An Interspecific Backcross of *Lycopersicon esculentum* × *L. hirsutum*: Linkage Analysis and a QTL Study of Sexual Compatibility Factors and Floral Traits

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Manuscript received February 7, 1997

Accepted for publication July 10, 1997

ABSTRACT

A BC₁ population of the self-compatible tomato *Lycopersicon esculentum* and its wild self-incompatible relative *L. hirsutum* f. *typicum* was used for restriction fragment length polymorphism linkage analysis and quantitative trait loci (QTL) mapping of reproductive behavior and floral traits. The self-incompatibility locus, *S*, on chromosome 1 harbored the only QTL for self-incompatibility indicating that the transition to self-compatibility in the lineage leading to the cultivated tomato was primarily the result of mutations at the *S* locus. Moreover, the major QTL controlling unilateral incongruity also mapped to the *S* locus, supporting the hypothesis that self-incompatibility and unilateral incongruity are not independent mechanisms. The mating behavior of near-isogenic lines carrying the *L. hirsutum* allele for the *S* locus on chromosome 1 in an otherwise *L. esculentum* background support these conclusions. The *S* locus region of chromosome 1 also harbors most major QTL for several floral traits important to pollination biology (e.g., number and size of flowers), suggesting a gene complex controlling both genetic and morphological mechanisms of reproduction control. Similar associations in other flowering plants suggest that such complex may have been conserved since early periods of plant evolution or else reflect a convergent evolutionary process.

IN angiosperms numerous mechanisms are known to determine whether a population reproduces by cross-pollination (SI), self-pollination (SC) or a combination of the two. Genetic self-incompatibility encompasses a group of mechanisms that prevent self-pollination in plants via pollen-pistil interactions (DE NETTANCOURT 1977; THOMPSON and KIRCH 1992; SIMS 1993). Genetic self-incompatibility can be controlled by a single multiallelic locus (*S* locus) or by several loci and may be determined sporophytically (SSI) or gametophytically (GSI) (CORRENS 1913; PRELL 1921; DE NETTANCOURT 1977).

Genetic self-incompatibility in plants, in particular that controlled by a single locus, is well characterized and displays an extraordinary degree of polymorphism comparable to the major histocompatibility complex (MHC) in mammals (RIVERS *et al.* 1993). For example, in *Trifolium repens* over 200 alleles have been identified (DE NETTANCOURT 1977). Genes from the *S* locus have been cloned and characterized in several species. In most families with GSI, an *S* locus gene product is a glycoprotein with ribonuclease activity (*S*-RNase) expressed in the stigma and style and involved in the determination of pollen rejection (ANDERSON *et al.* 1989; MCCLURE 1989, 1990). Ribonuclease activity has not been detected in Papaveraceae (FRANKLIN-TONG

and FRANKLIN 1992). For SSI, as in Brassica, the *S* locus contains genes coding for a secreted glycoprotein as well as a transmembrane protein kinase (TANTIKANJANA *et al.* 1993), but it is still unclear how these two components interact to mediate the *S* locus response. Less known are the pollen-specific components of either GSI or SSI systems. It is proposed that selectivity in GSI may be based on the expression of pollen-specific kinases that would interact with the style-specific proteins (KUNZ *et al.* 1996).

Plants not only display intraspecific self-incompatibility, but also interspecific incompatibility. Unlike the situation with self-incompatibility, the genetic basis of incompatibility between plant species is not well understood. A common phenomenon observed in crosses between related species is unidirectional cross-compatibility, also known as unilateral incongruity (UI), in which only one species can serve as the female parent (LEWIS and CROWE 1958). A series of models, often contradictory, have been proposed for the control of UI. LEWIS and CROWE (1958) observed that UI is most commonly manifested when pollen from a SC species is rejected by the style of a SI species. Most exceptions to this rule would involve SC species that only recently diverged from SI progenitors and thus have imperfect compatibility systems (LEWIS and CROWE 1958). This is an important point since it implies a common genetic basis for SI and UI (LEWIS and CROWE 1958). Working with *Nicotiana*, PANDEY (1968, 1969, 1970) extended this model and provided genetical evidence that

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pointed to the *S* locus in the control of UI. He also suggested that different genes within the *S* locus complex were controlling interspecific compatibility and intraspecific compatibility. A similar model connecting SI and UI has recently been proposed in Brassicaceae (HISCOCK and DICKSON 1993) and Solanaceae (TROGNITZ and SCHMIEDICHE 1993). Others have either rejected the involvement of the *S* locus in UI (ABDALLA 1974; HOGENBOOM 1975) or proposed a multigenic model for the control of UI (MARTIN 1963, 1964, 1967; HARDON 1967).

In addition to genetic barriers, there are physiological, morphological and ecological factors that influence the balance between self-pollination and cross-pollination in plant populations (GRANT 1971). Cross-pollination can be promoted by asynchrony of pollen shedding and stigma receptivity (protogyny and protandry), or by spatial separation as in flower heteromorphy, dioecy or stigma exertion. In turn, selfing may be promoted by cleistogamy, non-shedding pollen and proximity of female and male parts. Reproduction may also be affected by factors affecting plant-pollinator interaction, such as flower display, color cues, chemical attractants and flower shape and size (GRANT 1971).

Several studies have investigated the relationship between genetic incompatibility and morphological features affecting reproductive behavior. A classic example comes from some families with SSI (Primulaceae, Oxalidaceae, Linaceae, Rubiaceae, Apocynaceae) that also show flower heteromorphism (distyly and tristly). Genetic studies have shown that floral heteromorphy control is closely linked to genetic self-incompatibility in many instances (for a review see DE NETTANCOURT 1977). In contrast, studies in the insect-pollinated genus *Layia* and *Potentilla* failed to show any genetic linkage between genetic self-incompatibility and floral traits.

The tomato genus, *Lycopersicon*, is ideal for genetic studies of self-incompatibility, unilateral incongruity and floral variation associated with pollination behavior. All species in this genus are interfertile and encompass the full range of mating behaviors from small-flowered self-pollinators (e.g., *L. parviflorum*, *L. cheesmanii*) to large-flowered SI obligate outcrossers (e.g., *L. pennellii*, *L. peruvianum*, *L. hirsutum*). Exceptions exist to these pattern of reproductive behavior, with SC accessions of characteristically SI species often being present in the periphery of the distribution ranges. As with all Solanaceae, SI *Lycopersicon* species have monofactorial gametophytic self-incompatibility controlled by the *S* locus on chromosome 1 (LAMM 1950; TANKSLEY and LOAIZA-FIGUEROA 1985). Moreover most SC $\times$ SI crosses between *Lycopersicon* species are unilaterally incongruous succeeding only if the self-compatible parent acts as female (MACARTHUR and CHIASSON 1947).

In this article we report results from a genetic mapping study of SI, UI and several floral traits believed to

be related to mating behavior. The study was performed on a BC₁ population produced from a cross between the self-compatible tomato inbred *L. esculentum* cv. E6203 (recurrent parent) and the self-incompatible wild tomato *L. hirsutum* f. *typicum* accession LA1777. *L. hirsutum* and *L. esculentum* are highly differentiated with respect to flower morphology. *L. hirsutum* flowers are characteristic of an insect-pollinated obligate outcrossing species, producing many large showy flowers, with broad petals forming a minimally indented corolla often folding over backward. Its sepals reach midway up the anther cone and also fold over backward at the tip. Its stigmas are always well exerted beyond the anther cone and provide easy access to insect pollination. In contrast, the cultivated tomato has fewer, less conspicuous, flowers with smaller corollas and well indented petals. Its sepals fully embrace the flower bud and its stigmas are typically flush or recessed with respect to the anther cone insuring self-pollination. Several of these traits are believed to be associated with the frequency of insect visitation: corolla diameter, total number of flowers, length of the inflorescence and bud type (RICK 1988). Crosses between these two species are possible only if *L. hirsutum* acts as the staminate parent, thus falling under the (SI $\times$ SC) categorization of UI (LEWIS and CROWE 1958). The goals of this study were to determine the genetic basis of the following: (1) SC in *L. esculentum* vs. SI in *L. hirsutum*, (2) the UI response observed in crosses between the two species, (3) floral traits that differentiate the species and that are likely to be involved in pollination. To conduct this experiment we constructed a molecular linkage map, the first reported from a cross between these two species.

MATERIALS AND METHODS

Plant material and population structure: Half-sib seed of *L. hirsutum* f. *typicum* LA1777, hereafter referred to as H, was provided by C. M. RICK, University of California, Davis. H seed was grown at 10°, 8 hr photoperiod, in a growth chamber. The seven most vigorous seedlings were saved and a single individual was selected to serve as the staminate parent in a cross to *L. esculentum* cv. E6203, hereafter referred to as E. Approximately 70% of the F₁ seeds germinated and were confirmed to be hybrids based on intermediate leaf morphology as well as vegetative vigor. A single F₁ plant was used as the staminate parent in backcrosses to E. BC₁ seed germination averaged 90%. Three hundred ninety-five seedlings were produced from randomly selected seed to generate a BC₁ population. These BC₁ seedlings were then screened with the restriction fragment length polymorphism (RFLP) marker *CT109* to select individuals homozygous for E alleles at the *sp* locus on chromosome 6 (PATERSON *et al.* 1988; GRANDILLO and TANKSLEY 1996). *sp/sp* individuals possess a determinate growth habit that facilitates management in the greenhouse and field. A total of 149 individuals were determined to be homozygous *E/E* at *CT109* locus and formed the population that is the subject of this report. We hereafter refer to this population as the BC₁.

RFLP characterization and linkage analysis: DNA from the

H and E parents was screened with >400 probes from the tomato high density molecular map (PILLEN *et al.* 1996). Two restriction enzymes, *Eco*RI and *Hind*III, were employed for this survey. One hundred thirty-five informative clones, covering the entire tomato genome, were selected for analysis on the BC₁ population. RFLP procedures follow those described in GRANDILLO and TANKSLEY (1996). After completion of the RFLP linkage map, three *S*-protein cDNA clones (*Sm*1, *Sm*2 and *Sc*) were kindly provided by R. BERNATZKY, University of Massachusetts, for incorporation into this study. *Sm*1 and *Sm*2 are different functional *S*-alleles from the SI *L. peruvianum* accession LA2163, and *Sc* is a nonfunctional *S*-allele from the SC accession of the same species, LA2157. These clones were mapped using 40 BC₅ plants containing H introgressions of varying length at the *S* locus. These 40 plants derived from one BC₁ individual *E*/*H* at the *S* locus. Mapping in advanced populations (BC₅) can result in underestimation of recombination frequencies, yet despite this limitation and the fact that only 40 individuals were available, this population was the only material available for mapping the *S*-protein cDNAs at the time they were obtained.

Chi-square goodness of fit tests were used to compare single locus segregation against the expected 1:1 ratio. Linkage relations among markers were derived using MapMaker v.2 running on a Macintosh workstation (LANDER *et al.* 1987). Loci were initially grouped using the "group" command (LOD $\geq$ 4). Marker order within groups was established with multipoint analyses and confirmed using the "ripple" routine (LOD $\geq$ 3). The Kosambi mapping function was used for conversion of recombination frequencies into cM map distances (KOSAMBI 1944).

The proportion of *L. hirsutum* and *L. esculentum* genome present in each BC₁ individual was calculated as a function of marker genotype and map distance using the computer program QGENE (NELSON 1997) and were used to calculate the population average.

Phenotypic evaluations: Two forms of sexual cross-compatibility were evaluated, self-compatibility and unilateral incongruity. For the evaluation of the self-compatibility seven to 19 flowers from each BC₁ plant were manually self-pollinated by means of a hand held vibrator and direct hand-pollination in case the flowers showed exerted stigmas. An index of self-incompatibility, SI, was calculated as the proportion of self-pollinated flowers that produced fruit with seeds.

For the evaluation of unilateral incongruity reaction, E pollen was used to manually pollinate from four to 26 emasculated flowers from each of the 149 BC₁ plants. A unilateral incompatibility index, UI, was calculated as the proportion of pollinated flowers that resulted in fruit with seeds. Thus both self-incompatibility (SI) and unilateral incongruity (UI) were indirectly estimated by the respective rates of pollination success. Pollen viability was not evaluated. Fruits affected by the physiological disorder blossom end rot were excluded from the analysis.

Seven floral traits were evaluated on each BC₁ plant in the greenhouse (Figure 1). Stigma exertion (SE) refers to the position of the stigma relative to the tip of the anther cone. For this trait each plant was given a numerical rating of 1 = stigma recessed; 2 = stigma flush with anther cone tip, or 3–5 = increasing stigma exertion beyond the anther cone tip. Flower size (FLS), as represented by corolla diameter, was rated on a scale of 1–5 (1 = small, 5 = large). Corolla indentation (CI) (depth of the interpetal notches) and number of flowers per plant (NF) were rated on the same scale. The length of the inflorescence raquis (RL) from stem to tip was scored from 1 (long) to 3 (short). Flower bud type (BT) was rated from 1 (*L. hirsutum* type) to 5 (*L. esculentum* type) based

FIGURE 1.—*L. esculentum* (E) and *L. hirsutum* (H) floral buds and opened flowers. (A) Stigma exertion (SE). Petals and sepals (in E only) were removed. (B) BT, CI and FLS.

on length of sepals relative to corolla. *L. hirsutum* flower buds have sepals reaching only midway up the length of the anther cone with their tips curled backward, while in *L. esculentum* the flower buds have sepals considerably longer than the anther cone and they show no curling (Figure 1). Finally, the presence of a vegetative meristem in the inflorescence (IVM) was scored on a scale of 1–3 (1 = without expanded leaf, 2 = without expanded leaf and 3 = with vegetative meristem). Histograms for each trait are presented in Figure 2. Pearson's product coefficients of correlation were determined for all possible trait combinations with the software JMP v. 3.0 (SAS INSTITUTE 1994).

QTL analysis: The degree of association between phenotype and marker genotype was investigated by both interval analysis (LANDER and BOTSTEIN 1989) and single point linear models (TANKSLEY *et al.* 1982) using the application QGENE (NELSON 1997). Results from both methods were in close agreement. Hence only results from linear regression are reported with the exception of chromosome 1 for which both are presented. A $P \leq 0.001$ or its equivalent LOD ≥ 2.4 were used as exclusion thresholds for declaring the presence of a QTL at a marker locus (LANDER and BOTSTEIN 1989). The proportion of observed phenotypic variance attributable to a particular QTL was estimated by the coefficient of determination (R^2) from the corresponding linear model analysis. The *CT109-Sp* locus region of chromosome 6 is excluded from the

FIGURE 2.—Frequency distribution for each trait for 149 BC₁ individuals derived from *L. esculentum* cv. E6203 × *L. hirsutum* LA1777. E and H indicate the phenotypic class corresponding to the *L. esculentum* and *L. hirsutum* parental types, respectively.

QTL analysis due to the fixation of genotype *E/E* at *CT109*. The resolution of QTL mapping in flanking regions of skewed segregation is also likely to be adversely affected.

Epistasis analysis: For the traits SI and UI, two-way interactions were evaluated for each significant QTL locus and all segregating loci via two-way ANOVA using QGENE with a significance threshold of $P \leq 0.005$. Interactions involving the *S* locus region of chromosome 1 were further studied by performing QTL analysis on the subpopulations either homozygous or heterozygous for the *S* locus. All plants showing recombination events within the interval containing the

S locus (*TG301-CT81*) were excluded from the subpopulation analyses.

Production and evaluation of near isogenic lines (NILs): NILs were developed that carry single introgressions of H chromosomes at regions associated with reproductive behavior in an otherwise *E/E* genetic background. A total of eight NILs for the *S* locus region on chromosome 1 were selected from three original BC₁ individuals determined to be heterozygous for marker *CT209*, which is linked to the *S* locus on chromosome 1. Three backcross generations with positive (for *CT209*) and negative (against other unlinked H alleles)

TABLE 1
Skewed marker segregation in *L. esculentum* ×
L. hirsutum BC₁

Locus	Chromosome	Genotype	
		E/E	E/H
CT191A	1	55	87
CT267	1	44	88
TG260	1	47	100
CT255A	2	50	81
TG620B	2	54	88
TG251	3	50	97
CT170	3	51	95
TG417	3	52	95
TG164 ^a	6	89	57
TG356B ^a	6	102	44
CT174 ^a	6	117	30
TG162E ^a	6	128	17
CT109 ^a	6	147	0
TG275B ^a	6	135	11
TG279 ^a	6	136	10
TG477 ^a	6	132	15
TG642 ^a	6	126	21
TG482 ^a	6	122	25
CT20	10	55	88
CT95	10	41	102
TG233	10	49	96
TG557	11	88	57
CT168	11	100	45
TG523	11	106	41
CT182	11	103	43
TG400B	11	91	56
CT99	12	93	53

Loci deviating from expected 1:1 ratio ($P \leq 0.001$) in BC₁ generation. E, *L. esculentum* allele; H, *L. hirsutum* allele.

^a Loci affected by marker assisted selection against *sp*⁺ locus on chromosome 6.

marker-assisted selection (MAS) were needed to identify heterozygous NILs for this segment of chromosome 1. Selected BC₄ plants were further evaluated with additional markers on chromosome 1 to determine the extent of the introgressed segments. Mating behavior was evaluated for each BC₄ NIL line. In addition, 12 homozygous NILs were produced for regions other than chromosome 1 that harbored factors putatively associated with SI or UI. These NILs were derived from segregating BC₃F₂ seedlings in a single step of MAS. Each of these NIL plants was allowed to self-pollinate and also used as staminate and pistillate parents in crosses with *L. esculentum*. The number of selfed fruits per plant (nf), the proportion of crosses resulting in fruit (xp) and average number of seeds per fruit (spf) were recorded for each NIL plant in two consecutive generations (S₁ and S₂). Whenever that total number of crosses made was not available the total number of fruits per plant is reported.

RESULTS

Marker segregation: Nine different chromosomal regions deviated significantly ($P \leq 0.01$) from the expected 1:1 segregation ratio in the interspecific BC₁ population (Table 1, Figure 3). As expected, marker

selection against the *L. hirsutum* *sp* allele on chromosome 6 introduced skewedness in this region of the genome. The distortion extended from the point of selection, CT109, 3 cM from *sp*, to TG164, which is 50.3 cM away toward the centromere, and to TG482, 22.5 cM away toward the telomere of the long arm. Eight other unlinked chromosomal segments, totaling ~18% of the genome, were also significantly skewed. Two segments had an excess of E/E homozygotes: chromosome 11 (TG557 to TG400B) and chromosome 12 (CT99). The other six regions were skewed in favor of the heterozygote E/H class: chromosome 1 (CT191A to TG260), chromosome 2 (CT255A and TG620B), chromosome 3 (TG251 to TG417) and chromosome 10 (CT20 and CT95 to TG233). Despite this skewing, the average proportion of E genome was 0.75 ± 0.05 , which is that expected for a BC₁ generation.

Linkage analysis and map construction: The linkage map constructed from the 135 RFLP markers scored on 149 BC₁ plants is shown in Figure 3 (E × H). Twelve linkage groups were identified covering a total map distance of 1356 cM with an average interval length of 12 cM. Marker order was in agreement with the high density linkage map of tomato based on a *L. esculentum* × *L. pennellii* (E × P) F₂ population that has total map distance of 1287 map units (PILLEN *et al.* 1996).

Forty-eight adjacent marker intervals (42%), representing 22 chromosomal segments, were identified throughout the genome for which recombination frequencies differed between E × H and the E × P map by greater than twofold (Figure 3). Thirty-two of these (68%) showed increased map distance in the E × H map. Five centromeric regions (chromosomes 1, 3, 4, 8 and 12) were among the segments with increased map distance in the E × H map while the centromeric region of chromosome 7 and to a lesser extent chromosome 2 and chromosome 9 showed reduction in recombination. A total of 16 marker intervals (32%) showed reduced recombination in the E × H map. For individual chromosomes the ratio E × H cM/E × P cM varied from 1.47 (chromosome 1) to 0.64 (chromosome 11), with an overall average of 1.1 (Table 2). Additional comparisons between existing tomato linkage maps also show variation in the distribution of recombination across chromosomes. Other linkage maps have been reported for an interspecific cross *L. esculentum* × *L. pimpinellifolium* (PM) (GRANDILLO and TANKSLEY 1996) and for an *L. peruvianum* (PE) interspecific population (VAN OOIJEN *et al.* 1994). The chromosome length ratios for E × PM cM/E × P cM varied from 0.84 for chromosome 2 to 1.24 for chromosomes 10 and 12 (Table 2). The ratios E × PE cM/E × P cM varied from 0.82 for chromosome 2 to 1.81 for chromosome 12 (Table 2).

Correlations among traits: Strong correlations were found among a number of traits (Table 3). The strongest correlation, $r = 0.47$, corresponded to RL and flower

FIGURE 3.—Linkage map derived from *L. esculentum* × *L. hirsutum* BC₁ population (E × H). Comparative linkage groups and cM distances are also depicted for the high density tomato map (PILLEN *et al.* 1996) derived from *L. pennellii* × *L. esculentum* (E × P). Lines connect common loci. Solid lines indicate segments that vary between the two maps by a factor >2×. Shaded segments in the E × H map indicate segregation distortions toward homozygote E/E class; striped segments indicate areas enriched in heterozygote E/H class. Centromeric locations (●) are as described by GRANDILLO and TANKSLEY (1995). Shading (increasing) indicates level of significance for $0.01 \geq P > 0.001$; $0.001 \geq P > 0.0001$; $0.0001 \geq P > 0.00001$ and $P \leq 0.00001$, respectively. Most likely position of QTL is indicated by QTL name (italics) and point to position of greatest significance. A negative sign (–) indicates that the wild allele had an effect opposite of that predicted by the parental phenotypes. SI, self-incompatibility; UI, unilateral incompatibility; SE, stigma exertion; BT, bud type; CI, corolla indentation; RL, inflorescence raquis length; FLS, flower size; NF, number of flowers; IVM, inflorescence vegetative meristem. *Sm1* and *Sc* indicate the approximate mapping position of cDNA clone *Sm1* (functional *S*-allele from SI *L. peruvianum* LA2163) and cDNA clone *Sc* (nonfunctional *S*-allele from SC *L. peruvianum* LA2157) (BERNATZKY *et al.* 1995, 1996).

FIGURE 3.—Continued.

number (NF). SI and UI showed the second strongest association ($r = 0.39$). SI and UI were also strongly correlated with BT, RL, IVM and FN. BT was significantly associated with CI. Stigma exertion did not show a significant correlation with any other character.

QTL analysis: Results for QTL analyses for all traits are summarized in Table 4 and in Figure 3. Significant QTL ($P \leq 0.001$) were detected for all traits. Individual QTL explained 8.2–37% of the corresponding phenotypic variance (Table 3). Results for individual traits are summarized below. Interval mapping results for the *S* locus area are represented in Figure 4.

Self-incompatibility: SI was most strongly associated with CT62 near the centromere of chromosome 1 for which presence of the H allele conferred a strong self-incompatible reaction ($R^2 = 0.3$). This region of chromosome 1 has been shown previously to contain the

self-incompatibility locus, *S* (TANKSLEY and LOAIZA-FIGUEROA 1985; BERNATZKY 1993). The related *S* alleles *Sm1* and *Sc*, as indicated by BERNATZKY (personal communication), detected homologous sequences in the H genome. Genetic mapping revealed that both clones cosegregate with markers CT62, CT98 and CD76, when 40 segregating BC₅ individuals were screened (data not shown). At the high stringency conditions used, *Sm2* showed no homology to the DNA of the BC₅ individuals.

Three additional chromosomal regions were associated with SI but at significance levels below the declared threshold for QTL detection ($0.01 \geq P \geq 0.001$): chromosome 2 (TG308), four different markers on chromosome 5 (CT167, TG503, TG60 and CT138) and chromosome 11 (TG400B). For most of these loci the wild allele increased self-incompatibility. However, for TG308 on chromosome 2 the effect was reversed: heterozygosity

TABLE 2
A comparison of map distances (cM) derived from different interspecific crosses within *Lycopersicon*

Chromosome	E×H map	E×P map ^a	E×H/ E×P ratio	E×PM map	E×P map ^a	E×PM/ E×P ratio	PE×PE map	E×P map ^a	PE×PE/ E×P ratio	E×H/ E×PM ratio ^b
1	179.5	121.6	1.5	149.6	136.4	1.10	128	118	1.08	1.19
2	129.2	124.4	1.0	98.2	128.0	0.84	78	95	0.82	1.26
3	110.2	130.5	0.8	116.6	113.9	1.02	90	71	1.26	0.94
4	103.1	100.9	1.0	97.2	102.8	0.95	55	54	1.00	1.05
5	130.1	96.0	1.3	108.2	96.4	1.12	38	44	0.86	1.20
6	109.9	93.0	1.2	85.2	94.8	0.92	83	93	0.89	1.29
7	102.0	91.2	1.1	116.4	99.8	1.21	91	71	1.28	0.87
8	79.5	92.8	0.8	86.1	87.2	0.99	77	70	1.10	0.92
9	110.1	92.0	1.2	104.2	102.9	1.01	115	89	1.29	1.05
10	101.0	83.3	1.2	101.5	81.9	1.24	109	90	1.21	1.00
11	68.8	105.1	0.6	107.0	100.8	1.06	44	27	1.62	0.64
12	132.9	108.1	1.2	105.2	84.6	1.24	165	91	1.81	1.26
Avg			1.1			1.05			1.18	1.05

Map units per chromosome for E×H; E×PM and E×P tomato linkage maps and total chromosome length ratios. E, *L. esculentum*; H, *L. hirsutum*; PM, *L. pimpinellifolium*; PE, *L. peruvianum*; P, *L. pennellii*. Map units are centiMorgans.

^a Based on orthologous markers only.

^b Based on assayed markers only.

at *TG308* resulted in enhanced self-fertility as compared to the homozygous *E/E* class for the same marker.

Unilateral incongruity: Unilateral incongruity was significantly associated with three chromosomal regions. As with SI, the *S* locus region on chromosome 1, referred to as *ui1.1*, showed by far the strongest association with UI ($R^2 = 0.3$). However two additional QTL were identified for UI: *ui3.1* (*TG417*) on chromosome 3 ($R^2 = 0.1$) and *ui12.1* (*TG380*) on chromosome 12 ($R^2 = 0.1$). In both cases presence of the H allele was associated with reduced fruit set when pollinated with E pollen. A multiple regression model containing all three QTL simultaneously explains 45% of the UI variance. Three additional loci were associated with UI at significance below the declared threshold ($0.01 \geq P \geq 0.001$): chromosome 2 (*TG620B*), chromosome 11 (*CT182*) and chromosome 1 (*CT190*). Those on chromosome 2 and 11 had the predicted effect for which

presence of the H allele reduced compatibility. *CT190* on chromosome 1 had the opposite effect with the H allele being associated with higher compatibility ratings.

Stigma exertion: A single QTL, *se2.1*, centered at *TG169* on chromosome 2 was detected for stigma exertion, accounting for 19.7% of the trait variance.

Bud type: Bud morphology was affected by seven QTL dispersed across four chromosomes. In all cases, presence of the H allele was associated with reduced sepal length relative to the anther cone and H bud type. Three separate QTL, *bt1.1*, *bt1.2* and *bt1.3*, were identified on chromosome 1 each explaining 16% of the phenotypic variance. *bt1.1* mapped to the *S* locus. Two additional QTL were identified on chromosome 7 separated by 40 map units: *bt7.1* and *bt7.2*, each individually controlling 13% of the trait variance. The remaining two QTL were identified on chromosome 12, *bt12.1* and on chromosome 2, *bt2.1* (both $R^2 = 0.1$).

TABLE 3
Trait correlations in *L. esculentum* × *L. hirsutum* BC₁

Trait	BT	CI	RL	FS	IVM	FN	SI	UI
CI	0.35**							
RL	0.38***	0.01						
FS	0.09	0.26*	0.27*					
IVM	-0.01	0.02	-0.19*	-0.18				
FN	-0.39***	-0.10	-0.47***	-0.27*	0.15			
SI	0.22	-0.08	0.36***	0.11	-0.29*	-0.26*		
UI	0.37**	0.12	0.33**	0.19	-0.26*	-0.32**	0.39***	
SE	-0.06	-0.05	-0.06	-0.18	0.13	-0.03	0.08	-0.11

Pearson's correlation coefficient for all trait combinations in E × H BC₁ population. BT, bud type; CI, corolla indentation; RL, inflorescence raquis length; FS, flower size; IVM, inflorescence vegetative meristem; FN, flower number; SI, self incompatibility; UI, unilateral incongruity; SE, stigma exertion. * $P \leq 0.01$; ** $P \leq 0.001$; *** $P \leq 0.0001$.

TABLE 4
QTL analysis for SI, UI and morphological traits

Trait	QTL	Locus	Chromosome	R ²	P value	E/E	E/H
SI	<i>S locus</i>	<i>CT62</i>	1	0.3	6.27E-13	0.22 ± 0.02 (82)	0.00 ± 0.00 (65)
		(<i>TG308</i>)	2	0.045	0.01	0.08 ± 0.02 (75)	0.17 ± 0.03 (72)
		(<i>CT167, TG503, TG60, CT138</i>)	5	0.061	0.00264	0.17 ± 0.03 (76)	0.07 ± 0.02 (71)
		(<i>TG400B</i>)	11	0.062	0.00231	0.16 ± 0.02 (91)	0.06 ± 0.02 (56)
UI	<i>ui1.1</i>	<i>CT62</i>	1	0.343	4.21E-12	0.44 ± 0.04 (77)	0.02 ± 0.01 (60)
		<i>TG417</i>	3	0.099	0.0002	0.40 ± 0.06 (49)	0.17 ± 0.03 (87)
		<i>TG380</i>	12	0.1	0.00017	0.35 ± 0.05 (78)	0.12 ± 0.03 (59)
		(<i>CT190</i>)	1	0.063	0.0045	0.17 ± 0.04 (61)	0.38 ± 0.06 (66)
		(<i>TG620B</i>)	2	0.07	0.00219	0.42 ± 0.07 (51)	0.19 ± 0.04 (81)
		(<i>CT182</i>)	11	0.05	0.00821	0.33 ± 0.05 (97)	0.13 ± 0.04 (39)
SE	<i>se2.1</i>	<i>TG169</i>	2	0.197	4.95E-08	1.30 ± 0.09 (77)	2.38 ± 0.18 (60)
BT	<i>bt1.1</i>	<i>TG58</i>	1	0.157	0.00003	3.39 ± 0.16 (51)	2.42 ± 0.16 (53)
		<i>TG310</i>	1	0.16	0.00003	3.50 ± 0.16 (44)	2.51 ± 0.16 (59)
		<i>TG260</i>	1	0.175	0.00001	3.56 ± 0.15 (39)	2.50 ± 0.15 (68)
		<i>TG620B</i>	2	0.097	0.00130	3.43 ± 0.20 (37)	2.64 ± 0.14 (66)
		<i>TG639</i>	7	0.141	0.00013	3.44 ± 0.18 (39)	2.50 ± 0.15 (60)
		<i>TG331</i>	7	0.12	0.00025	3.33 ± 0.16 (51)	2.48 ± 0.15 (56)
		<i>CT211</i>	12	0.107	0.00059	3.26 ± 0.17 (57)	2.46 ± 0.15 (50)
		<i>CT211</i>	12	0.107	0.00059	3.26 ± 0.17 (57)	2.46 ± 0.15 (50)
CI	<i>ci2.1</i>	<i>TG140</i>	2	0.123	0.00017	3.58 ± 0.14 (60)	2.76 ± 0.16 (50)
		<i>CT88</i>	8	0.109	0.00033	3.57 ± 0.14 (60)	2.80 ± 0.16 (54)
RL	<i>rl1.1</i>	<i>CT62</i>	1	0.37	4.40E-14	2.93 ± 0.03 (69)	2.11 ± 0.10 (57)
		<i>CT167</i>	5	0.145	0.00001	2.82 ± 0.05 (62)	2.33 ± 0.10 (61)
		<i>CT52</i>	7	0.107	0.00018	2.77 ± 0.06 (65)	2.33 ± 0.10 (61)
FLS	<i>fls1.1</i>	<i>CT231</i>	1	0.114	0.00019	2.54 ± 0.09 (52)	2.02 ± 0.10 (66)
		<i>CT62</i>	1	0.095	0.0006	2.48 ± 0.09 (63)	2.00 ± 0.11 (56)
		<i>TG251</i>	3	0.089	0.00097	2.58 ± 0.11 (40)	2.09 ± 0.09 (79)
NF	<i>nf1.1</i>	<i>CT62</i>	1	0.284	7.19E-12	1.98 ± 0.06 (80)	2.95 ± 0.13 (63)
IVM	<i>ivm4.1</i>	<i>TG182</i>	4	0.082	0.00173	2.70 ± 0.07 (61)	2.30 ± 0.11 (56)
		<i>CT79B</i>	12	0.097	0.00091	2.72 ± 0.07 (58)	2.29 ± 0.11 (52)

QTL analysis results ($P \leq 0.001$, based on one-way ANOVA) in $E \times H$ BC₁ population. SI and UI loci associated at $0.001 \geq P \geq 0.01$ are also reported (in brackets). Locus = marker showing strongest association with trait. Model estimates include the following: R^2 and associated P value, mean $\pm$ SE for genotypic classes E/E and E/H ; the number of individuals in each genotypic class is in parentheses. Trait units are described in MATERIALS AND METHODS.

Corolla indentation: Two QTL were detected for corolla indentation. *ci2.1* on chromosome 2 ($R^2 = 0.12$) and *ci8.1* on chromosome 8 ($R^2 = 0.1$). Heterozygotes for these loci displayed the H type corollas.

Rachis length: Three QTL were identified for inflorescence rachis length. The most significant QTL was *rl1.1*, which mapped coincidental with the *S* locus and accounted for 37% of the trait variation. Two other QTL were also identified on chromosome 5, *rl5.1* ($R^2 = 0.14$), and on chromosome 7, *rl7.1* ($R^2 = 0.1$). The corresponding three-factor multiple regression model accounted for 45% of the trait variance.

Flower size: Three QTL were identified for flower size. *fls1.1* ($R^2 = 0.11$) and *fls1.2* ($R^2 = 0.1$) mapped to the *S* locus region of chromosome 1, while *fls3.1* mapped to chromosome 3 ($R^2 = 0.08$). In all cases presence of the H allele was associated with larger corolla diameters. A multiple regression model containing all three loci explained 20% of the phenotypic variance.

Number of flowers: A single QTL, *nf1.1*, coincident

with the *S* locus was identified for this trait that accounted for 28% of the trait variance. Plants containing the H allele at this locus produced on average 50% more flowers than homozygotes E/E .

Inflorescence vegetative meristem: Two QTL were associated with the occurrence of vegetative growth in the inflorescence: *ivm12.1* on chromosome 12 ($R^2 = 0.09$) and *ivm4.1* on chromosome 4 ($R^2 = 0.08$). In both instances presence of the H allele conditioned an increase in the production of vegetative meristems in the inflorescence axis.

Figure 4 depicts LOD scores for traits mapping to chromosome 1 and better illustrates the clustering of floral trait QTL in the *S* locus region of the chromosome. The map position for the major QTL controlling RL, NF, BT and FLS appears to be coincident with the locus controlling SI and UI, all reaching their strongest association at, or close to, marker *CT62*. No other QTL were detected elsewhere in the genome for number of flowers. BT showed the most number of independent

FIGURE 4.—Lod score plots for traits associated with the *S* locus region on chromosome 1. SI, self incompatibility; UI, unilateral incongruity; RL, inflorescence requis length; NF, number of flowers; BT, bud type; FLS, flower size. X axis represents chromosome 1 linkage group. CT62 represents most likely position of *S* locus. Y axis is interval mapping LOD scores. LOD ≥ 2.36 marks threshold for QTL identification. Inserted legend contains trait name, key and LOD score for CT62.

QTL (seven) spread over four chromosomes, while flower size had QTL in chromosomes 1 and 3. Flower size may be controlled by two linked loci (*fls1.1* and *fls1.2*) mapping to the *S* locus region.

Epistatic interactions: *Self-incompatibility:* The *S* locus (marked by CT62) showed no significant interactions with any other loci in the control of self-incompatibility.

When only plants homozygous *E/E* at the *S* locus were included in the QTL analysis three regions showed significant association to SI: chromosome 5 (TG60, $P = 0.02$), chromosome 11 (TG400B, $P = 0.04$) and chromosome 3 (CT234, $P = 0.04$) (Table 5). In all three cases the H allele was associated with reduced self-compatibility. Plants containing the H allele at the *S* locus displayed complete self-incompatibility.

Unilateral incongruity: Five significant interactions between UI QTL and other loci in the genome were identified (Table 5). *ui1.1*, which maps to the *S* locus, interacted with both *ui3.1* (chromosome 3, $P = 0.0001$) and *ui12.1* (chromosome 12, $P = 0.002$). The pattern of association was similar in both cases. The UI response mediated by the H alleles at the *S* locus region QTL (*ui1.1*) is strong and is not affected by *ui3.1* or *ui12.1*. In the absence of H alleles at the *S* locus, these QTL (*ui3.1* or *ui12.1*) individually effect a moderate reduction in cross-compatibility.

ui3.1 showed additional significant interactions with a distal locus on chromosome 3 (CT171, $P = 0.003$),

another on chromosome 9 (CT74, $P = 0.0008$) and one on chromosome 11 (TG523, $P = 0.003$). In the interactions of *ui3.1* with CT171 or with CT74, double homozygous *E/E* showed reduced cross-compatibility (UI = 0.35) while heterozygote plants for either CT171 or CT74 alone had high cross-compatibility (UI = 0.76). Heterozygosity at *ui3.1* alone or combined heterozygosity at *ui3.1* and either CT171 or CT74 caused strong cross-incompatibility (UI = 0.12, UI = 0.23 and UI = 0.26, respectively). Finally *ui3.1* showed a significant interaction with TG523 on chromosome 11 ($P = 0.003$). In this case heterozygosity at either locus alone or at both jointly caused a similar reduction in cross-compatibility.

Analysis of the *S*-selected subpopulations revealed the following: (1) *ui3.1* and *ui12.1* retained their association with UI in the subpopulation comprised of individuals *E/E* at the *S* locus, (2) out of the three regions marginally associated with UI (CT190, TG620B and CT182) only CT190 maintained its effect in the *E/E* subpopulation and (3) in the *E/H* subpopulation the only locus that significantly affected UI was TG620B on chromosome 2 ($P = 0.005$) for which the H allele results in reduced cross-compatibility (Table 5).

Compatibility reactions of near isogenic lines:

NILs for the S locus region: All three NILs (TA1116, TA1117 and TA1118) containing a copy of H alleles at the *S* locus marker (defined by CT62) were self-incompatible (Figure 5, Table 6). In addition, NIL TA1118 failed to function as pistillate parent in crosses to *E*. When *E* was used as the male parent, NILs TA1116 and TA1117 yielded only one and two fruits with reduced seed set, respectively (Table 6). Four out of five other NILs for chromosome 1 (TA1119, TA1120, TA1121 and TA1122), which did not contain the H allele at the *S* locus, yielded fruit and seed both by self-pollination and when pollinated with the *E* parent (Figure 5). The exception was NIL TA1123, which yielded selfed fruit but failed in crosses with *E*, both as male and female.

NILs for QTL ui3.1 and ui12.1: *ui3.1* was represented by the NIL TA1107 and *ui12.1* by the NILs TA1115 and TA1114. TA1107 failed to produce any selfed seed in two separate evaluations and functioned very poorly as staminate parent in a single evaluation. This NIL was also characterized by severe morphological deformations (observed in both *S*₁ and *S*₂) including short stature, contorted stems and leaves and brittle dark tissue. It seems likely that this severe condition affected overall fertility. In all cases the NILs TA1115 and TA1114 produced selfed fruit and seed and accepted *E* pollen readily with normal seed production.

NILs for chromosomal regions marginally associated with SI and UI: NIL TA1113 was available for the CT182 region of chromosome 11, which was marginally associated with both SI and UI (Table 3). TA1113 flowered poorly, setting two and eight selfed fruits for the *S*₁

TABLE 5
S locus epistatic interactions and subpopulation analysis

QTL marker (chromosome)	BC ₁ population		Subp. E/E at S area ^a			Subp. E/H at S area ^a		
	F	P	F (marker)	P	Δ	F	P	Δ
TG308 (2)	6.79	0.01		ns			ns	
TG60 (5)	9.36	0.0026	5.57 (TG358)	0.02	-0.15		ns	
TG400B (11)	9.62	0.0023	4.16 (TG36)	0.04	-0.15		ns	
CT243 (3)	5.36	0.02	4.16	0.04	-0.14		ns	

Interaction term ^b	QTL/marker involved (chromosome)	P	Mean AaBb		Mean AABb		Mean AaBB		Mean AABB	
			N		N		N		N	
CT62 × TG380	ui1.1 × ui12.1	0.002	0.01	28	0.22	31	0.02	32	0.63	46
CT62 × TG251	ui1.1 × ui3.1	0.0001	0.01	42	0.32	49	0.03	18	0.73	28
TG251 × CT171	ui3.1 × CT171 (3)	0.003	0.14	56	0.87	8	0.26	32	0.40	35
TG251 × CT74	ui3.1 × CT74 (9)	0.0008	0.09	34	0.66	21	0.23	52	0.30	23
TG251 × TG523	ui3.1 × TG523 (11)	0.003	0.17	29	0.06	9	0.18	61	0.49	37

QTL marker (chromosome)	BC ₁		Subp. E/E at S ^c			Subp. E/H at S ^c		
	F	P	F	P	Δ	F	P	Δ
ui3.1 (TG251)	15.64	0.00012	6.61	0.01	-0.40		ns	
ui12.1 (TG380)	14.94	0.00017	8.24	0.006	-0.46		ns	
CT190 (1)	8.39	0.0045	5.74	0.02	+0.40		ns	
TG620B (2)	9.77	0.0021		ns		15.5	0.0005	-0.10
CT182 (11)	7.2	0.0082		ns			ns	
TG417 (3)	9.64	0.0023		ns		6.1	0.018	-0.06

No significant two-way interactions were identified for SI ($P \leq 0.005$). For genotypes: upper case, E alleles; lower case, H alleles. A/a corresponds to first marker of interaction term and B/b to second marker. N, class size. Subpopulations (E/E and E/H) lack recombinants within the area TG301-CT81 in the S locus region of chromosome 1. Δ, phenotypic change associated with the presence of the H allele (E/H - E/E). F, F statistic for single point ANOVA.

^a Subpopulation QTL analysis for SI.

^b Two-way interactions involving the UI QTL.

^c Subpopulation QTL analysis for UI.

and S₂ generations, respectively, and functioned as a pistillate parent in crosses with E.

Four markers on chromosome 5 showed marginal association with SI (CT167, TG503, TG60 and CT138). Five NILs were obtained for these areas: TA1110, TA1108, TA1109, TA1111 and TA1112. TA1111 and TA1112 were evaluated only at the S₁ generation and produced normal quantities of fruit and seed upon selfing and crossing with E in either direction. TA1108, which carried the same introgressed segment as TA1109, had severely contorted leaves and irregular fruit with overall poor fertility and crossability. In contrast, TA1109 selfed and crossed normally in both S₁ and S₂. TA1110 was also only evaluated in S₁, displaying poor self-fertility and normal crossability with E. Plants for all chromosome 5 NILs showed reduced numbers of flowers.

Three NILs were evaluated that carried introgressions for segments of chromosome 2: TA1104 and TA1105 carried a fragment marginally associated with reduction of SC (TG308), and TA1106 carried a fragment marginally associated with reduced UC (TG620B).

QTL analysis showed that H introgressions at TG308 were associated with increased self-fertility. The evaluation of NILs TA1104 and TA1105, which carried introgressions at TG308, had a normal load of selfed fruit (Table 5). Interestingly, the introgressed segment carried by TA1104 and TA1105 spans TG308 and also contains marker TG169, site of the only QTL for stigma exertion identified in this study. Both NILs TA1104 and TA1105 had well exerted stigmas without any apparent reduction in self-fertility. Results for NIL TA1106 were not conclusive since S₁ selfing was normal, while S₂ selfing failed to produce fruits.

DISCUSSION

Marker segregation: Deviations from expected segregation ratios have been observed repeatedly in interspecific crosses in plants (STEPHENS 1949; RICK 1969b, 1972; VALLEJOS and TANKSLEY 1983; ZAMIR and TADMOR 1986 and BONIERBALE *et al.* 1988). Studies by PATERSON *et al.* (1988, 1991) using interspecific populations of tomato showed marker segregation distortions in 68%

TABLE 6
Mating behavior of NILs

NIL	Chromosome	QTL	Reaction	BC ₄					
				Self		xE		Ex	
				spf	nf	spf	xp	spf	xp
TA1116	1	<i>s</i>	SI/UC	0	0	13	0.2	5	0.8
TA1117	1	<i>s</i>	SI/UC	0	0	2	0.2	30	0.8
TA1118	1	<i>s</i>	SI/UI	0	0	0	0	21	1
TA1119	1	<i>s</i>	SC/UC	16	6	25	0.4	16	1
TA1120	1	<i>s</i>	SC/UC	24	3	45	0.4	30	0.6
TA1121	1	<i>s</i>	SC/UC	64	1	25	0.8	22	0.6
TA1122	1	<i>s</i>	SC/UC	17	6	24	0.8	23	0.6
TA1123	1	<i>s</i>	SC/UI	19	5	0	0	0	0

NIL	Chromosome	QTL	Reaction	NIL S ₁						NIL S ₂					
				Self		xE		Ex		Self		xE		Ex	
				spf	nf	spf	nf	spf	nf	spf	nf	spf	xp	spf	xp
TA1104	2	SI		13	6	0	0	—	—	41	8	56	0.8	30	0.5
TA1105	2	SI		10	9	20	1	—	—	36	9	38	0.6	—	—
TA1106	2	UI		13	9	31	9	—	—	0	0	—	—	9	0.3
TA1107	3	<i>ui3.1</i>		0	2	7	12	—	—	0	0	—	—	—	—
TA1108	5	SI		0	2	15	1	—	—	6	6	14	0.7	—	—
TA1109	5	SI		50	6	27	3	—	—	17	5	21	0.7	0	0
TA1110	5	SI		20	1	35	4	7	22	—	—	—	—	—	—
TA1111	5	SI		51	7	50	3	16	31	—	—	—	—	—	—
TA1112	5	SI		51	8	42	7	13	27	—	—	—	—	—	—
TA1113	11	SI, UI		10	2	30	5	—	—	38	8	29	0.7	—	—
TA1114	12	<i>ui12.1</i>		19	6	16	7	—	—	—	—	—	—	—	—
TA1115	12	<i>ui12.1</i>		16	3	15	8	—	—	20	5	50	0.9	40	0.7

SI, self-incompatible; SC, self-compatible; UI, unilaterally incongruous; UC, unilaterally congruous; QTL, QTL introgressed in NIL (if below QTL threshold only the trait symbol is given); self, selfed pollination; xE, NIL $\times$ *L. esculentum*; Ex, *L. esculentum* $\times$ NIL; spf, average number of seeds per fruit; nf, total number of fruits; xp, proportion of crosses made that produced fruit. S₁ and S₂ represent two independent evaluations of homozygous NILs.

of the loci in an interspecific BC₁ with *L. chmielewskii* ($P \leq 0.05$) and 51% of the loci surveyed in an interspecific F₂ with *L. cheesmanii* ($P \leq 0.05$). In studies of a BC₁ population with *L. pimpinellifolium* GRANDILLO and TANKSLEY (1996) detected distortions at 8.3% of the loci ($P \leq 0.05$). An extreme case of 80% distortions ($P \leq 0.05$) is reported by DE VICENTE and TANKSLEY (1993) in a study of a F₂ population derived from *L. esculentum* $\times$ *L. pennellii*. Overall, it has been proposed that greater genetic distance between the parental lines results in increased segregation distortion (PATERSON *et al.* 1991; GRANDILLO and TANKSLEY 1996). The comparatively low percentage of markers showing segregation distortion in the current study (15% at $P \leq 0.05$) would not support that contention since *L. hirsutum* is phylogenetically very distant from *L. esculentum*.

A comparison of the chromosomal locations and direction of skewedness detected in this and previous studies with *Lycopersicon* species revealed several common features. Four out of the eight chromosomal re-

gions skewed in the current study were also similarly skewed in other studies. For example a BC₁ population derived from a cross E $\times$ *L. pimpinellifolium* (GRANDILLO and TANKSLEY 1996) showed a similarly inflated heterozygous class for markers on the distal part of the long arm of chromosome 1 (Table 1, Figure 3). Also, in both studies a similar increase in the homozygous *E/E* class was detected for markers on the short arm of chromosome 11 (Table 1, Figure 3). In a BC₁ derived from E $\times$ *L. chmielewskii* (PATERSON *et al.* 1990), a part of chromosome 2 was also enriched in heterozygotes as it was in the current study. ZAMIR and TADMOR (1986) reported an enrichment of homozygotes for *L. pennellii* alleles in an F₂ population derived from a cross between E $\times$ P for the same region of chromosome 2. The middle of chromosome 10 was similarly enriched for heterozygotes in a BC₁ derived from a E $\times$ P cross (TANKSLEY *et al.* 1982). For the other areas skewed in the current study, previous reports show no significant skewing.

FIGURE 5.—NILs. Full line for each designation represents the unique *L. hirsutum* introgression present in the NIL in an otherwise completely homozygous *E/E* genome as determined by complete genome assay of molecular markers. Chromosome 1 NILs contained heterozygous (*E/H*) introgressions, while all other NILs had homozygous (*H/H*) introgressions. The position of all major QTLs found for SI and UI are indicated to the left of the chromosomes. Areas associated at lesser significance are in brackets. All chromosomes and NIL introgressions are drawn to scale (see bar).

Among the studies cited above there are backcross as well as F_2 populations and the proportion of genome surveyed from study to study varied greatly. Both these factors hinder the ability to compare results in regard to location and direction of skewed areas of the genome. In general the F_2 interspecific populations show more skewing (average 70%) than BC_1 interspecific populations (average 40%). This difference may result from increased manifestation of deleterious and subdeleterious allelic combinations in the F_2 populations, possibly associated with recessive epistatic factors. Particular allelic combinations in interspecific populations of distantly related parents may have reduced or increased fitness resulting in selection in favor or against genotype or allele, independently of the population structure and the diversity between the parental lines.

Distribution of recombination frequencies along chromosomes: Several studies in the genus *Lycopersicon* have reported considerable variation in recombination rates for common chromosomal intervals in different populations (PATERSON *et al.* 1991; VAN OOIJEN *et al.* 1994; GRANDILLO and TANKSLEY 1996). Similar to segregation distortion, variation in recombination has

also been attributed to the genetic distance between the parental lines (PATERSON *et al.* 1990; WILLIAMS *et al.* 1995; GRANDILLO and TANKSLEY 1996).

A review of *Lycopersicon* linkage data now available does not support the existence of a relationship between parental genetic distance and recombination. Rather, there appears to be cross-specific hot spots of depressed or increased recombination, some of which may be conserved. The *E* × *H* map reported in this study, which involved two distantly related species, is comparable in marker saturation, population structure (BC_1) and population size to that reported from a cross between the more closely related *Lycopersicon* species *L. esculentum* and *L. pimpinellifolium* (*E* × *PM*) (MILLER and TANKSLEY 1990; GRANDILLO and TANKSLEY 1996). The total map units for both maps were very similar (1356 and 1279 cM, respectively). More variation in recombination is observed when comparing specific chromosomes or chromosomal segments rather than whole maps (Table 2). Comparing single chromosomes between the *E* × *H* and the *E* × *PM* maps, we see that the ratio *E* × *H* cM/*E* × *PM* cM varies from 1.29 (chromosome 6) to 0.64 (chromosome 11) with an av-

erage of 1.05. Considering differences in total map units >10%, five chromosomes (1, 2, 5, 6 and 12) are larger in the E $\times$ H map and two larger in the E $\times$ PM map, with the remaining differing by <10%. Orthologous comparisons for each chromosome between the high density E $\times$ P tomato map and the E $\times$ H map reported here, the E $\times$ PM map and the *L. peruvianum* $\times$ *L. peruvianum* map (VAN OOIJEN *et al.* 1994) all show chromosomes with reductions, as well as chromosomes with increases, in recombination scattered in the genome (Table 2). This variation is also present when comparing the intraspecific PE map with the more divergent E $\times$ P map. Though parental diversity may play a role in regulating recombination, *Lycopersicon* linkage maps developed thus far show localized variation in recombination rates apparently independent of the genetic distance between the parents.

Relationship of the *S* locus to self-incompatibility and unilateral incongruity: It is generally accepted that most self-compatible plant species derived from ancestral self-incompatible forms (WHITEHOUSE 1950; STEBBINS 1957). Self-compatibility would then have evolved as a consequence of breakdown events in the self-incompatibility system (NETTANCOURT 1977). For *Lycopersicon* MCGUIRE and RICK (1954) and RICK (1977, 1978, 1988) concluded that the patterns of mating behavior across the range of the genus (which has both SI and SC species) were consistent with the notion of SC being derived from SI types.

Results from the current QTL mapping study indicate that the genetic determinant for the difference between SI in H and SC in E maps to the *S* locus. We find no evidence for any other significant loci controlling this difference. This result is supported by the observation that a single copy of the H allele at the *S* locus (in NILs) is sufficient to restore self-incompatibility to the cultivated tomato. These results are in contrast to those of BERNATZKY *et al.* (1995, 1996) in which the segregation of *S* locus stylar proteins was monitored in a segregating BC₁ population derived also from a *L. esculentum* $\times$ *L. hirsutum* cross. While most BC₁ individuals carrying the H-type stylar proteins were SI, a few were self-fertile. They also developed NILs for the *S* locus region of chromosome 1 (in a E background) and reported that most plants were self-fertile and that a few segregated for self-incompatibility. They interpreted these results as indicating that other loci, independent from *S*, were also responsible for the loss of SI in *L. esculentum*, and that *S*-related proteins can be recombined with SI/SC response. Most studies, including the current one, have so far evaluated SI indirectly by measuring fruit and seed set. Given the multiple processes that may impact self-fertility independently of GSI, loci showing association with an index of self-pollination, such as SI, may be loci that affect self-fertility independently of the *S* locus, especially those detected at lower

significance. The susceptibility of GSI systems to stressful environmental conditions may represent another source of variation in results from independent studies. The understanding of the relations between the *S* locus and other loci involved in fertilization and reproductions should allow to clarify the role of the different factors and facilitate the eventual utilization of SI for breeding purposes.

Results from this study also implicate the *S* locus as the major factor controlling unilateral incongruity. By far the strongest QTL for UI also mapped to the *S* locus. NILs for the *S* locus were not entirely UI but had significantly reduced function as pistillate parents in crosses with E, producing few if any seeds. They did however function well as males in crosses to *L. esculentum* genotypes. Two other QTL for UI (*ui3.1* and *ui12.1*) have a much less significant impact and apparently only act to enhance UI in the presence of the functional allele at the *S* locus. Alone, neither QTL is capable of generating a UI reaction in the NIL.

The fact that the *S* locus region controls both SI and UI supports the hypothesis that, like SC, UI resulted from modifications at the *S* locus. In fact the possibility exists that SC and UI are different manifestations of the same mutation event(s). Though there is still insufficient data to elucidate the exact molecular nature of *S* locus reactions, models have been proposed that can accommodate a common origin for SI and UI such as the antigen-antibody model of LEWIS and CROWE (1958) or the key-lock model of TROGNITZ and SCHMIEDICHE (1993). Several scenarios could be imagined with a common basis for SI and UI that would be consistent with current observations. For example, (1) SC species that manifest UI when pollinating a SI style could have undergone a mutation(s) that inactivated their stylar arrest proteins (becoming self-compatible) jointly with a mutation(s) that rendered its pollen factors nonspecific to stylar arrest (triggering UI by any and all functional stylar factors). This situation would explain the compatibility of SC $\times$ SI crosses. (2) SC species, as described by LEWIS and CROWE (1958), may have lost their pollen specificity (ability to trigger arrest or to be arrested), thus becoming self-compatible, yet triggering UI if crossed as pistillate parent with SC pollen since their style arrest factors would still be functional. (3) UI between strictly SI species could be explained as involving a species whose pollen factor is nonselectively arrested outside its species boundary. The correct hypothesis may only be identified after it is determined whether the SI reaction results from selective uptake; nonselective uptake and selective activation or nonselective uptake and selective deactivation of style components by the growing pollen tube.

While the major control of UI can be attributed to the *S* locus, two additional QTL of lesser effect on chromosome 3 (*ui3.1*) and chromosome 12 (*ui12.1*) appear

to enhance UI. These two loci appear not to be epistatic modifiers of *S*, but rather to behave in an additive manner, enhancing only modestly the UI reaction. Neither *ui3.1* nor *ui12.1* can cause UI alone as demonstrated by the analysis of the NILs that carried these alleles that did not display UI.

Secondary loci associated with UI have also been reported by CHETELAT and DEVERNA (1991) in crosses involving *L. pennellii*, *L. esculentum* and *Solanum lycopersicon*. They found that the *S* locus region, as well as areas of chromosome 6 and 10, were associated with the ability to overcome UI. The only factor of UI identified in common with the current study was the *S* locus. We propose that *S*-associated unilateral incongruity, which shows a qualitative (compatible or incompatible) pattern, should be distinguished from reduced cross compatibility induced by factors other than *S*. In this study, *ui3.1* and *ui12.1* may encode such factors. These secondary loci may control any of a number of pre- or post-zygotic stages of interspecific fertilization that, in studies like the current one, would be confounded with *S*-associated UI. These factors could also be cross-specific.

The *S* locus region on chromosome 1 contains QTL controlling a number of key floral traits: The most significant QTL for four floral traits mapped to the *S* locus region of chromosome 1. These include FLS, NF, BT and RL. Presence of the *H* allele at the *S* locus of chromosome 1 was associated with more flowers per plant, larger flowers, longer inflorescence and *H*-type flowers buds, increasing the overall flower display. Thus, the *S* locus region of chromosome 1 controls not only GSI and UI but also a number of floral characteristics that probably affect reproductive behavior. DEVICENTE and TANKSLEY (1993) also mapped a QTL for total number of flowers per plant to the *S* locus in progeny from a *E* × *L. pennellii* cross.

It seems unlikely that these diverse effects are pleiotropic manifestations of a single gene. The more plausible explanation for these results is that the *S* locus region of chromosome 1 contains a complex of tightly linked genes affecting separate aspects of reproductive biology. The observation that rare self-compatible populations of otherwise self-incompatible species in *Lycopersicon* retain the original *SI*-type flower morphology is consistent with the concept of several tightly linked genes (RICK 1988). RICK (1978, 1982) has also reported on studies to test the genetic associations between reproductive behavior and floral morphology in tomato. These involved studies between the small flowered *SC L. pimpinellifolium* and both large flowered *SI L. hirsutum* and *L. pennellii*. In both instances *SI* was associated with longer anther tube length and marginal associations were observed between *SI* and corolla size and inflorescence axis length.

In the current study stigma exertion is a major ex-

ception to the clustering of floral trait QTL at the *S* locus region. Exserted stigmas, believed to promote cross pollination (RICK and DEMPSEY 1969a), were found to segregate independently from *SI* by RICK (1978, 1982). Our results confirm this finding by failing to identify common QTL for these traits. In the *E* × *H* population stigma exertion is controlled by a major gene on chromosome 2, though some additional marginal associations were detected with loci on chromosome 3. The fact that stigma exertion is unlinked from the *S* locus suggests that it is an adaptation independent of gametophytic self-incompatibility.

Ancestral gene complex for genetic and morphological control of reproduction? It has been proposed that independent adaptations that serve a common purpose or have a particular adaptive value often may be inherited together (GRANT 1966, 1967, 1971). A good example is the tight association of the genes controlling distyly with the *S* locus as in *Primula* (GREGORY 1915) and *Fagopyrum* (DAHLGREEN 1922; SHARMA and BOYES 1961). Crosses succeed only if pin flowers (short anthers-long style) cross with thrum flowers (long anthers-short style). This form of flower heteromorphism occurs exclusively in families with sporophytic self-incompatibility (DE NETTANCOURT 1977). Crosses fail between flowers of the same type indicating that morphology factors and incompatibility factors are tightly linked.

Cases of linkage of floral morphology or viability factors to the *S* locus have also been reported in families with homomorphic self-incompatibility systems as in the case of the pollen mutant *pl* of *Oenothera* (EMERSON 1941), the *grandiflora* gene in *Petunia* (BIANCHI 1959), and the anther spotting and sepal marks in *Brassica* (THOMPSON and TAYLOR 1965). The fact that *S* locus-floral morphology associations have been identified in a variety of diverse families suggests the possibility of a common conserved ancestral gene complex containing the *S* locus as well as genes controlling floral traits involved in pollination. The fact that the *S* locus complex resides near the centromere of chromosome 1 of tomato, where crossing over is suppressed, would tend to preserve linkage disequilibrium in potentially adaptive allelic configurations.

This research was supported in part by grants from the National Research Initiative Cooperative Grants Program, U.S. Department of Agriculture Plant Genome Program (58-1908-5-001) and by the Binational Agricultural Research and Development Fund (US-2427-94) to S.D.T. D.B. was also funded in part by a Fulbright Scholarship.

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Communicating editor: K. J. NEWTON