Fine Mapping of Quantitative Trait Loci Using Selected Overlapping Recombinant Chromosomes, in an Interspecies Cross of Tomato

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ABSTRACT

Quantitative trait loci (QTLs) have been mapped to small intervals along the chromosomes of tomato (*Lycopersicon esculentum*), by a method we call substitution mapping. The size of the interval to which a QTL can be mapped is determined primarily by the number and spacing of previously mapped genetic markers in the region surrounding the QTL. We demonstrate the method using tomato genotypes carrying chromosomal segments from *Lycopersicon chmielewskii*, a wild relative of tomato with high soluble solids concentration but small fruit and low yield. Different *L. chmielewskii* chromosomal segments carrying a common restriction fragment length polymorphism were identified, and their regions of overlap determined using all available genetic markers. The effect of these chromosomal segments on soluble solids concentration, fruit mass, yield, and pH, was determined in the field. Many overlapping chromosomal segments had very different phenotypic effects, indicating QTLs affecting the phenotype(s) to lie in intervals of as little as 3 cM by which the segments differed. Some associations between different traits were attributed to close linkage between two or more QTLs, rather than pleiotropic effects of a single QTL: in such cases, recombination should separate desirable QTLs from genes with undesirable effects. The prominence of such trait associations in wide crosses appears partly due to infrequent reciprocal recombination between heterozygous chromosomal segments flanked by homozygous regions. Substitution mapping is particularly applicable to gene introgression from wild to domestic species, and generally useful in narrowing the gap between linkage mapping and physical mapping of QTLs.

CONTINUOUS variation in phenotype, observed for most traits in nature and agriculture, is due to independent segregation of many different "polygenes" which each have a small effect (JOHANSSEN 1909; NILSSON-EHLE 1909; EAST 1915), together with environmental variation. In the past half-century, numerous investigators have inferred particular polygenes to occur at discrete "quantitative trait loci" (QTLs), by fortuitous genetic linkage to easily detected markers. Densely populated genetic linkage maps, based on putatively selection-neutral DNA markers (BOTSTEIN *et al.* 1980), have recently made possible the systematic mapping of QTLs to ~20 centiMorgan (cM) regions throughout the genomes of sexually-propagated higher organisms (PATERSON *et al.* 1988, LANDER and BOTSTEIN 1989).

Study and manipulation of quantitative traits are greatly facilitated by mapping QTLs to particular genomic regions, but resolution of 20 cM is insufficient for many purposes. In plant breeding, for example, a 20 cM region of an interspecies cross often includes genes with undesirable effects, which supercede agricultural utility of resident desirable genes (TANKSLEY and HEWITT 1988). In physical terms, 20 cM of the tomato genome represents an average of 12 million base pairs of DNA (GANAL, YOUNG and TANKSLEY 1989), a quantity beyond the capability of current systems for manipulating intact DNA sequences. The resolution of QTL mapping might be improved by using larger populations (LANDER and BOTSTEIN 1989), with more recombinant chromosomes contributing to more accurate placement of QTLs. However, incremental increases in investment yield diminishing returns of new information.

Herein, we demonstrate substitution mapping, a method for fine mapping of QTLs previously localized to chromosomal regions, using tomato as a model system. Substitution mapping is analogous to deletion mapping of genetic loci in human (KUNKEL *et al.* 1986; PAGE 1986), in which altered phenotype is associated with loss of a specific chromosomal region. However, substitution mapping utilizes meiotic recombinants, rather than deletions. By prior QTL analysis (such as that employed in PATERSON *et al.* 1988), a restriction fragment length polymorphism (RFLP) is identified which lies near a QTL of interest. Different chromo-

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somal segments carrying the RFLP are identified, and their regions of overlap determined using all available genetic markers. Phenotypic effects of each chromosomal segment are determined by QTL analysis of segregating progeny populations. Effects shared by different segments are attributed to QTLs in regions shared by those segments, while effects unique to a segment are attributed to QTLs in a region unique to that segment. Consequently, the resolution of substitution mapping is determined by the number and spacing of genetic markers available to distinguish overlapping recombinant segments.

For the current study, six genetic stocks were developed. These stocks were heterozygous for 2 to 4 chromosome segments hypothesized to carry QTLs increasing fruit soluble solids concentration. Effects of each recombinant chromosome on soluble solids and other fruit traits were determined, in segregating populations of about 150 self-pollinated progeny from each genetic stock. Many overlapping chromosomal segments had very different phenotypic effects, indicating QTLs affecting the phenotype(s) to lie in intervals of as little as 3 cM by which the segments differed. Some associations between different traits were attributed to close linkage between two or more QTLs, rather than pleiotropic effects of a single QTL: in such cases, recombination should separate desirable QTLs from genes with undesirable effects. The prominence of such trait associations in wide crosses appears to result from diminished reciprocal exchange, between heterozygous chromosomal segments flanked by homozygous regions. Substitution mapping is particularly applicable to gene introgression from wild to domestic species, and generally useful in narrowing the gap between linkage mapping and physical mapping of QTLs.

MATERIALS AND METHODS

Construction of genetic stocks: The genetic composition of 237 backcross progeny of *L. esculentum* cv. UC82B (E; recurrent) × *L. chmielewskii* accession LA1063 (CL), was determined previously using 70 DNA markers spanning 852 cM at intervals averaging 14.3 cM. Together with phenotypic data, this was used to construct a first-generation map of genomic regions affecting soluble solids concentration, mass per fruit and fruit pH (PATERSON *et al.* 1988).

About 20 BC1 individuals, each carrying different CL segments associated with elevated soluble solids concentration but not with reduced mass per fruit, were selected. [For example, plant #121 (Figure 1a) carried CL segments on chrs. 1, 2, 5, and 6 which were associated with increased soluble solids, but was free of CL segments on chromosomes 4, 6, 7, 9, and 11 which were associated with reduced mass per fruit.] From each of these BC1 plants, 10 self-pollinated (BC1F2) progeny were grown in the field. Several of the BC1F2 families showed partial or complete sterility, and were discarded. From the remaining families, each plant was genotyped. Individuals retaining the target CL segments, but carrying a minimal number of nontarget segments, were selected. [For example, plant #121.08 carried each of the four target segments from plant #121, and was heterozygous at each of three nontarget segments (Figure 1b), making it feasible to eliminate all of the latter by another backcross.] A total of six BC1F2 plants, from five different BC1 families, were selected and backcrossed (as males) a second time to E. Each plant in the six BC2F1 populations (12–64 plants each; total ~200) was genotyped. From each population, one plant was selected which retained the target CL segments but was free of the nontarget segments. [For example, one of 64 individuals grown from the backcross of 121.08 to E carried each of the four target segments, and none of the remaining nontarget segments known (Figure 1c).]

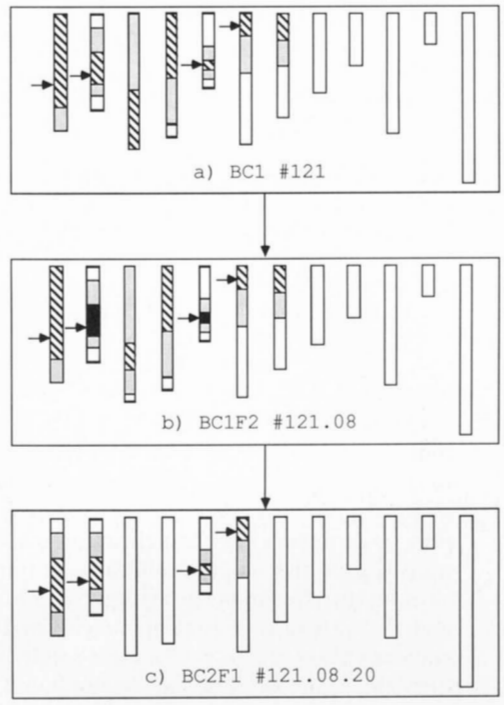

FIGURE 1.—Pedigree of a single BC2F1 plant, based on genotype at DNA markers. Genotype of an interval is inferred from genotype at markers flanking the interval. Open bars represent homozygosity for the recurrent parent (E) genome, hatched bars indicate heterozygosity, and solid bars are homozygosity for the donor parent (CL) genome. Dotted bars indicate intervals in which a recombination has occurred, as evidenced by different genotype at adjacent markers. Arrows indicate target regions for substitution mapping.

Six BC2 individuals with different genotypes, each from a different BC2 population, and derived from five different BC1 plants, were used in substitution mapping. These individuals were known to retain two to four unlinked segments from CL. These segments were characterized in detail, using all available DNA markers polymorphic in this cross. Further, in gaps between markers of ≥10 cM (based on the BC1 map; PATERSON *et al.* 1988), additional markers were scored (where available) to verify the genotype inferred for the interval.

Phenotyping: Self-pollinated progeny (125–151 individuals) from six BC2F1 plants (above) were transplanted to the field at Davis, California, on 18 April 1988, in a completely randomized design with 35 cm between plants and 175 cm between rows. Average mass per fruit, soluble solids concentration (°Brix), and fruit pH were determined as described in TANKSLEY and HEWITT (1988). Plant yield was measured as total weight of fruit on each plant, when most fruit were mature.

Genotyping and linkage analysis: RFLP genotypes were determined as described in TANKSLEY and HEWITT (1988), except that probes were labeled by random primer extension (FEINBURG and VOGELSTEIN 1983). Linkage analysis was performed on a VAX 8530, using MapMaker (LANDER *et al.* 1987).

Quantitative trait analysis: Effects of each CL segment on quantitative traits were determined using SAS (SAS Institute 1988), by analysis of variance. The two degrees of freedom for genotype at each locus were partitioned into the additive effect {(CC-EE)/2}, and the dominance deviation {EC-[(CC-EE)/2]}, where EE and CC represent the E and CL homozygotes, and EC the heterozygote. The 4 d.f. for two-way interactions between unlinked fragments were partitioned into additive × additive, additive × dominant, dominant × dominant, and dominant × additive effects. Log transformation of fruit size was used to improve normality.

To compare recombinant segments which overlap only in a small region, we determined effects at a single marker in the overlapping region, rather than using interval analysis at flanking markers (LANDER and BOTSTEIN 1989). Most segments showed little recombination, hence underestimation of allele effects due to recombination between marker and QTL (LANDER and BOTSTEIN 1989), is small. For an experimentwise confidence level of 95% (*e.g.*, ≤5% chance of ≥1 false positive in n independent trials), the single-comparison confidence level x must be such that $1 - (x)^n \geq 0.05$. In substitution mapping, n is the number of independent segments assayed in a population, since inference is based on genotype at a predetermined point on each segment. For $n = 2$, x is ~97.5%, and for $n = 4$, x is ~99%, for 95% confidence experimentwise.

RESULTS

Segregation: Although segregation of some CL segments followed the Mendelian expectation, regions of chromosomes 2, 5, and 7 showed significant deviations (Figure 2). Such segregational distortion is common in matings across species boundaries (TANKSLEY 1983; ZAMIR and TADMOR 1986; PATERSON *et al.* 1988), and suggests reduced fitness of particular combinations of factors from the distantly-related parents. By substitution mapping, such factors can be localized to small chromosomal regions. For example, one segment of chromosome *2* shows an excess of heterozygotes (Figure 2b–far right column of data). The segment segregating abnormally (F) includes the chromosomal region marked by *Cab-1*, while an overlapping segment (G) is free of this region and segregates normally. Hence, we infer a factor distorting segregation to lie near *Cab-1*, in the region unique to F.

Recombination: Recombination between pairs of markers was generally less in the BC2F2 (herein) than in the BC1 generation (PATERSON *et al.* 1988). The intervals measured in both studies totaled 134 (±10) cM in BC2F2, *vs.* 209 (±10) cM in BC1, a highly significant reduction. The two studies differ, in that biparental transmission of recombinant gametes could be measured in BC2F2, while only paternal transmission could be measured in BC1. This difference is unlikely to explain the observed reduction, as we have previously found biparental (F_2) transmission to yield a *larger* recombination fraction than paternal (BC) transmission (PATERSON *et al.* 1988). However, reduced recombination in regions heterozygous for donor (*e.g.*, CL) chromosomal segments has been previously associated with elimination of much of the donor genome by backcrossing (RICK 1969), such as was done to develop the stocks used herein.

It may be noteworthy that the only putatively intact chromosome studied, chromosome *5*, showed similar recombination in both generations (44 cM in BC2F2 *vs.* 52 cM in BC1, a nonsignificant difference). In contrast, heterozygous chromosome segments flanked by homozygous regions showed reductions in recombination of fourfold or more in BC2F2 (Figure 2b, 6 cM *vs.* 23 cM for *CAB1-CD35*; Figure 2d, 2 cM *vs.* ~15 cM for *CD67-TG118*). We postulate that chiasmata formation may be more common in homozygous regions, at the expense of recombination in heterozygous segments (see DISCUSSION).

QTL allele effects: Small QTL allele effects reached statistical significance in this study, due to fewer QTL segregating and a smaller penalty for experimentwise error. Effects of as little as 0.38°Brix and 0.07 pH units reached significance in BC2F2 (Figure 2), *vs.* 0.83°Brix and 0.096 pH in the BC1 (PATERSON *et al.* 1988).

Allele effects on mass per fruit are more difficult to compare across generations, because these effects are proportional to absolute fruit size (WRIGHT 1968). In absolute units, the smallest effect on mass per fruit to reach significance here was −3.7 g, *vs.* −3.5 g in BC1. In the BC1, with average mass per fruit of 17.5 g, the reduction of 3.5 g represented −20%. However, because average mass per fruit in BC2F2 was 39.5 g, a reduction of 3.7 g represented only −9%, a smaller relative effect. Similarly, a QTL might have different absolute effects, but similar relative effects, in populations with different average mass per fruit. For example, chromosome 2 factors inferred to be common to segments F and G (Figure 2b–also see below), showed additive effects of −8.1 and −4.4 g, respectively. Average mass per fruit of the corresponding populations was 44.6 and 25.7 g, respectively, hence this region reduced fruit mass by ~20% in each case.

QTL substitution maps: By observing the shared versus unique properties of overlapping recombinant chromosomes, several QTLs could be assigned to small CL intervals by which the chromosomes differed. Because each of the five chromosomal regions studied showed effects on more than one trait (Figure 2), substitution mapping also provided evidence useful in evaluating whether close linkage or pleiotropy caused these associations.

The *TG19-TG27* region of chromosome 1 was previously associated with a nonsignificant increase in

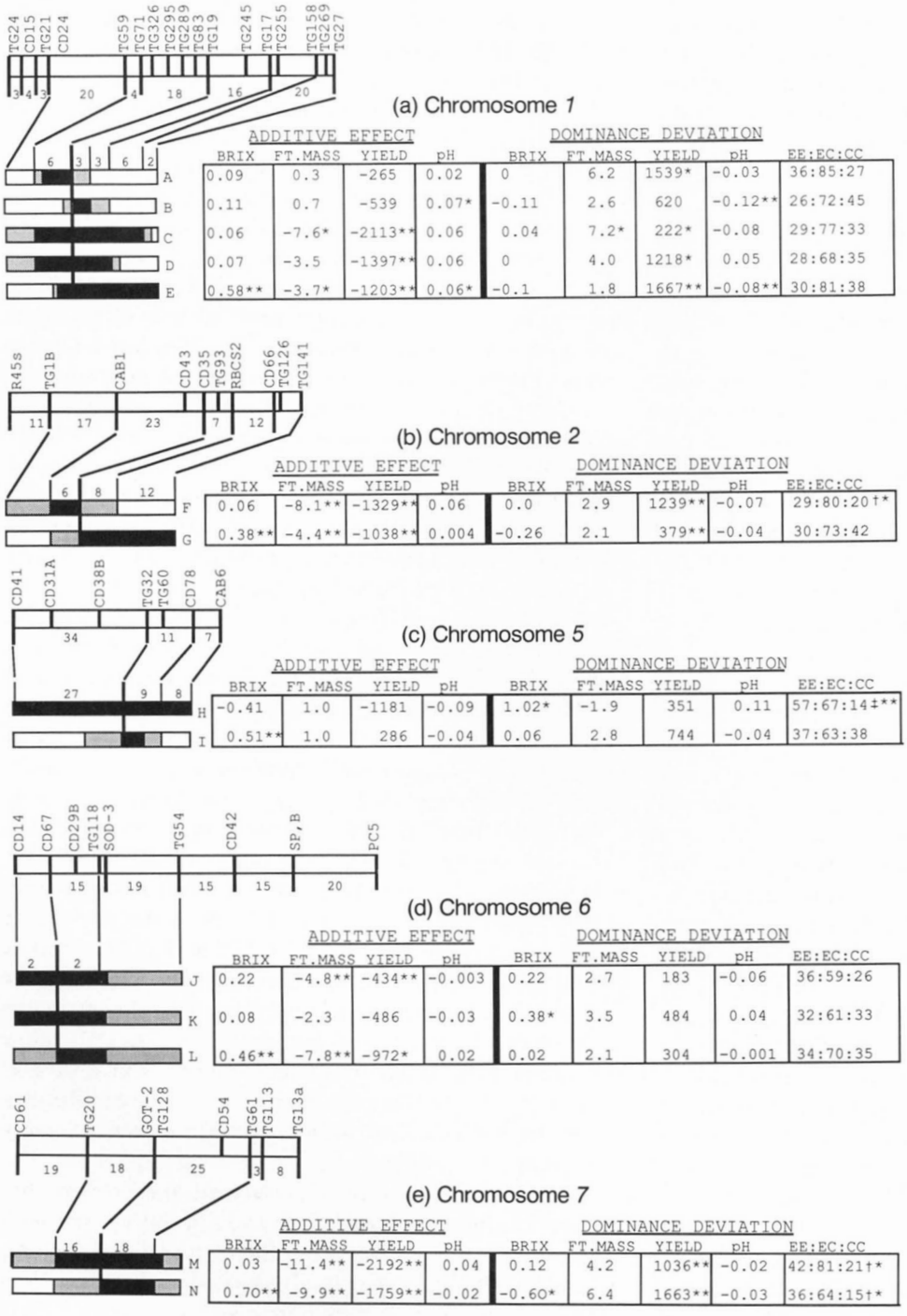

FIGURE 2.—Substitution mapping of QTLs, in 5 genomic regions of *L. esculentum* × *L. chmielewskii* BC2F2. (a) Effects at *TG19* linked to different chromosome *1* recombinants. (b) Effects at *CD35* linked to different chromosome *2* recombinants. (c) Effects at *TG32* linked to different chromosome *5* recombinants. (d) Effects at *CD67* linked to different chromosome *6* recombinants. (e) Effects at *TG128* linked to different chromosome *7* recombinants. Recombinant chromosome segments are designated A–N, immediately to the right of each segment. Genetic maps of each intact chromosome are presented in upper left of each subfigure (a–e), with BC1 map distances in KOSAMBI (1944) cM (26). Map distances in BC2, averaged over all informative populations, are presented along the uppermost recombinant chromosome of each set (A, F, H, J, M). Some markers were not mapped in BC1, but were used in the present study for detailed characterization of CL recombinant segments: these are presented along the chromosome maps, in the linear order determined from prior mapping in *L. esculentum* × *L. pennellii* F_2 (our unpublished data). Based on genotype at the flanking markers, open intervals are inferred to be homozygous for *L. esculentum*, solid intervals are segregating for *L. chmielewskii* in the BC2F2, and dotted intervals contain a recombination site. For each set of recombinant segments, the marker used to determine segment effects is indicated by a vertical line through the corresponding segments. Additive effects and dominance deviations for soluble solids concentration are expressed in °Brix, mass per fruit and plant yield in grams (g), and pH in original units. * and ** indicate significance at experimentwise error rates of ≥5% and ≥1%, respectively. † and ‡ indicate significant deviation from expected allele (1:1) and genotype (1:2:1) frequencies, respectively.

solids of about 0.6°Brix (PATERSON *et al.* 1988). In the present study, phenotypic effects were determined for five CL segments which overlapped at *TG19* (Figure 2a). A significant additive effect on solids of +0.58°Brix was unique to the only segment (E) carrying the CL region from *TG158* to beyond *TG27*, a distance of at least 2 cM. Reductions of fruit mass and yield were found for three segments (C, D, E), which differ from the two segments (A, B) not showing these effects, in that they carry a CL interval of about 3 cM, from *TG245-TG255*. Significant dominance deviation (but nonsignificant additive effect) for increased yield is found for segments A, C, D, and E, overlapping in the *TG295-TG19* region. The effect of segment B is large but not significant; if it also carries the factor, the overlapping region would exclude *TG295*. Elevated pH is significant for two segments (B, E) and just shy of significance for two more (C, D). These differ from the only segment not affecting pH (A), in that they carry the CL interval from *TG19-TG17*, about 6 cM. Thus, soluble solids is elevated by one or more CL factor(s) between *TG158* and the *TG27* end of chromosome *1*, mass per fruit and/or yield are reduced by factor(s) between *TG245* and *TG255*, yield

of heterozygotes might be increased by factor(s) near *TG19*, and pH might be increased by factor(s) between *TG19* and *TG17*. Effects of this chromosome *1* region on these four traits are attributed to at least four QTLs, separable by recombination.

Chromosome *2* showed no effect on solids in BC1 (PATERSON *et al.* 1988), but the region near *CD35* was associated with elevated solids in selfed progeny of the BC1 (our unpublished data). This suggested a recessive factor from CL, the donor parent. Each of two CL chromosome *2* segments, (Figure 2b–F, G) which overlap near *CD35*, reduce fruit mass and yield. Only G significantly affects soluble solids, with an additive effect of +0.38°Brix per CL allele: the dominance deviation of −0.26°Brix is just shy of the significance threshold, but again suggests recessiveness of the CL factor. A factor affecting solids is inferred to lie distal to *CD35*, in a CL region unique to G. Factors affecting fruit mass and yield are inferred to lie near *CD35*, where F and G overlap. Effects of this chromosome 2 region on these three traits are attributed to at least two QTLs, separable by recombination.

In the BC1 study, the *TG32* region of chromosome 5 showed a normal (1:1) ratio of EE homozygotes to heterozygotes, and a nonsignificant increase in solids of 0.52°Brix (PATERSON *et al.* 1988). In the present study, an intact CL chromosome 5 (Fig. 2c; H) showed significant segregation distortion near *TG32*, with only 14 of 138 progeny being CL homozygotes. The same CL chromosome exhibited a dominance deviation of +1.02°Brix near *TG32*, detected with marginal confidence ($P = 0.05$) due to the paucity of CL homozygotes. The heterozygotes averaged 0.61°Brix higher than the E homozygotes ($P < 0.001$), giving a more reliable approximation of the effect of this region. Elimination of CL genome at each end of chromosome *5* generated a small segment (I) marked by *TG32*, which segregated normally, and showed a highly significant additive effect on solids of +0.51°Brix. Segment H is sufficiently long that effects detected at *TG32* could be due to distant QTLs. However, the short segment (I) has a similar effect on solids, suggesting that QTLs accounting for much of the effect lie on segment I. Further, the factor(s) causing segregational distortion appear distinct from factor(s) affecting solids, as the traits have been separated by recombination.

Chromosome *6*, previously dominated by the massive effects of *sp* on all traits (PATERSON *et al.* 1988), carries additional QTLs revealed after eliminating CL segments which carry *sp*. Two CL chromosome *6* segments (Figure 2d–J, L), sharing the *CD67-TG118* region, share significant reductions in fruit size and yield. A third segment (K) sharing the *CD67-TG118* region, also shows a marginally nonsignificant reduction in yield. Of these segments, only L shows a significant additive effect on soluble solids (+0.46°Brix). Segment K shows a significant dominance deviation (+0.38°Brix) but no additive effect. The apparently different mode of inheritance could indicate different QTLs, perhaps beyond *TG118* on L and outside *CD14* on K: at present, the segments cannot be uniquely characterized in these regions, due to lack of RFLPs. Alternatively, mode of inheritance may be modified by differences in the segments or epistatic relationships beyond the resolution of our data (see below), making it unreliable evidence that the segments carry different QTLs. New markers and more studies of this genomic region are needed.

The *TG61-TG128* region of chromosome 7, previously showing single-allele effects of +0.83°Brix, +0.096 pH units, and −3.7 g (20%) mass per fruit (PATERSON *et al.* 1988), again affected several traits. In the present study, we found additive effects of about −10 g (20%) in mass per fruit, greatly reduced yield, and aberrant segregation at *TG128* for both segments studied (Figure 2e; M, N). Segment N, shorter than M at the *TG128* end, shows a significant elevation of solids in the CL homozygote: no such effect is exhibited by segment M. N could carry unique CL QTL at the *TG61* end, but the solids effect at *TG61* in the current study (not shown) was not significant, suggesting that QTLs by which the segments differ lie near *TG128*. Alternatively, a double crossover in the 18 cM interval between *TG128* and *TG61* could have eliminated part of M: this cannot presently be verified, due to a lack of RFLPs in the region. In a previous study, both the *TG128* and *TG61* regions of chromosome *7* persisted through several generations of backcrossing CL to E, with selection for high solids (RICK 1974). However, only the *TG128* region was shown to actually increase solids (TANKSLEY and HEWITT 1988). New markers and additional studies are needed to better understand the effects of chromosome *7* on these traits.

Epistasis: Only infrequently were interactions between unlinked genomic regions significant, suggesting that epistasis had little effect on the traits which were studied. Since each population carried from two to four segments, it was possible to test from 1 to 6 two-way interactions between segments in each population. Each of these interactions was partitioned into four contrasts, corresponding to additive × additive, additive × dominant, dominant × additive, and dominant × dominant epistasis. From the total of 25 two-way interactions, one (4%) was significant for solids, five (20%) for fruit mass, and three (12.5%) for pH, at the nominal 0.05 significance level (*e.g.*, not adjusted for experimentwise error). Further, of the 100 possible single d.f. contrasts, four (4%) were significant for solids, ten (10%) for mass per fruit, and four (4%) for pH, at the nominal 0.05 level. Only for mass

per fruit were significant interactions consistently more frequent than the random expectation of 5%. Single-locus additivity and dominance appear to explain much of the genetic variation in our populations. Use of more than 150 individuals would provide a more powerful test of epistasis, however previous marker-based studies using larger populations have also detected minimal epistasis (EDWARDS, STUBER, and WENDEL 1987; PATERSON *et al.* 1988).

DISCUSSION

Substitution mapping reduces the size of the interval to which a QTL can be assigned, by determining the phenotypic effects of chromosomal segments with small differences. Prior mapping of QTLs to ~20 cM intervals (PATERSON *et al.* 1988) serves as a starting point, from which QTLs can be mapped with much finer resolution. In this study, we were able to assign QTLs to intervals of as little as 3 cM. Resolution of QTLs may be improved even further, by subsequent study of recombinants in these small intervals. After several iterations, QTLs might be assigned to intervals sufficiently small for physical mapping and map-based cloning, especially in a relatively simple (for higher organisms; 555 Mbp, and diploid) genome such as tomato.

In some cases, genes with desirable effects (increased solids) mapped to different intervals than genes with undesirable effects (reduced mass per fruit). Close linkage, rather than pleiotropy, appears responsible for trait associations in such cases. Experiments in progress are studying recombinants in such regions, to attempt to identify progeny carrying only the desirable trait (or, with somewhat less enthusiasm, the undesirable trait), which would verify that the association had indeed been due to linkage.

Because genetic variation in the BC2F2 populations had been reduced by eliminating much of the donor genome, small effects previously not significant were inferred with confidence to indicate QTLs. This supports a model for polygenic trait inheritance wherein the magnitude of effect attributable to different genomic regions follows a continuum (SHRIMPTON and ROBERTSON 1988). Consistent with this model, we found reductions in mass per fruit not previously detected, which had likely been obscured by QTLs of larger effect. As a corollary, small effects deemed nonsignificant in the present experiment may prove to represent additional QTLs of still smaller effect.

The QTLs studied herein continue to show phenotypic effects in isolation from much of the donor genome, and show little evidence of epistasis with the remaining donor genome. Use of larger populations, and testing of QTLs in multiple backgrounds (TANKSLEY and HEWITT 1988), will be important in assessing the role of epistasis in quantitative inheritance. However, both the current results and previous evidence (EDWARDS, STUBER, and WENDEL 1987; PATERSON *et al.* 1988) suggest that some QTLs can function independently of others. This is of major importance in QTL manipulation, and extraction of valuable traits from wild germplasm, as it is far easier to identify and transfer single QTLs than pairs, trios, *et cetera.*

While the present results largely corroborate results from a prior generation in a different environment (PATERSON *et al.* 1988), it is important to note that the present results are based on a single-environment test. In a different environment, a particular chromosomal segment might have different phenotypic effects, due to different expression of the same genetic factors, or to expression of different factors. While this might alter our description of particular chromosomal segments, the general approach to mapping QTLs would remain valid. Experiments in progress are investigating environmental sensitivity of QTLs, by conducting trials in locally different environments, and on different continents.

Mechanics of substitution mapping: The resolution of substitution mapping is determined primarily by ability to distinguish overlapping chromosomal segments. These segments can be obtained relatively simply. Genotyping about 150 F2 or 300 BC progeny, enough to establish ~20 cM QTL likelihood intervals (PATERSON *et al.* 1988, LANDER and BOTSTEIN 1989) as targets for substitution mapping, also gives 95% confidence of obtaining a recombinant within any 1 cM interval. For practical reasons, a single population was employed both for determining likelihood intervals, and as a source of chromosomal segments for substitution mapping in later generations. It could be argued that this constitutes a bias, since the gametic array of subsequent generations is influenced by that of the first generation. It is unclear whether this bias would have much effect on phenotype: plant breeders almost invariably study different generations descended from a particular population, with reasonable success.

To take full advantage of substitution mapping, the overlapping regions of different chromosomal segments must be determined as precisely as possible, using genetic markers whose relative order is known. Thus, the resolution at which segments can be distinguished is determined by the resolution at which markers have been mapped. The ability to order closely spaced markers by linkage analysis depends on the size of the largest informative mapping population available (for a homosequential genome). Many current linkage maps, based on less than 100 individuals, can only accurately order markers which are several cM apart. Use of larger populations, or recombinant

inbred strains (HALDANE and WADDINGTON, 1931; BURR *et al.* 1988), might permit one to determine orientation of markers as little as 1 cM apart. Physical mapping of genetic markers (COULSON *et al.* 1988; GANAL, YOUNG and TANKSLEY 1989), should improve resolution of both genetic maps and substitution mapping, to less than 1 cM.

Recombinant chromosomes may be assayed either in segregating populations as we did, or in true-breeding near-isogenic lines which differ only by overlapping recombinant segments. Making isogenic lines requires several generations of backcrossing and genotyping small populations, but eliminates the need for subsequent genotyping. Such lines can be replicated extensively to detect small effects, and can be tested as heterozygotes by mating to the recurrent parent. Using near-isogenic lines incurs a risk that small donor chromosome segments (independent of the target segment) have persisted by chance; such "relics" are randomized in segregating populations, but are confounded with treatment effects in otherwise near-isogenic lines. Several backcrosses, or study of several different lines which are putatively near-isogenic for a segment, should factor out effects of any relics.

Recombination shrinkage: Most CL segments showed reduced recombination in the BC2F2, however the only putatively intact CL chromosome (chr. 5) largely retained its BC1 recombinational length. Each of the most prominent models for meiotic recombination (MESELSON and RADDING 1975; SZOSTAK *et al.* 1983) require single-stranded exchanges between homologous chromosomes. Such exchanges between the sequence-identical homologs in homozygous regions may more frequently yield reciprocal exchange than those between the sequence-divergent strands in heterozygous regions. Increased chiasmata formation in homozygotes has been observed in a number of plant species (reviewed in GRANT, 1958). In yeast, introduction of 9 restriction site polymorphisms into a 9-kb interval reduced reciprocal exchange by about 50%, and increased the frequency of nonreciprocal repair of DNA mismatches (BORTS and HABER, 1987). Mismatch repair might also partly explain the rather high frequency of variants in fruit color which we have observed in these populations.

Reduced reciprocal exchange, together with positive interference, might explain both the pronounced recombination shrinkage we found in heterozygous segments, and the minimal shrinkage of a heterozygous intact chromosome. The lower likelihood of a reciprocal exchange in a heterozygous chromosomal segment may be accentuated by positive interference, from more frequent reciprocal exchanges in flanking homozygous regions. When the heterozygous segment represents a largely intact chromosome, such as our CL chromosome *5*, fewer flanking "preferred sites" would be present, and less shrinkage would be observed.

Recombination shrinkage may be particularly pronounced in wide crosses such as we have studied here, where greater sequence-divergence would result in stronger preference for homozygous regions. Such restrictions on genetic exchange might be less pronounced in crosses between less diverse parents, such as elite crop varieties.

Recombination shrinkage increases the quantity of DNA and number of genes transmitted as a unit, reducing the precision at which individual genes (or traits) can be selected for. Even without recombination shrinkage, theoretical models suggest that large chromosomal regions may remain intact through numerous meioses (HANSON 1959). Reduced recombination in such regions may contribute greatly to maintaining trait associations by "linkage drag" (ZEVEN, KNOTT and JOHNSON 1983) or "genetic hitchhiking" (BIRKY and WALSH 1988). This may partly explain why previous efforts to transfer solids from CL to E, with selection against undesirable wild traits (RICK 1974), lost many CL regions we found to increase solids (TANKSLEY and HEWITT 1988). For example, the small region of chromosome *1* which we found to carry closely linked QTLs for increased solids and reduced fruit mass, would likely have been selected against. Recombination shrinkage may account for many such losses, in attempts to transfer specific attributes from wild to domestic species. Substitution mapping defines regions in which recombination can prevent such losses, and DNA markers identify the rare desirable recombinants.

Fine mapping of quantitative trait loci amplifies the utility of DNA markers in study and manipulation of quantitative traits. Distinction of close linkage from pleiotropy, in causing associations of valuable traits with undesirable properties, should invigorate efforts to transfer an abundance of attributes from wild species to domestic strains. Assigning a QTL to a small interval, such as an interval between two RFLP markers which are on a single yeast artificial chromosome (COULSON *et al.* 1988), would be a major step toward cloning the genetic factor(s) lying at the QTL. Consequently, identification of small target regions for physical manipulation of QTLs may extend the reach of molecular biology into this class of genes fundamental to evolution, agriculture, and medicine.

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