirsutum* f. *glabratum* allele at only one of the two marker loci (4.3 and 4.8, respectively; Table 3). For both loci, dominance was in the direction of the *L. esculentum* allele. We propose to designate the QTLs affecting type IV trichome density on chromosome 5 and chromosome 9 as *TriIV-1* and *TriIV-2*, respectively.

For type VI trichome density, a significant test statistic ($K^* = 18.6$; $P<0.0001$) was found only for TG27 on chromosome 1. Mean type VI trichome densities were 2.8, 3.8 and 4.1 for plants with the *e/e*, (homozygous for the *L. esculentum* allele), *e/h* (heterozygous) and *h/h* (homozygous for the *L. hirsutum* f. *glabratum* allele) genotypes at TG27, respectively. We propose to designate the QTL for type VI trichome density as *TriVI-1*.

Table 2 Means of oviposition rate (OR) of the greenhouse whitefly on F_2 plants classified according to their genotype for TG142 and TG296

	TG296			
	e/e	*e/h*	*h/h*	Overall
TG142				
e/e	4.1 (8)	3.5 (29)	3.5 (11)	3.6 (51)
e/h	3.9 (27)	2.9 (65)	2.7 (34)	3.1 (131)
h/h	2.9 (22)	2.9 (30)	2.7 (12)	2.9 (67)
Overall	3.6 (58)	3.0 (128)	2.9 (61)	3.1 (273)

Plant numbers are indicated in parentheses.
Differences between the overall numbers and the sum of the genotype class numbers are caused by dominant (*e/?* or *h/?*) and/or missing scores for one of both markers.
e/e: homozygous for the *Lycopersicon esculentum* allele; *e/h*: heterozygous; *h/h*: homozygous for the *L. hirsutum* f. *glabratum* allele.

Table 3 Means of type IV trichome densities (number of trichomes/mm^2) of F_2 plants classified according to their genotype for TG379 and TG223

	TG223			
	e/e	*e/h*	*h/h*	Overall
TG379				
e/e	0.1 (5)	1.2 (19)	0.8 (9)	0.8 (37)
e/h	0.5 (28)	1.0 (56)	2.4 (40)	1.3 (133)
h/h	1.0 (11)	3.7 (33)	8.0 (28)	4.8 (81)
Overall	0.6 (47)	1.8 (117)	4.3 (82)	2.5 (278)

Plant numbers are indicated in parentheses.
Differences between the overall numbers and the sum of the genotype class numbers are caused by dominant (*e/?* or *h/?*) and/or missing scores for one of both markers.
e/e: homozygous for the *Lycopersicon esculentum* allele; *e/h*: heterozygous; *h/h*: homozygous for the *L. hirsutum* f. *glabratum* allele.

Selection of F_2 plants for progeny testing

To confirm the presence of the QTLs for whitefly resistance, F_2 plants were selected which represented, on the basis of the RFLP analysis, the four possible homozygous QTL genotype classes: homozygous for the *L. hirsutum* f. *glabratum* allele at both resistance loci (*Tv-1 Tv-1/Tv-2 Tv-2*), homozygous for the *L. hirsutum* f. *glabratum* allele at one locus and for the *L. esculentum* allele at the other (*Tv-1 Tv-1/tv-2 tv-2* and *tv-1 tv-1/Tv-2 Tv-2*) and homozygous for the *L. esculentum* allele at both loci (*tv-1 tv-1/tv-2 tv-2*).

An important aspect in marker-aided selection for a particular QTL genotype is the length of the map segment to be considered. In a simulation study, a QTL explaining 5 per cent of the total variance in an F_2 of 200 individuals was enclosed in 92 per cent of 2-LOD support intervals (Van Ooijen, 1992). Ideally, only plants would be selected with the desired genotype for the markers flanking the 2-LOD support interval and all markers within the interval. However, the 2-LOD support intervals for *Tv-1* and *Tv-2* corresponded to regions of 40 cM and at least 38 cM, respectively (Fig. 1). Thus, only one F_2 plant was found to be homozygous for the whole length of the 2-LOD support interval and the markers flanking the support interval for both QTL regions, so less stringent selection criteria (and, as a consequence, a higher risk of including other QTL genotypes) had to be applied. Additional F_2 plants were selected for homozygosity in the interval between TG295 and TG17 of chromosome 1 and for the interval between TG360 and TG350 of chromosome 12. For the *Tv-1 Tv-1/tv-2 tv-2* group two plants were selected which were homozygous at the marker loci TG350 and TG296 and heterozygous at TG360.

F_3 clip-on cage test

The OR values of plants from the *Tv-1 Tv-1/Tv-2 Tv-2* F_3 lines were smaller than those of plants from the *tv-1 tv-1/tv-2 tv-2* F_3 lines but higher than the value of the resistant parent. Plants from *tv-1 tv-1/tv-2 tv-2* F_3 lines had a higher OR than the susceptible parent and the other QTL genotypes. *Tv-1 Tv-1/tv-2 tv-2* and *tv-1 tv-1/Tv-2 Tv-2* genotypes were intermediate between *Tv-1 Tv-1/Tv-2 Tv-2* and *tv-1 tv-1/tv-2 tv-2* genotypes (Table 4). Linear regression of OR on QTL genotype showed that QTL genotype accounted for 24.8 per cent of the total variance in the F_3. The additive effects of the QTLs on chromosomes 1 and 12 were estimated as 0.54 and 0.61, respectively.

F_3 compartment test

The sizes of the whitefly populations on F_3 lines and controls grown under commercial cultivation conditions were evaluated in isolated compartments. Large differences were found between the population sizes of the whiteflies on the different F_3 lines. The numbers of whiteflies observed on *Tv-1 Tv-1/Tv-2 Tv-2*, *Tv-1 Tv-1/tv-2 tv-2* and *tv-1 tv-1/Tv-2 Tv-2* genotypes were much smaller than on *L. esculentum* but higher than on the resistant parent (Table 5). One of the *tv-1 tv-1/tv-2 tv-2* F_3 lines showed the smallest whitefly population size of all F_3 lines tes-

Table 4 Means and standard errors of the means for oviposition rate (OR) of the greenhouse whitefly on F_3 lines of *Lycopersicon esculentum* cv. Moneymaker (P_1) × *L. hirsutum* f. *glabratum* (P_2) and controls

		OR	Significance
Controls			
P_1	(12)	4.3 ± 0.2	cd
P_2	(12)	2.3 ± 0.2	a
F_1: $P_1 \times P_2$	(12)	3.4 ± 0.1	bc
QTL gentotype classes F_3 lines			
Tv-1 Tv-1/Tv-2 Tv-2	(66)	3.3 ± 0.2	b
tv-1 tv-1/Tv-2 Tv-2	(12)	4.3 ± 0.4	d
Tv-1 Tv-1/tv-2 tv-2	(31)	4.5 ± 0.2	d
tv-1 tv-1/tv-2 tv-2	(23)	5.6 ± 0.2	e

Plant numbers are indicated in parentheses.
Tv-1: QTL for whitefly resistance on chromosome 1. *Tv-2*: QTL for whitefly resistance on chromosome 12.
Significance: genotypes which have no letter in common are significantly different with respect to OR based on least significant differences ($P < 0.05$).

Table 5 Sizes of whitefly populations propagated on F_3 lines of *Lycopersicon esculentum* cv. Moneymaker (P_1) × *L. hirsutum* f. *glabratum* (P_2) and controls in the F_3 compartment test

		EP/plant ($\times 10^3$)
Controls		
P_1	(60)	7.1 ± 0.6
P_2	(39)	0.5 ± 0.2
F_1: $P_1 \times P_2$	(20)	3.7
QTL gentotype classes F_3 lines		
Tv-1 Tv-1/Tv-2 Tv-2	(50)	2.1
tv-1 tv-1/Tv-2 Tv-2	(19)	3.5
Tv-1 Tv-1/tv-2 tv-2	(58)	2.3
tv-1 tv-1/tv-2 tv-2	(58)	5.9

Plant numbers are indicated in parentheses.
EP/plant: estimated number of empty pupae per plant, 94 days after inoculation.

ted. This was not expected from the QTL genotype and does not agree with the results of the F_3 clip-on cage test. The whitefly populations on both of the other *tv-1 tv-1/tv-2 tv-2* genotypes were much larger and in agreement with their assumed QTL genotype.

Discussion

Segregation of the marker loci

Segregation distortion occurred for 16 of the 64 marker loci. In all cases the *L. hirsutum* f. *glabratum* allele was favoured over the *L. esculentum* allele. For chromosome 7 the distortion was so extreme that it could not be explained by pollen selection alone and it must be assumed that selection occurs for both the male and female gametes or that selection against the *L. esculentum* allele occurs at the diploid level. In a similar F_2 population, Helentjaris *et al.* (1986) also demonstrated a prevalence of the *L. hirsutum* f. *glabratum* allele for 16 of 50 RFLP loci. However, Zamir *et al.* (1984) reported an excess of *L. esculentum* homozygotes for five of seven skewed isozyme and morphological markers, including one on chromosome 7. In an F_2 of a cross between *L. esculentum* and *L. pennellii* deVicente & Tanksley (1993) found distorted segregation in favour of the wild parent allele for most markers.

QTL mapping

Two QTLs (on chromosomes 1 and 12) affecting whitefly OR were found in the F_2. The effects of these QTLs explain only part of the variation between the parents, even though there were large differences between the means of the QTL genotypes, also in comparison with the parental means. Probably, other genes with smaller effects contribute to the resistance. The detection of such genes would require a larger population or a reduction of the environmental error in the resistance test. This could be achieved by replication of genotypes or by improving the conditions in the test. For instance, synchronization of the whitefly population could reduce differences in fitness between individual insects and, hence, improve the detection of genetic differences between the plants.

Although the difference between *L. esculentum* and *L. hirsutum* f. *glabratum* in PS of the whiteflies was large and the heritability estimate high, no QTLs were detected in the F_2. Possibly many genes contribute small effects to the resistance component. The variation for AS in the F_2 was very small and no genetic differences with respect to AS could be detected.

Insect resistance has been found to be associated with the density of type IV trichomes (Carter & Snyder, 1985). In our study two QTLs for type IV trichome density, *TriIV-1* and *TriIV-2*, mapped to chromosomes 5 and 9, respectively, whereas the two QTLs affecting whitefly oviposition rate mapped to chromosomes 1 and 12. So the whitefly resistance mapped in this experiment was clearly independent of the presence of type IV trichomes. This opens the possibility of incorporating whitefly resistance from *L. hirsutum* f. *glabratum* into cultivated tomato with-

out the undesirable type IV trichomes, which is of major importance for the growers.

It is noteworthy that in a backcross population of potato (dihaploid *Solanum tuberosum* × *Solanum berthaultii*) × *Solanum berthaultii* (Bonierbale *et al.*, 1994) the region around TG379 on chromosome 5 had a very large effect on type B trichome density and sucrose ester level and was associated with oviposition rate and leaf consumption by the colorado potato beetle. The QTL with the largest effect on oviposition rate of the colorado potato beetle was detected on chromosome 1 in the same region as *Tv-1*. However, the other QTLs detected for trichome densities and insect resistance did not correspond. Comparisons of the two insect–plant systems should be considered with caution as the resistance mechanisms are still obscure.

The type VI trichome density QTL, *TriVI-1*, mapped to chromosome 1 close to TG27 at a map distance of 28 cM from TG142, where *Tv-1* was mapped. Linkage between these QTLs was not obvious from the phenotypic data as the correlation between OR and type VI density in the F_2 was small ($r = -0.11$). However, it cannot be excluded that the detected QTL for type VI trichome density is associated with a QTL for whitefly resistance. The overall correlation may be insignificant because of the possible presence of unlinked QTLs, differences in environmental error for both characters and recombination events between the QTLs.

The F_3 results confirmed the existence of QTLs for OR at the loci on chromosomes 1 and 12 in that F_3 lines with a *Tv-1 Tv-1/Tv-2 Tv-2* genotype had a smaller OR than F_3 lines with a *tv-1 tv-1/tv-2 tv-2* genotype. However, only one of the *Tv-1 Tv-1/Tv-2 Tv-2* lines showed a level of resistance comparable to that of the resistant parent.

The approach to QTL mapping applied in this study provides a tool for unravelling the genetics of complex traits such as insect resistance. Until now, plant breeders were reluctant to incorporate such traits into their breeding programmes because of the risks, the amount of work involved in insect resistance tests and the poor prospects of maintaining the resistance throughout successive generations (Bas *et al.*, 1992). When markers tightly linked to the resistance genes become available, marker-based selection offers several advantages in circumventing large insect trials and minimizing environmental variation. Moreover, selection of plants homozygous for the desired alleles can be controlled and linkage drag can be minimized. Finally, assumptions with regard to pleiotropic effects are amenable to a more precise investigation. In this study, for example, we have been able to demonstrate that QTLs for whitefly resistance and type IV trichome density from *L. hirsutum* f. *glabratum* are unlinked.

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