Genetic Analysis of Traits Distinguishing Outcrossing and Self-Pollinating Forms of Currant Tomato, *Lycopersicon pimpinellifolium* (Jusl.) Mill.

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ABSTRACT

The evolution of inbreeding is common throughout the angiosperms, although little is known about the developmental and genetic processes involved. *Lycopersicon pimpinellifolium* (currant tomato) is a self-compatible species with variation in outcrossing rate correlated with floral morphology. Mature flowers from inbreeding and outcrossing populations differ greatly in characters affecting mating behavior (petal, anther, and style lengths); other flower parts (sepals, ovaries) show minimal differences. Analysis of genetic behavior, including quantitative trait locus (QTL) mapping, was performed on representative selfing and outcrossing plants derived from two contrasting natural populations. Six morphological traits were analyzed: flowers per inflorescence; petal, anther, and style lengths; and lengths of the fertile and sterile portions of anthers. All traits were smaller in the selfing parent and had continuous patterns of segregation in the F_2. Phenotypic correlations among traits were all positive, but varied in strength. Quantitative trait locus mapping was done using 48 RFLP markers. Five QTL total were found involving four of the six traits: total anther length, anther sterile length, style length, and flowers per inflorescence. Each of these four traits had a QTL of major (>25%) effect on phenotypic variance.

EVOLUTIONARY change in mating system from outcrossing (cross-pollination) to inbreeding (self-pollination) has occurred frequently throughout the flowering plants. Indeed, it has been described as the most common evolutionary trend in angiosperm reproduction (Stebbins 1957, 1970). Although the majority of angiosperms possess hermaphroditic flowers, many are highly outcrossed. This outcrossing can be regulated by a variety of mechanisms, including genetic self-incompatibility and temporal and physical separation of male and female functions in a self-compatible flower. These systems can and do break down in nature, leading to a condition in which a plant is able to pollinate and fertilize itself (Wyatt 1983). At present, relatively little is known about the genetic and developmental mechanisms involved in this change in mating system. This study is an investigation into the genetics and development associated with the evolution of inbreeding in one species, currant tomato, *Lycopersicon pimpinellifolium* (Jusl.) Mill.

The evolution of inbreeding in flowering plants typically involves a syndrome of changes affecting several morphological characters (Ornduff 1969). Many simple cases of plant evolution, in which one or two genes seem to control a given trait, have been described (Gottlieb 1984); the existence of such genes of major effect in plants has been known at least since the time of Mendel. But many of the characters involved in mating system evolution, such as sizes of floral organs or amount of pollen produced, are quantitative in nature. They do not show the discrete segregation that is typical of monogenic or digenic systems; these are termed "quantitative traits" and are regarded as having a more complex genetic basis.

The genetic control of flower development has been the subject of considerable research in recent years, mainly involving the identification and characterization of major regulatory genes. A number of genes controlling meristem and floral organ identities have recently been discovered and characterized. Several of these genes appear to control the differentiation of floral organs produced during the development of flowers (Weigel and Meyerowitz 1994). These organ identity genes are part of a hierarchy of regulatory genes controlling the organization of flowers; many are MADS-box genes that code for transcription factors. It is not currently known if these regulatory genes are involved in quantitative changes in plant form. Relatively little is known about the control of quantitative characters involved in flower development. The development of quantitative trait locus (QTL) mapping techniques (Lander and Botstein 1989) has provided a means of identifying regions of a genome affecting phenotypic traits. This technique can provide information on the map location, relative effect, gene action, and dominance properties of each identified locus, as well as

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provide a basis for eventual isolation of the actual genes. QTL mapping has been used in the study of maize domestication and has aided in the detection of several genes of major effect in maize morphological evolution (Doebley 1992; Doebley *et al.* 1997).

Mating system evolution in plants has been the subject of several studies over the past decade (Macnair and Cumbes 1989; Shore and Barrett 1990; Hill *et al.* 1992; Holtsford and Ellstrand 1992; Sherry 1994; Fenster *et al.* 1995). These studies either dealt exclusively with morphology and development or used biometrical techniques to analyze genetics. More recently, QTL mapping has been employed to gain insight into the genetic basis of mating system evolution. Bradshaw *et al.* (1995) used QTL mapping to investigate the differences between two species of Mimulus, one bee pollinated, the other hummingbird pollinated. They found at least one major QTL of at least 25% effect for each phenotypic trait. More recently, Lin and Ritland (1997) performed QTL mapping on two species of Mimulus, one outcrossing and the other selfing. The QTL found in their study were of smaller effect, with only one exceeding 20%.

Species of Lycopersicon range from self-incompatible outcrossers to self-compatible selfers (Rick *et al.* 1977; Miller and Tanksley 1990). Several self-compatible species vary in their rates of outcrossing, making them ideal candidates for the study of mating system evolution. In a study by Rick *et al.* (1977), variation among populations of *L. pimpinellifolium* was documented in terms of outcrossing rate, anther length, and degree of stigma exsertion. These characters were found to be positively correlated and the floral features were found to be inherited in a quantitative fashion. Self-incompatible species of Lycopersicon, which are obligate outcrossers, also differ from self-pollinating types by the same characters (Rick 1982). Rick *et al.* (1976) have also documented a closely related pair of species differing mainly in mating system, *L. parviflorum* and *L. chmielewskii* (collectively referred to as the *L.* "*minutum*" complex). This species pair is distinguished by the same floral characters that differentiate the selfing and cross-pollinating populations of *L. pimpinellifolium* and that are typical of many such pairs of closely related taxa throughout the angiosperms. Rick *et al.* (1977) also noted that the self-pollinating forms are found typically at the periphery of the native range of the species, which is compatible with the hypothesis that they are derived from the more highly outcrossed forms.

Several studies have addressed the genetics of floral form in relation to pollination and fruit characters in tomato, using classical methods of genetic analysis (Levin *et al.* 1994) and QTL mapping (Grandillo and Tanksley 1996). However, they used cultivated tomato varieties and examined only those aspects of mature flowers considered important in agriculture, without regard for the developmental basis of floral form or for the natural range of mating system variation.

To establish the best morphological characters to use for genetic study of the evolution of inbreeding in *L. pimpinellifolium,* an analysis of developmental differences between selfing and outcrossing flower types in this species was undertaken (Georgiady and Lord 2002). On the basis of this developmental analysis and previous work on the pollination biology of this species (Rick *et al.* 1977, 1978), six characters were chosen for genetic analysis: number of flowers per inflorescence and petal, anther (three characters), and style lengths. The number of flowers per inflorescence is one of the factors affecting the extent of floral display and, by inference, pollinator attraction. This character has also been examined in other QTL mapping experiments (Grandillo and Tanksley 1996), so it provides a basis for comparison. Petal length is used as a measure of corolla size, also a factor in floral display. The developmental study (Georgiady and Lord 2002) revealed that anther differentiation was the first indication of divergence in the two flower forms. Anther length is used as a measure of both anther-tube length and anther size; in tomato, anthers add to floral display, produce pollen, and control pollination by their proximity to the stigma. The fertile portion of the anther represents the site of pollen production, and the sterile portion, which is a unique and defining feature of Lycopersicon species (Tal 1967), represents a functional part of the anther tube. Style length is important for pollination, as is the relative position of the anthers and stigma (Rick and Dempsey 1969; Rick *et al.* 1978). Differences in pistil length in this species are due to differences in style length alone (Georgiady and Lord 2002).

In this study on the evolution of inbreeding flower form in *L. pimpinellifolium*, the central question is: what is the genetic basis for the differences in flower form in this species? Different populations of a single species, which show divergence in mating system from outcrossed to inbred forms, are examined. The plants used are derived from natural populations and are not cultivars. Environmental variation is controlled by growing plants together, under the same conditions, in a climate-controlled greenhouse. It has been shown that outcrossing rate correlates with floral morphology in tomato (Rick *et al.* 1978), so morphological characters are used as an indirect measurement of outcrossing rate.

MATERIALS AND METHODS

Plant material: Seed for *L. pimpinellifolium* (Jusl.) Mill. of contrasting mating system was obtained from the Tomato Genetics Research Cooperative (TGRC, Davis, CA). Two accessions were selected to represent selfing and outcrossing types: LA1237, the "selfer," and LA1581, the "outcrosser" (Rick *et al.* 1977). These two lines were originally obtained from wild populations that were estimated to have outcrossing rates of 0 and 37%, respectively, and that represented the extremes

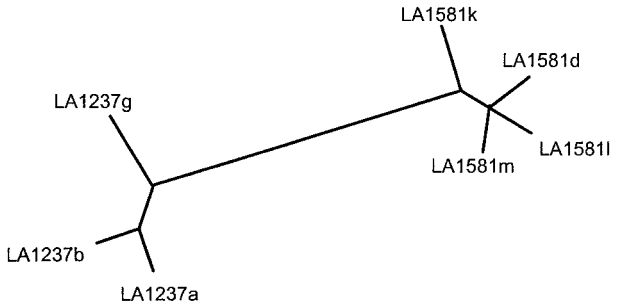

FIGURE 1.—UPGMA tree based on RFLP data for the seven *L. pimpinellifolium* individuals selected as potential parents for the mapping cross. The TGRC accession number for the outcrosser (LA1581) and selfer (LA1237) are shown, along with a lowercase letter suffix indicating an arbitrary designation for each individual within the accession. The tree was generated with the PHYLIP program NEIGHBOR (FELSENSTEIN 1993).

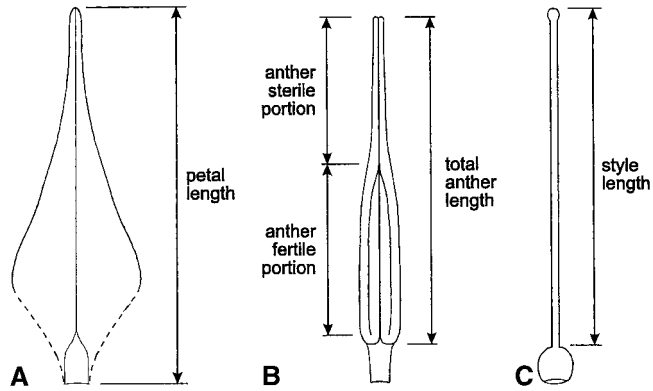

FIGURE 2.—Morphological characters used for genetic analysis. (A) Petal length was measured as the length of the petal from its apex to its adaxial junction with the receptacle. (B) Anther length was measured as the entire length of the anther. Fertile length was measured as the length of the stomia, from their basal end to their junction at the midline of the anther. Sterile length was measured as the distance from the junction of the stomia to the apical end of the anther. (C) Style length was measured from the junction of the style and ovary to the tip of the stigma.

for this species in terms of morphology and outcrossing rate. Plants were grown in a greenhouse at Riverside, California, and periodically propagated by cuttings.

Three selfing and four outcrossing individuals were initially surveyed. The pair showing the greatest degree of genetic polymorphism was crossed, producing F_1 hybrids. The choice of parents was largely arbitrary, since the individuals within each accession were very similar in terms of their restriction fragment length polymorphism (RFLP) fragment patterns, and the two accessions were quite distinct (Figure 1). A few probes showed heterozygosity in one parent and were resurveyed in 28 F_1 plants. An F_1 individual having the most informative genotype was selfed and used to generate a mapping population of 147 F_2 plants. This F_2 population was used for both linkage analysis and QTL mapping.

Scoring of phenotypic characters: Measurements of phenotypic traits for all plants were made on fresh material. Flowers were harvested at midday on the day of anthesis. Each flower was dissected with the aid of a stereoscope and length of individual parts (sepals, petals, anthers, filaments, styles, and ovaries) was measured to the nearest 0.1 mm using an ocular reticle. In addition, for anthers, lengths of fertile and sterile regions were estimated by measuring stomium length, which corresponds well to pollen-sac length (Figure 2). Whole anthers were stained in 0.2% aqueous crystal violet for 30 sec and destained in dH_2O to aid in visualizing the stomia. Thirty flowers from parents and F_1 and five from each F_2 plant were measured.

The number of flowers produced per inflorescence was counted, 30 inflorescences each for parents and F_1 and 10 each for F_2 individuals. Counts were made using inflorescences that were fully developed, *i.e.*, in which the youngest (most distal) bud was at or near anthesis; often a final bud was initiated but did not develop fully, and these buds were not counted. Branched inflorescences, and those bearing foliage leaves, were not used.

Statistical analysis of phenotypic data: Means, standard deviations, Pearson correlation coefficients, and covariances were calculated for each phenotypic trait using the general linear model (GLM) procedure of SAS (SAS INSTITUTE 1989). Genetic variances and covariances were calculated by subtracting the phenotypic variances and covariances of the F_1 population from those of the F_2 population (FALCONER 1989); this makes the assumption that genotype-environment interactions are not a factor.

Southern blotting and hybridization: Genomic DNA was extracted from fresh young shoots via a modified cTAB extraction (OKADA *et al.* 1997). Southern transfers were produced by digestion of genomic DNA with one of four restriction endonucleases (*Eco*RI, *Eco*RV, *Hin*dIII, and *Dra*I), agarose gel electrophoresis, and solvent transfer to nylon membrane (WHITKUS *et al.* 1992).

Tomato genetic probes (TANKSLEY *et al.* 1992) were obtained as plasmid DNA from Steven Tanksley at Cornell University and prepared as described in OKADA *et al.* (1997). Briefly, probe DNA for hybridization was generated by bacterial transformation, miniprep extraction, and insert excision via gel isolation. Alternatively, direct amplification of plasmid inserts was used to prepare some probes. Oligolabeling of probe DNA, hybridization, and washing of Southern blots were done as described in OKADA *et al.* (1997).

Marker selection, map construction, and QTL mapping: Of 117 probes surveyed, 54 appeared to be polymorphic between the two parents. Behavior of questionable probes was rechecked in parents and F_1. Forty-seven probes were deemed usable in the F_2 and were used for mapping. Of these, 5 were dominant and 42 were codominant. Segregation ratios were checked using a chi-square test (WEAVER and HEDRICK 1989). A linkage map was constructed using MAPMAKER/EXP v3.0 (LANDER *et al.* 1987; LINCOLN *et al.* 1992). QTL Cartographer v1.13 (BASTEN *et al.* 1999) was used for single-marker regression analysis and interval mapping and for permutation tests to determine experiment-wide significance levels for each of the traits.

RESULTS

Phenotype of parents, F_1, and F_2: Results of phenotypic measurements for parents, F_1, and F_2 are summarized in Figure 3. The parents possessed large and distinct differences for each character, the outcrosser consistently being the larger of the parents. All characters showed quantitative inheritance in the F_2 generation.

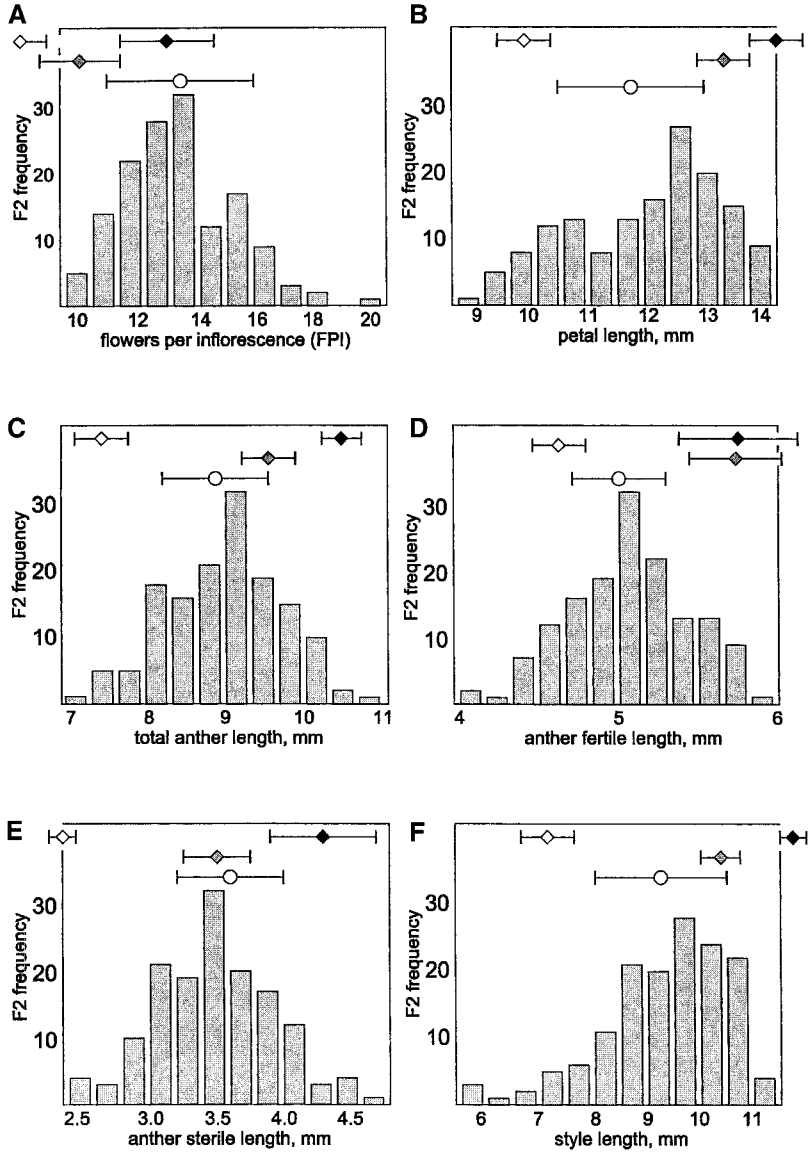

FIGURE 3.—Phenotypic data for parents, F_1, and F_2. At the top of the graphs, open diamonds represent selfing parent means, solid diamonds represent outcrossing parent means, and shaded diamonds represent F_1 means. Means for the entire F_2 population are shown by open circles. Error bars represent ± 1 SD. In the vertical bar graphs, bar lengths indicate relative frequencies of F_2 phenotypes in 12 arbitrary classes.

The number of flowers per inflorescence was 4.8 (60%) greater in the outcrosser than in the selfer, although with a substantial amount of variation, particularly in the outcrosser (Figure 3A). Relative to their parents, the F_1 was intermediate in this character, with essentially no dominance toward either parent. F_2 plants exhibited significant transgressive segregation, with the mean of the F_2 population slightly exceeding the larger parent. The smaller parent inflorescence size was not recovered in the F_2 population.

Petals were 4.5 mm (46%) longer in the outcrosser than in the selfer; they were intermediate in the F_1, although with a moderately high degree of dominance toward the larger parent (Figure 3B). The mean petal length in the F_2 population was very close to the mean of the parents. A small portion of the F_2 population had petals shorter than the smaller parent, and the larger parent length was not recovered. Two widely spaced peaks occur in the F_2 distribution, a large peak between 12 and 13 mm and a smaller peak between 10 and 11 mm.

Anther length showed a similar pattern of inheritance to petal length. Anthers, and their fertile and sterile portions, were greater in the outcrosser by 3.0 mm (41%), 1.1 mm (24%), and 1.9 mm (77%), respectively. Total anther length in the F_1 was intermediate with some dominance toward the larger parent, and the F_2 mean was exactly intermediate between the parents (Figure 3C). There are also two peaks in the F_2 distribution, although they are closer together than those in petal length. The dominance in the F_1 appears to be due to the dominance in the fertile portion of the anther (Figure 3D), which is nearly complete. In contrast, the sterile portion of the anther (Figure 3E) shows very little dominance. The F_2 mean for fertile length is well below the mean parental length, and the mean F_2 sterile length is greater than that of the parental mean by an equal amount. The secondary peak in overall anther length is also seen in sterile length, but not in fertile length. All parental lengths were recovered in the F_2, with the exception of sterile tip length for the outcrosser.

TABLE 1

Means, variances, and estimated broad-sense heritabilities of traits in the F_2 population

	FPI	Petal	Total anther	Anther fertile	Anther sterile	Style
Phenotypic mean	13.30	11.75	8.87	5.02	3.55	9.34
Phenotypic variance	4.68	1.63	0.422	0.115	0.206	1.36
Genetic variance[a]	2.63	1.42	0.344	0.031	0.153	1.18
Heritability (H^2)	0.562	0.871	0.815	0.270	0.743	0.868

All traits are given as lengths in millimeters, except flowers per inflorescence (FPI).
[a] See MATERIALS AND METHODS for explanation.

Styles in the outcrosser were 4.8 mm (66%) longer than those in the selfer and were of intermediate length in the F_1, with some dominance toward the larger parent (Figure 3F). The F_2 mean was close to the mean of the parents. As with petal length, a small portion of the F_2 population had styles shorter than the smaller parent, and the larger parental length was not recovered.

Means and variances for the F_2 population are given in Table 1, and correlations among traits are given in Table 2. Flowers per inflorescence is poorly correlated with all other traits in terms of phenotype; this lack of correlation is reflected in the poor statistical significance for this trait. The best correlation for flowers per inflorescence is with style length, but only with a coefficient of 0.208. Overall, the best correlations were total anther length *vs.* length of anther sterile portion (0.870) and petal length *vs.* style length (0.839). All correlations among the floral (noninflorescence) traits are statistically highly significant, and all are above 0.5, with the exception of fertile *vs.* sterile portions of the anther (0.329).

Linkage behavior of markers: Initial processing of the marker data produced 14 linkage groups (Figure 4); 2 of the 12 chromosomes had linkage groups spaced far enough apart that MAPMAKER did not recognize them as being linked, and their position was inferred from TANKSLEY *et al.* (1992). In all, 44 of the 47 markers mapped to locations essentially the same as given in TANKSLEY *et al.* (1992). The remaining 3 were apparently previously unmapped secondary loci. Using the Haldane map function to convert recombination frequencies to map distances, the total map length was 1296 cM, and the average distance between markers was 37 cM.

Segregation distortion was significant in 4 of the 47 markers, in all cases toward the selfing parent. All 3 markers on chromosome 4 showed this distortion, as well as TG96 on chromosome 5. In general, the use of markers exhibiting segregation distortion is to be avoided; however, particularly in the case of chromosome 4, there would seem to be little alternative here. This should be kept in mind when interpreting the results.

Quantitative trait loci: Initially, 18 QTL were identified for the six traits (Table 3), using an arbitrary LOD threshold of 2.4 (GRANDILLO and TANKSLEY 1996). Subsequent permutation analysis incorporating 1000 resamplings was used to estimate the genome-wide significance of each QTL, and those exceeding the 0.05 level of significance were retained. Five QTL met these crite-

TABLE 2

Correlations among traits

	FPI	Petal	Total anther	Anther fertile	Anther sterile	Style
FPI	1	0.206	0.193	0.142	0.158	0.208
		0.013	*0.019*	*0.086*	*0.056*	*0.012*
Petal	—	1	0.741	0.607	0.617	0.839
			0.0001	*0.0001*	*0.0001*	*0.0001*
Total anther	—	0.808	1	0.748	0.870	0.707
				0.0001	*0.0001*	*0.0001*
Anther fertile	—	1.118	1.088	1	0.329	0.519
					0.0001	*0.0001*
Anther sterile	—	0.781	1.066	1.071	1	0.631
						0.0001
Style	—	0.896	0.727	0.845	0.731	1

The two values in each entry above the diagonal are the phenotypic correlation coefficient and its associated probability (in italics). Entries below the diagonal are the derived genetic correlation coefficients. Estimates of genetic correlation are not available for flowers per inflorescence due to the lack of correspondence between this trait and the others in the single F_1 genotype used. Genetic correlations >1 are due to the method of estimation of environmental variance. FPI, flowers per inflorescence. Other measures are lengths in millimeters.

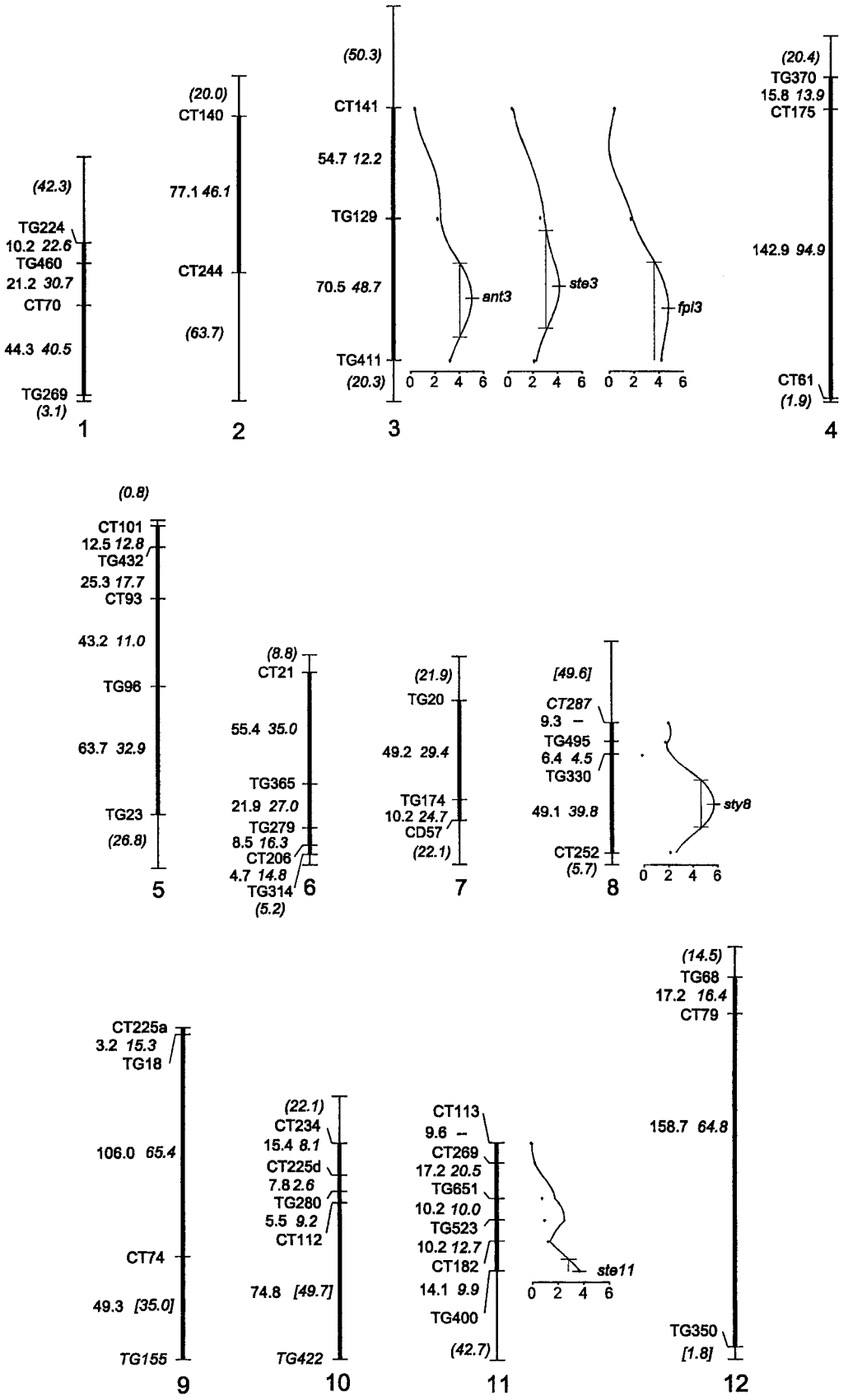

FIGURE 4.—Linkage map with QTL. Marker designations are from TANKSLEY *et al.* (1992); those in italics are unique to this study. Map distances (Haldane) from data in this study are given to the left of each chromosome; values in italics are comparable distances from TANKSLEY *et al.* (1992), and distances for terminal portions of that map not covered by markers in this study are in parentheses. Distances for intervals covering markers unique to this study are in brackets. All distances are in centimorgans. To the right of each chromosome containing a putative QTL detected in this study, a graph of the test statistic from interval mapping is given, with LOD scale. The five significant QTL identified in this study are shown, along with a one-LOD confidence interval. Dots on the graphs aligned with each marker indicate the results of linear regression of the trait on that marker.

TABLE 3

QTL detected for each trait via interval mapping

QTL	Nearest marker	Interval length	QTL pos.	LOD	Significance	%VE	Additive	Dominance
				Flowers per inflorescence				
fpi2.1	CT244.2	77.3	71.2	3.06	0.142	12.1	0.85	−0.72
fpi3.1	TG411.3	70.5	44.1	4.80	0.005	29.8	1.57	−0.70
fpi5.1	CT93.5	25.3	13.4	3.58	0.057	13.4	1.08	0.23
				Petal length				
pet1.1	CT70.1	44.3	20.2	3.12	0.999	22.0	0.54	−0.93
pet4.1	CT175.4	15.8	14.8	3.98	0.995	12.6	0.28	−0.67
				Total anther length				
ant3.1	TG129.3	54.7	45.7	2.50	0.209	10.3	0.29	0.14
ant3.2	TG411.3	70.5	39.5	5.07	0.001	35.2	0.53	−0.10
ant6.1	CT21.6	55.4	3.6	3.12	0.070	36.7	−0.10	0.77
				Anther fertile length				
fer3.1	TG411.3	70.5	47.8	2.73	0.118	19.6	0.20	−0.08
fer5.1	CT93.5	25.3	24.9	2.49	0.205	7.6	0.09	−0.13
				Anther sterile length				
ste3.1	TG129.3	54.7	51.5	2.87	0.092	9.6	0.19	0.10
ste3.2	TG129.3	70.5	32.5	4.12	0.008	31.0	0.35	−0.04
ste10.1	CT234.10	15.5	7.2	2.79	0.105	10.2	0.19	−0.10
ste11.1	TG523.11	10.2	7.4	2.59	0.164	8.7	0.11	−0.20
ste11.2	TG400.11	14.1	14.1	3.81	0.013	11.3	0.22	−0.02
				Style length				
sty4.1	CT175.4	15.8	8.1	3.81	0.231	16.5	0.60	−0.33
sty8.1	TG330.8	49.1	24.1	5.84	0.004	42.2	0.88	−0.99
sty9.1	TG18.9	3.2	2.6	2.42	0.893	7.4	0.60	−0.33

For each QTL, data are given for nearest marker to the QTL, length of interval, position within interval of the QTL, LOD score, significance estimate from permutation analysis, %VE (percentage of phenotypc variance explained by the QTL), and additive and dominance effects.

ria; 2 were associated with anther sterile length and 1 each with total anther length, style length, and flowers per inflorescence. No QTL were detected in this way for petal length or anther fertile length. Four of the 5 QTL were of major effect, explaining >25% of phenotypic variance. The QTL were found on three chromosomes (Figure 4), with 3 QTL on chromosome 3 and 1 each on chromosomes 8 and 11.

Results from single-marker analysis (Figure 4) confirm the interval mapping results. As expected, QTL close to markers were identified by linear regression, while those not near a marker were not detected as reliably. In the one case in which a QTL occurred coincident with a marker (*ste11*, TG400), results for interval mapping and linear regression were identical, as expected.

The QTL for flowers per inflorescence had a large additive component and a large but negative dominance component that correspond well to the small inflorescences of the F_1 and very large inflorescences of the F_2 (Figure 2A). This QTL is in a region of the long arm of chromosome 3 near two other major QTL. One QTL was found for total anther length (*ant*), also on chromosome 3. Two QTL were found for anther sterile length (*ste*), on chromosomes 3 and 11. It should be noted that these two anther-related traits each have a QTL on the long arm of chromosome 3. The QTL of largest effect found in this study, explaining 42% of the variance in style length (*sty*), is located on chromosome 8.

A substantial degree of dominance was associated with two of the QTL, *fpi3* and *sty8*. The dominance effect was of greater magnitude in *sty8* than the additive effect. All of the QTL had negative dominance components, although all six traits had some degree of positive dominance in the phenotypic analysis. These negative dominance components indicate dominance of the selfing parent or, alternatively, recessiveness in the outcrossing parent, given that it is presumed to be the ancestral form.

DISCUSSION

The two most striking results of this study are the variation in genetic behavior of the traits examined and

the presence of QTL of large effect on phenotypic variance for four of the six traits: total anther length, anther sterile length, style length, and flowers per inflorescence. The differences between the fertile and sterile portions of the anther, and the QTL of large effect on variance in style length, are of particular interest, given their importance in the mechanics of pollination.

Anther length: The lengths of fertile and sterile regions of anthers are controlled by substantially different genetic mechanisms, as evidenced by low phenotypic correlation, dominance behavior in F_1 phenotype, and transgressive segregation in the fertile region in the F_2. A QTL for total anther length was found on chromosome 3 and explained ~35% of phenotypic variation for this trait. The QTL has a small additive component and a small negative dominance component. As the F_1 shows phenotypic dominance toward the larger outcrossing parent, other factors must be involved in the regulation of total anther length. Two QTL were found for anther sterile length, one of major effect (31.0%) and the other of lesser (11.3%) effect on phenotypic variance. The major QTL is located on chromosome 3; given its location and effects, it may be the same as the QTL on that chromosome affecting overall anther length.

It should be noted that Grandillo and Tanksley (1996) examined anther length in a backcross between *L. esculentum* and *L. pimpinellifolium*. They found two QTL affecting this trait, on chromosomes 2 and 7, which accounted for only 24% of the phenotypic variation. Neither of those loci corresponds to any of the three found in this study. This could be due to a monomorphic state for such loci in the plants used, given the species involved in each.

The sterile anther tip has been used as a defining character for the genus Lycopersicon, although *L. pennellii* (formerly *Solanum pennellii*) has the typical Solanum-type anther, which lacks this feature. Tal (1967) performed a crossing experiment between *L. esculentum* and *L. pennellii* and attempted to characterize the genetic basis for several traits, including the sterile anther tip. Other than documenting the quantitative nature of this trait, however, he shed little light on this interesting aspect of tomato flower morphology. The interspecific cross produced a wide range of intermediates, but quantitation of the intermediates was not done due to their unusual morphology. From purely qualitative observations, he estimated that two major genes controlled this trait.

The use of component characters here, namely lengths of component portions of the anther, allowed for detection of a QTL (*ste11*) that would not have been found using total anther length alone. This might be expected, since the more complex a character is, the more genes would be involved in its regulation, and the less effect each would have, making detection more difficult. This situation points up the complexity of doing genetic analyses and the importance of judicious character selection.

Style length: The presence of a QTL of very large effect (42.2%) for style length is somewhat of a surprise. It is well documented that continuous variation in stigma exsertion exists among wild populations of this species (Rick *et al.* 1977). From this, one might expect a larger number of genes of lesser effect to control style length. However, if several modifier genes are also present, with significant diversity in dominance effects, this would not be a limiting factor. Given the large negative dominance component of *sty8* and the positive dominance seen in the F_1, other factors are apparently modifying the effect of the QTL on the phenotype to a substantial degree.

It is interesting to note that the outcrossing parental phenotype was not recovered in the F_2. This may be due in large part to chance, since relatively few F_2 individuals would be expected to be homozygous for all outcrossing parent alleles, given that several unlinked genes affect the trait. The low level of environmental variance, as evidenced by the parents and F_1, and dominance effects also may have played a part.

Flowers per inflorescence: Flowers per inflorescence differed from all the other traits in the dominance of the selfer in the F_1 and the large degree of transgressive segregation of the F_2. It is not surprising that behavior of flowers per inflorescence in the F_1 and F_2 is very different from the other traits. The inflorescence is a product of an indeterminate shoot apical meristem, which produces foliage leaves and then branch floral meristems. The floral meristems are determinate in nature and produce only the floral organs (which comprise the other traits) and are thus very different in character from the inflorescence meristem. Thus, one would expect the genetic basis of these two types of traits to be very different, as was seen here.

The large inflorescences in the F_2 seem to be due to the breakdown of normal regulation rather than to the heterotic increase in vigor. Inflorescences with multiple branches were common in some of the F_2 plants, but are almost never seen in nature. This behavior has, however, been described in cultivated "Multiflor" plants and *anantha-3* mutants (Stancheva *et al.* 1997). The atypical branching and pronounced transgressive segregation in the F_2 provide evidence that large inflorescences are under significant negative selection pressure in wild populations. Although a large inflorescence provides a larger floral display for pollinators and a greater possibility of fruit production, there are limiting factors that are also involved. These could include the ability of the plant to support large inflorescences, both physiologically and mechanically, and the effect on outcrossing rate.

The QTL detected for flowers per inflorescence (*fpi3*) corresponds to a major QTL for the same basic character (number of fruits per truss) in a *L. esculentum* × *L.*

pimpinellifolium backcross (Grandillo and Tanksley 1996). As differentiation of the shoot meristem is one of the earliest events in embryogeny, one would expect genes affecting shoot growth to be evolutionarily conserved. Also, since flowers are always a product of shoot growth, genes affecting flower development always operate downstream of those affecting shoot growth and would be expected to be more prone to evolutionary change. Thus some commonality for this trait with another species (*L. esculentum* in this case) is not surprising.

Correlations between traits: Flowers per inflorescence did not show significant correlation with any of the other traits. Again, this was not unexpected, as FPI is different in nature from the other traits. All the remaining traits showed significant positive phenotypic correlation between one another. The low phenotypic correlation between fertile and sterile portions of the anther, as noted above, is particularly striking and provides good evidence that they are distinct components of the anther that warrant separate attention. The high correlation between petal and style is also notable, since they are separated spatially and temporally during flower development by the anthers. The excessively large values derived for the genetic correlation coefficients in 4 of the 10 comparisons indicate that the assumption of no genotype-environment interaction is not well supported in this experiment.

Comparison with other tomato QTL mapping experiments: QTL mapping has been used by plant breeders to identify loci affecting traits for yield and disease resistance (Leonards-Schippers *et al.* 1994; Alpert and Tanksley 1996). Several QTL mapping experiments have been performed on various tomato species; a few of these have included morphological traits affecting mating system (de Vicente and Tanksley 1993; Grandillo and Tanksley 1996; Bernacchi and Tanksley 1997; Fulton *et al.* 1997). However, interpreting these results in an evolutionary light presents some difficulty. Relatively wide, interspecific crosses between *L. esculentum* cultivars and other species were used, which tend to obscure the evolutionary significance of morphological differences. Wide crosses also produce major changes in the background in which genes operate, making analysis of their function more difficult (Bernacchi *et al.* 1998; Allard 1999). The use of complex traits (flower size, stigma exsertion) and subjective scoring of traits in these studies also reduces their usefulness. Still, they provide some basis for comparison with our study, and results from these other studies have been included for comparison as appropriate.

Monogenic traits: There does not seem to be good correspondence between any of the QTL found in this study and genes of known effect on tomato floral morphology (Tanksley and Mutschler 1990). For example, many genes of the *ms* (male sterile) series can alter the size of anthers as well as reduce pollen production. One might expect some of them to be involved in the reduction of those traits in the selfer in this study. However, all the *ms* genes are on different chromosomes from the QTL for anther fertile length found in this study, except for *ms-9* on chromosome 3; it is at the extreme opposite end of the chromosome from *ant3*, so it would seem an unlikely candidate.

Several monogenic mutants that affect inflorescence architecture, including *anantha*, *conjunctiflora*, and *multifurcata*, are also known. These generally have conspicuous effects on vegetative organs also, so it would seem unlikely that the vegetatively normal plants used in this study would have significant variation in these genes. An exception might be *sft* (single-flower truss), which can reduce the number of flowers per inflorescence. It has been linked to chromosome 3, but its position is not known; this leaves open the possibility that it is the gene responsible for *fpi3*.

Other effects: Most previous studies of mating system evolution in plants have dealt exclusively with phenotypic data, which made analysis of dominance more difficult. In two studies of the genus Mimulus, involving different species, Macnair and Cumbes (1989) found dominance toward the outcrossing parent, but Fenster and Ritland (1994) found dominance toward the selfer. In the only other QTL mapping study done to date, Lin (1996) found a mixture of dominance effects, but many QTL had dominance toward selfing.

Latta and Ritland (1993) attempt to model conditions under which a stable mixed mating system can occur. They concluded that mixed mating was more likely if selfing alleles were dominant and when multiple genes are involved in controlling outcrossing rate. Both those conditions would seem to be present in the *L. pimpinellifolium* system. However, the monotonic decline in fitness from inbreeding that was assumed in their model does not seem to be realistic; populations with little or no outcrossing are found through the native range of *L. pimpinellifolium* (Rick *et al.* 1977) and seem to be quite successful. Both selfing and mixed mating systems appear to be stable in this species, which indicates that both long- and short-term elements of fitness may be involved.

Based on the additive and dominance values found for the QTL, predictions of F_1 phenotypes are always smaller than those observed in the actual F_1 plants. This could be due to error in the estimates, the action of undetected loci, or more complex genetic interaction than the simple additive-dominance model assumes. The data here do show that F_1 phenotype may not be a reliable indicator of the dominance of individual genes controlling quantitative traits such as these.

Transgressive segregation has been documented in tomato crosses and attributed to complementary gene action and dominance effects (Rick and Smith 1953; de Vicente and Tanksley 1993). Each of the five floral traits in this study shows a small amount of transgressive

segregation. This is probably due to normal environmental variation, although the underdominance of several QTL could also be factors. The transgressive segregation seen in flowers per inflorescence is particularly striking. The question here is perhaps not why the transgressive segregation is occurring in this trait, but why the inflorescences are apparently constrained to smaller numbers of flowers in the outcrossing parent and F_1. This is an interesting situation that deserves further investigation.

General observations: The most interesting result of this study is that a QTL of major effect on phenotypic variance exists for four of the six traits. This is in agreement with several other studies in which major QTL were found for floral traits (Paterson *et al.* 1988, 1991; Doebley and Stec 1991; Bradshaw *et al.* 1995; Grandillo and Tanksley 1996; Fulton *et al.* 1997) and stands in contrast to the study of Lin and Ritland (1997) in which primarily QTL of lesser effect were found. This last study is of particular interest as it is the only other QTL mapping study that specifically addresses the phenomenon of the evolution of inbreeding in plants that differ by the same basic syndrome of floral traits as those in this study; the contrasting results may be due in part to the interspecific cross used or to character choice (*e.g.*, stigma-anther separation).

In most of the traits in our study, the genetic basis seems to be essentially that described by Grant (1975), with a macromutation and modifiers. This result is particularly interesting, both because there was relatively little existing work to base expectations on and because the debate over the mechanisms of evolution of characters is very much current. In this system at least, Darwin's "innumerable slight variations" (1859, p. 459) or change by means of many genes of small effect (Lande 1981) would not seem to be the mechanism by which evolutionary change has occurred. It should be noted that a QTL of large effect on phenotypic variance, such as *sty8*, could be made up of several closely linked genes of lesser effect, although this need not be the case; Frary *et al.* (2000) have recently shown that a single gene underlies a QTL of major effect on variation in tomato fruit weight.

Taken as a whole, these data indicate that slightly different genetic mechanisms have resulted in similar changes in each of the characters involved in the evolution of inbreeding in this species. The genetic regulation of development can be very complex; it remains to be seen what the functions of the specific genes involved are and to decipher the regulatory hierarchy of the genes controlling development.

This study and others, such as Lin (1996), show that many of the simplifying assumptions made in the theoretical modeling of mating system evolution are not supported by empirical data. Several loci can be involved in the control of mating system traits; these loci can be of large and unequal effect, and additive and dominance components can vary widely. There may be species with simple enough reproductive evolution that such models might be adequate. However, tomato seems to be a relatively simple system to deal with, because of its regular flower architecture and its low degree of environmental input into flower development, and yet the genetics appear to be relatively complicated. Without question, more empirical data on different species will be needed before a general understanding of mating system evolution in plants will be reached.

The main disadvantage of using a close cross, as in this study, is that marker polymorphism is difficult to find. That was certainly the case in this system, in which fewer than one in eight probe-enzyme combinations showed polymorphism in the parents. The lack of thorough coverage of the genome by markers leaves open the possibility that some QTL were not discovered or that the estimates of gene action and additivity are not very accurate. A standard caveat in studies of this type is that the effects of detected QTL are often overestimated (Lynch and Walsh 1998). The use of a larger mapping population to increase the power of analysis could also improve the quality of results, particularly in terms of detecting QTL of small effect (Beavis 1998). One study (Paterson *et al.* 1991) investigated plants grown in different environments and showed that different QTL were sometimes found; that approach could be used for further investigation also. The use of developmental traits such as growth rates would be laborious, but perhaps interesting, given that the duration of growth is a major factor in distinguishing the selfing and outcrossing flower types in a study of their development (Georgiady and Lord 2002). An example of this might be to perform floral organ allometry in the F_2, to see if the simple heterochronic changes suggested by our developmental analysis are regulated independently in each floral organ or on a global basis. And finally, the discovery and characterization of the individual genes involved is becoming a realistic prospect at this point.

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