Comparative Genetics of Potential Prezygotic and Postzygotic Isolating Barriers in a Lycopersicon Species Cross

LEONIE C. MOYLE

From the Department of Biology, Indiana University, 1001 East Third Street, Bloomington, IN 47405.

Address correspondence to L. C. Moyle at the address above, or e-mail: lmoyle@indiana.edu.

Abstract

I compare the genetic basis of quantitative traits that potentially contribute to pre- and postzygotic isolation between the plant species *Solanum lycopersicum* (formerly *Lycopersicon esculentum*) and *Solanum habrochaites* (formerly *Lycopersicon hirsutum*), using quantitative trait loci (QTL) mapping in a set of near-isogenic lines. Putative prezygotic isolating traits include flower size, flower shape, stigma exsertion, and inflorescence length, that can influence pollinator preferences and/or selfing rates, and therefore gene flow between divergent types. Postzygotic isolating traits are hybrid pollen and seed sterility. Three substantive results emerge from these analyses. First, the genetic basis of floral differentiation appears to be somewhat less complex than the genetic basis of postzygotic hybrid sterility, although these differences are very modest. Second, there is little evidence that traits for floral differentiation are causally or mechanistically associated with hybrid sterility traits in this species cross. Third, there is little evidence that hybrid sterility QTL are more frequently associated with chromosomal centromeric regions, in comparison to floral trait QTL, a prediction of centromeric drive models of hybrid sterility. Although genome-wide associations are not evident in this analysis, several individual chromosomal regions that contain clusters of QTL for both floral and sterility traits, or that indicate hybrid sterility effects at centromere locations, warrant further fine-scale investigation.

The evolution of barriers to gene flow between diverging taxa—speciation—can potentially involve genetic changes at many different stages of reproductive isolation. These range from alterations in environmental preference or amplitude that produce geographical or ecological isolation between taxa, through to genetic changes responsible for later generation hybrid breakdown (hybrid viability and/or sterility) (Coyne and Orr 2004). The relative importance of these different isolating barriers in the process of speciation is the subject of ongoing debate. Some have argued that traits responsible for prezygotic isolation, especially those conferring ecological differentiation, are likely to be the most important isolation barriers as they act before species come into reproductive contact, and because they appear to be very common as functional isolating mechanisms, especially among species that might otherwise freely hybridize (e.g., Kirkpatrick and Ravigne 2002; Ramsey et al. 2003). Conversely, barriers that act postzygotically to confer hybrid inviability and sterility are unlikely to be reversible, and therefore are more likely permanent barriers to gene flow between species (Muller 1942; Coyne and Orr 2004). As the “final arbiter” of species isolation, these stages are arguably the most important barrier.

One strategy for determining the relative importance of isolating barriers has been to quantify in detail their individual contributions to restricting gene flow among well-recognized species (e.g., Ramsey et al. 2003; see Coyne and Orr 2004, for other strategies). Alternatively, data on the comparative genetic basis and complexity of different isolating traits can also be useful in evaluating their relative contribution to reproductive isolation, for several reasons. First, these comparisons can suggest which traits are likely to evolve more readily. For example, trait changes that are based on one or few genes of major effect are potentially easier to evolve than genes based on many loci of small individual effect. This is both because fewer new mutations are required, and because the phenotypic effect of each change is individually larger, thereby conferring a larger selective differential per mutation (Orr 1998). Accordingly, genetic complexity could be considered a rough proxy for ease of evolutionary origin, and more readily evolvable traits might have been more important in the very first stages of speciation. Second, the comparative genetic basis of different isolating barriers might also indicate which genetic changes might have been most efficacious in restricting gene flow between diverging lineages. For example, large effect genes might have been the first changes
to significantly reduce gene flow between species (even if they were not the very first to accumulate between species) primarily by virtue of their individual large effect. Conversely, small effect genes—that have only an incremental influence on reducing potential gene flow—are arguably less important in speciation, even if they are the first to arise between species.

Finally, the specific genetic basis of different isolation barriers might provide clues about the evolutionary dynamics responsible for their fixation. For example, one group of models has proposed that reproductive isolating genes should be preferentially associated with genomic regions of low or no recombination (e.g., chromosomal rearrangements) if these traits have arisen between lineages that were still subject to ongoing gene exchange (Noor et al. 2001; Rieseberg 2001). This is because low recombination regions “shelter” isolating barriers that would otherwise be selectively removed via gene exchange between diverging lineages (Noor et al. 2001; Rieseberg 2001). Under this model, an association between chromosomal rearrangements and traits contributing to reproductive isolation indicates that these traits likely arose during a period of sympatry (i.e., under gene flow); no such association is expected when ongoing gene flow is absent (i.e., in allopatry). Another model of hybrid sterility invokes the action of segregation distorters and/or meiotic drive in the evolution of hybrid sterility (e.g., Henikoff et al. 2001; Henikoff and Malik 2002). If centromeric drive is a common cause of postzygotic hybrid incompatibility, quantitative trait loci (QTL) for these barriers are expected to be preferentially associated with centromeric regions, that is, the genomic location where drivers are most likely to be effective in distorting transmission during meiosis. There is no equivalent expectation for prezygotic isolating traits. In both these models of reproductive isolation, then, the preferential association between reproductive isolating traits (or some subset of these traits) and genomic structural features (e.g., inversions, centromeres) implies that specific modes of the evolution of reproductive isolation are more or less plausible. Similarly, associations between traits at different stages of reproductive isolation might suggest causal or mechanistic connections between them. For example, co-localization of QTL for species morphological differences and for hybrid incompatibility could indicate that selection for morphological trait differentiation lead to fixation of changes contributing to hybrid dysfunction, consistent with the Dobzhansky–Muller model of the evolution of hybrid inviability and sterility (Coyne and Orr 2004). In general, then, the comparative genetic basis of isolating traits acting at different stages might both suggest plausible models for their evolutionary origin and causal connections between these traits.

Here, I compare the genetic basis of traits that potentially contribute to different stages of reproductive isolation between 2 plant species: hybrid male and female fertility (postzygotic isolation) and floral morphology (prezygotic isolation, via the influence of floral traits on pollinator behavior and/or selfing, and therefore pollen flow between species—see Study System, below). To map QTL associated with differentiation in floral morphology, floral traits were assessed in a set of near-isogenic introgression lines (NILs) between the plant species Solanum lycopersicum and Solanum habrochaites. QTL for hybrid pollen (male) fertility and seed fertility were previously reported for the same NIL population (Moyle and Graham 2005). My goals in this paper are 4-fold: First, to identify genomic regions (QTL) associated with floral trait differences between the 2 parental species, and to quantify their number, genomic location, and individual effects. Second, to assess the relative genetic complexity of these traits in comparison to those already detected for hybrid pollen and seed fertility in this cross. Third, to evaluate the association (if any) between these different classes of isolating traits. Fourth, to evaluate the association between trait QTL and centromeric chromosomal regions. (Comparative mapping data indicate that Lycopersicon species are not differentiated by detectable chromosomal inversions; Quiros 1991.) It is important to note that these species do not co-occur in nature: S. lycopersicum (SL) is a domesticated species rarely found in the native range of the wild species S. habrochaites (SH). Accordingly, this comparison focuses on what might be inferred about the mode and tempo of evolution of different stages of reproductive isolation, by assessing the relative genetic complexity of, and relationships between, traits contributing to these different stages.

Materials and Methods
Study System
Lycopersicon is a relatively small plant group within the large and diverse Solanaceae family, that consists of 9–12 closely related diploid species, including the domesticated tomato SL (Mill.), (D’Arcy 1979; Peralta et al. 2005; Spooner et al. 2005). Recent taxonomic revision indicates that Lycopersicon is a monophyletic clade nested within the genus Solanum and renames Lycopersicon species accordingly (Peralta and Spooner 2001). Here, I use the revised nomenclature, but continue to refer to the clade as Lycopersicon to retain continuity with the large historical literature published on this group. (Note that the classical nomenclature was used in the previous analysis of hybrid incompatibility QTL [Moyle and Graham 2005].) Classical biosystematic analyses within Lycopersicon (Rick and Lamming 1955; Rick 1979) document the presence and relative strength of a variety of substantial, readily diagnosed, reproductive barriers among species. Nonetheless, all species are to some degree intercrossable (Rick 1979). The 2 parental species analyzed here differ in several biologically significant features. Solanum habrochaites (formerly Lycopersicon hirsutum) is a wild, short-lived herbaceous, perennial species that occurs predominantly from mid to high elevations in northwestern South America. Most populations of SH are obligately outcrossing—a mating system strictly enforced by the gametophytic self-incompatibility system—and exhibit high nucleotide diversity (Miller and Tanksley 1990; Stephan and Langley 1998). In contrast, SL (formerly Lycopersicon esculentum)—the cultivated tomato—is a domesticated, self-pollinating species with comparatively low genetic variation. The putative wild progenitor of SL is also predominantly selfing
and pollinator visitation patterns (Kearns and Inouye 1993, i.e., nectar guides) are known to influence flower recognition including flower number, size, shape, color, and petal patterns, with strong pollinator fidelity, changes in morphology (in- quency with which specific pollinators visit flowers, and the to strongly influence rates of gene flow between species. 

accompanying morphological changes) have the potential mating system shifts from outcrossing to selfing (and their marginal populations of SH (Rick et al. 1979). Because of this size, shape (corolla limb width), and SE are associated with species (e.g., Rick et al. 1977). In particular, reduced flower been inferred in intraspecific studies in several Lycopersicon (Rick et al. 1978). Similar strong associations between floral effects of floral morphology on propensity for selfing tentively correlated with SE and flower size, consistent with di-
rect effects of floral morphology on propensity for selfing (Rick et al. 1978). Similar strong associations between floral traits (including inflorescence size) and selfing rates have been inferred in intraspecific studies in several Lycopersicon species (e.g., Rick et al. 1977). In particular, reduced flower size, shape (corolla limb width), and SE are associated with shifts to self-compatibility (and reduced genetic variance) in marginal populations of SH (Rick et al. 1979). Because of this strong effect of morphological traits on outcrossing rates, mating system shifts from outcrossing to selfing (and their accompanying morphological changes) have the potential to strongly influence rates of gene flow between species.

Second, changes in floral morphology can affect the frequency with which specific pollinators visit flowers, and the effectiveness of pollen transfer during each visit. In species with strong pollinator fidelity, changes in morphology (including flower number, size, shape, color, and petal patterns, i.e., nectar guides) are known to influence flower recognition and pollinator visitation patterns (Kearns and Inouye 1993, and references therein). The most relevant traits for Lycopersicon species differentiation are likely flower size and shape; species color differences are negligible in Lycopersicon, and the existence of interspecific differences in UV-spectrum nectar guides has not yet been examined. With respect to visitation frequency, the most comprehensive study of natural intra-
specific cross pollination in wild Lycopersicon—among genotypes of S. pimpinellifolium—indicated that >95% of floral visits were from a single species of Exomalopsis solitary bee, de-
spite sporadic visits by other bee species (Rick et al. 1978); pollen samples collected from the bodies of this bee visitor indicated high foraging constancy in this species (Rick et al. 1978). In addition, although sample sizes were small, opportunistic pollinator collections on 4 wild Lycopersicon species in Peru indicated little to no overlap in pollinators collected on different species, suggesting distinct species-specific pollina-
tors on Lycopersicon species in this geographical region (Rick 1950). Pollinators have not been systematically studied in SH, however anecdotal accounts report floral visitors that in-
clude species of Bombiniid, Andrenid, Colletid, Meleponid, and Xylocopid bees (Rick et al. 1979), although there are also reports of interspecific foraging patterns in these bees (Rick et al. 1979).

There is also indirect evidence that flower size and other handling features may influence the efficiency of pollen collection from buzz-pollinated species (e.g., Harder 1990) and therefore the likelihood of between-species pollen flow. For example, smaller pollinators on Solanum species had consistently longer handling times per flower than larger pollina-
tors, suggesting that flower size might influence pollen gathering and transfer efficiency (Shelly and Villalobos 2000). In addition, studies in other buzz-pollinated species have shown that both floral morphology and buzz frequency (per second) of pollinating bees influences the amount of pollen released during flower visitation (e.g., King and Buchmann 1996). As such, the degree of “tuning” between specific floral morphologies and the buzz frequencies of their floral visitors could strongly affect the efficiency of interspecific pollen transfer (Harder and Barclay 1994). Because of these potential effects on both pollinator attraction and efficiency, therefore, it is plausible that changes in Lycopersicon floral morphology might significantly influence interspecific gene flow at prezygotic stages.

Floral Differentiation, Pollinator Behavior, and Prezygotic Barriers to Gene Flow

All species in Lycopersicon have radially symmetrical, yellow flowers, and morphological features consistent with “buzz pollination” by bee pollinators (Rick 1950, and see below). Nonetheless, species in the group are florally differentiated, primarily based on petal shape, flower size, and degree of stigma exertion (SE). The latter 2 traits are associated with mating system differences; larger flowered species with exerted stigmas retain ancestral gametophytic self-
incompatibility, whereas self-compatible species tend to be smaller-flowered with more inserted stigmas (Rick 1979). In this study, SH exhibits large showy flowers and a strongly exerted stigma, whereas SL has comparatively small flowers and inserted stigmas. The influence of these floral differences on rates of interspecific gene flow via pollen transfer has not been directly studied in Lycopersicon. Nonetheless, based on indirect data and analyses in other systems, this floral differ-
entiation can plausibly influence interspecific gene flow via 2 mechanisms.

First, increased selfing reduces the potential for pollen transfer between species. Floral morphology is known to be strongly associated with selfing rates in Lycopersicon. For example, comparisons among floral variants within Solanum (formerly Lycopersicon) pimpinellifolium showed that natural (field estimated) rates of cross pollination were strongly posi-
tively correlated with SE and flower size, consistent with di-
rect effects of floral morphology on propensity for selfing (Rick et al. 1978). Similar strong associations between floral traits (including inflorescence size) and selfing rates have been inferred in intraspecific studies in several Lycopersicon species (e.g., Rick et al. 1977). In particular, reduced flower size, shape (corolla limb width), and SE are associated with shifts to self-compatibility (and reduced genetic variance) in marginal populations of SH (Rick et al. 1979). Because of this strong effect of morphological traits on outcrossing rates, mating system shifts from outcrossing to selfing (and their accompanying morphological changes) have the potential to strongly influence rates of gene flow between species.

Second, changes in floral morphology can affect the frequency with which specific pollinators visit flowers, and the effectiveness of pollen transfer during each visit. In species with strong pollinator fidelity, changes in morphology (including flower number, size, shape, color, and petal patterns, i.e., nectar guides) are known to influence flower recognition and pollinator visitation patterns (Kearns and Inouye 1993,
and Tanksley 2000). From this population, 111 BC2 individuals were selfed through single-seed descent for 3 generations (Monforte and Tanksley 2000). To generate a population of NILs, this BC2 population was then subjected to 2 rounds of marker-assisted selection to minimize the number of SH-introgressed segments found within each line while maximizing genome-wide SH representation. Each resulting line carries one or more marker-characterized SH introgressions in homozygous form in an isogenic SL background. The complete set of developed NILs (99 lines) are publicly available from the Tomato Genetics Resource Center (TGRC) at UC Davis (http://tgrc.ucdavis.edu). In combination, they cover 85% of the wild species genome. Four genomic regions are known to account for the missing genomic coverage (Monforte and Tanksley 2000), and see below.

For this experiment, 71 introgression lines were chosen to maximize genomic representation of SH introgressions (i.e., 85% of the SH genome in total) in the SL genetic background, while avoiding undue repetition of any single introgressed region and minimizing the number of lines that contained >1 individual introgression. Based on estimates of introgression size (Monforte and Tanksley 2000; Moyle and Graham 2005), each of these 71 NILs contained, on average, 48.5 cM of introgressed SH genome (range 4.5–135 cM); this corresponds to an average of 3.86% SH genome per NIL (range 0.35–10.7%), assuming a genome size of ~1260 cM (Tanksley et al. 1992). (NILs with >100 cM of introgressed SH genome contain 2 or 3 introgressed regions on different chromosomes—see below.)

Line Cultivation and Handling

Each line and both parental accessions were evaluated in a replicated (3 plants per NIL, 10 plants per parental accession), randomized common garden experiment. All plants were propagated under the same standard greenhouse conditions, as detailed in Moyle and Graham (2005). Briefly, seeds were germinated on blotting paper, cultivated in soil-filled flats after germination, and then transferred to individual one-gallon pots containing greenhouse potting mix. Potted plants were placed in a climate-controlled greenhouse at the Division of Biological Sciences Greenhouse facility, UC Davis, watered daily via drip irrigation, and fertilized twice weekly. Individual plants were pruned and staked prior to flowering.

Phenotyping of Parental and Introgression Lines

Floral Morphology

Eight floral characters were measured on each of the 3 independent flowers (from separate inflorescences) per plant, using hand calipers (see Figure 1A). Traits were: corolla diameter (CD; the width of the corolla in millimeters at the widest point); SE (the distance in millimeters from the stigmatic surface to the lip of the anther cone); petal tip-to-notch (TN; the distance from the petal tip to the nearest interpetal notch); petal notch-to-base (the closest distance in millimeters from the petal notch to the base of flower); rachis length (RL; length from stem to pedicel of terminal flower, in centimeter); number of petals (NP); number of sepals (NS); inflorescence vegetative meristem (IVM; presence/absence of vegetative meristem within the inflorescence). Note that CD, SE, TN, and RL are similar to floral traits assessed in the BC1 population that was used as progenitor material to generate the NILs analyzed here (Bernacchi and Tanksley 1997), with the exception that these traits were estimated categorically in the previous study but are quantitatively measured here. (Note that TN is the quantitative equivalent to CI—corolla indentation—estimated in Bernacchi and Tanksley [1997]; IVM was also assessed in this previous analysis.) Because little genetic variation was detected for the 3 traits, NP, NS, and IVM, these were excluded from further analysis.

For analysis, 4 summary floral characters were used or generated from floral measurements: flower size (CD), SE, RL, and flower shape (FSH). The last character combines corolla and petal dimensions to give an overall estimate of the shape of a flower, using the equation:

\[ FSH = \frac{(TN - NB)}{CD/2}. \]

For SH, FSH values average 1.28, corresponding to a broader corolla surface (see Figure 1B), whereas for SL FSH values average 1.05, corresponding to narrower petals and a more star-shaped flower.

Pollen and Seed Fertility

 Procedures used for measuring pollen and seed fertility have been described in detailed elsewhere (Moyle and Graham 2005). Briefly, pollen fertility was estimated from 3 unopened flowers per plant by dissecting the entire anther cone from each flower into a microcentrifuge tube containing lactophenol aniline blue histochemical stain (Kearns and Inouye 1993), homogenizing the anther cones, and counting a subsample of
pollen solution using a hemacytometer. Pollen that fails to stain lacks functional cytoplasm and is classified as sterile. Total pollen counts and proportions of fertile and sterile pollen were quantified for each sample to generate per-plant averages. Two measures of pollen fertility were evaluated: total number of pollen grains per flower (PN) and proportion of pollen fertile (arc sine square root transformed) (PF). Seed fertility was quantified as the total seed count resulting from self-pollination (self-seed set [SSS]). In Moyle and Graham (2005), seed fertility was also evaluated by crossing each NIL with pollen from the SL parent (cross seed set—CSS), however, no QTL for reduced seed production were detected for this crossing treatment so these data are excluded from the current analysis. SSS was estimated from 3 naturally or manually selfed fruits per experimental plant, by hand harvesting, extracting, and counting the number of visible seeds per individual fruit. As previously noted (Moyle and Graham 2005), some observed reductions in selfed seed set were likely pleiotropic consequences of pollen infertility in specific NILs. Only SSS QTL that were found to be statistically independent of pollen fertility (see Moyle and Graham 2005) have been included in QTL comparisons here.

QTL Analysis

The degree of association between each floral trait and specific introgression lines was assessed using Dunnett’s mean contrast that evaluates the mean phenotype of each NIL against the control SL accession with an experimentwise alpha level of 0.05, that is, corrected for multiple comparisons (Dunnett 1955; Zar 1984). For lines that were significantly different from the control, results are presented as percentage difference (Δ%) from the isogenic SL control; this difference is defined as the phenotypic effect of the QTL hypothesized to reside within the introgressed segment, as in previous studies (e.g., Eshed and Zamir 1995, 1996), and analyses of other traits in this population (Moyle and Graham 2005).

The minimum number and genomic location of QTL underlying differences in each trait were inferred by comparing the positions of introgressed segments having different trait values from the control parent, using 3 assumptions (following Eshed and Zamir 1995; Tao et al. 2003): 1) a QTL is counted only if the relevant NIL is significantly different from the corresponding control, 2) each NIL affecting the trait carries only a single QTL, and 3) two overlapping introgressions with a significant effect on the trait, in the same direction relative to the control, carry the same QTL. In addition, for adjacent introgressions that contain overlapping regions but whose mean trait values differed significantly, the nonoverlapping portion of the introgression was declared the probable location of the QTL of interest. A trait shift between adjacent introgressions was evidenced by a significant difference in trait values between plants carrying each introgression, such that one line was significantly phenotypically different from SL whereas the others were not. In these cases, I also directly confirmed phenotypic differences between the adjacent introgression lines using standard pairwise *t*-tests. In at least one case, conflicting results from NILs with whole or partial overlapping regions made definitive assignment of a QTL to a specific genomic location difficult, perhaps due to violation of one or more of these assumptions (see Moyle and Graham 2005).

Associations between Traits and QTL for Hybrid Inviability and Floral Morphology and Hybrid Inviability

The strength of association between floral traits and previously quantified hybrid incompatibility traits was assessed in 2 ways. First, trait correlations among all floral and hybrid incompatibility traits were assessed with standard pairwise correlation analyses, using mean trait values for each of the 71 NILs. Second, the significance of association between detected QTL for floral morphology and QTL for hybrid pollen and seed fertility was assessed by evaluating the probability (*P*) that QTL for each pair of traits were genomically co-localized (found at the same or overlapping chromosomal locations) more frequently than expected by chance. As in a previous analysis of the association between hybrid incompatibility QTL and regions of marker transmission ratio distortion in this cross (Moyle and Graham 2006), this probability was estimated using the hypergeometric probability distribution function (see Paterson 2002) according to which:

\[
P = \frac{\binom{m}{s} \binom{n-s}{l-s}}{\binom{n}{l}},
\]

where *l* = the number of QTLs found in the larger sample (i.e., for the trait with more detected QTLs), *s* = the number of QTLs found in the smaller sample (i.e., for the trait with fewer detected QTLs), *m* = the number of matches between QTLs, and *n* = the number of intervals that can be compared. In essence, this statistic assesses the probability that an observed number of matches (co-localizations) between 2 different groups of QTLs could occur by chance alone, given the number of QTLs detected in each group and the total number of intervals across which they could be distributed (Paterson 2002). In the analysis, an interval was defined as 35 cM—approximately the average size of individual introgressions in the NIL study (Moyle and Graham 2005). Given this, and a whole genome size of 1260 cM in tomato, *n* = 36. When *P* is less than 0.05, it is considered significantly unlikely that the observed number of QTL matches would occur by chance. Tests were individually performed to assess the qualitative genomic association between each floral trait QTL and each of the QTLs for PN, PF, and SSS. The physical association between centromeric regions and QTL for all traits was similarly assessed with this approach.

Results

Floral Morphology

Introgression lines showed wide variation in floral morphology, in comparison to the SL parental lines (Figures 2A–D). For all traits, a subset of NILs deviated in the direction of the wild SH parent line (i.e., larger CD, SE, and FSH; longer RL), consistent with introgressed SH alleles conferring
phenotypes characteristic of this parent species. Nonetheless, different floral traits were affected somewhat differently. In particular, the NIL averages for CD and FSH were intermediate between the SL and SH parent lines, with few lines falling outside the parental values (Figures 2A and 2C). In comparison, NIL averages for SE and RL were indistinguishable from the SL parent and a substantial minority of NILs showed transgressive trait values (Figures 2B and 2D). NILs were particularly transgressive for RL, with some lines showing both smaller trait values than SL, and larger trait values than SH. For SE, transgressive NILs exclusively showed smaller trait values than both parents (i.e., highly inserted stigmas), possibly consistent with general developmental dysfunction of stigmas in these lines.

Corolla Diameter

Six QTL, located on 4 different chromosomes, were detected for CD (Table 1 and Figure 3). All were in the direction of increased flower size, consistent with SH alleles conferring increased CD; each QTL conferred a modest increase in flower size compared with the SL parent (Table 1). One of these QTL (cd1.1) appears to coincide with a genomic region previously identified as carrying floral size QTL in the BC1 population of this species cross (i.e., fls1.2 in Bernacchi and Tanksley 1997), whereas the other 5 QTL are not previously described between the 2 species. Similarly, we did not recover 2 QTL for flower size (on chromosomes 1 and 3) that were previously described in this earlier population; one of these (fls1.1 in Bernacchi and Tanksley 1997) could not be detected because the relevant region on chromosome 1 was not represented in the NIL mapping population.

Stigma Exsertion

Five QTL were detected for SE (Table 1 and Figure 3), 3 of which were in the direction of increased SE, consistent with SH alleles conferring exserted stigmas. The remaining 2 were transgressive—associated with stigmas that were more inserted than the SL parent—possibly suggesting developmentally compromised flowers. Of the 5 QTL detected here, 4 are previously undescribed, but one (se2.1) coincided with the single QTL previously detected in Bernacchi and Tanksley (1997). This locus has also been detected in several other crosses between wild outcrossing tomato species and SL (Solanum peruvianum: Fulton et al. 1997; S. pennellii: Chen and Tanksley 2004), and has been subject to fine mapping in one of these cases (Chen and Tanksley 2004). Detected

Figure 2. Distributions of NIL mean phenotypes for floral traits for 71 NILs. For each trait, the mean phenotypic value of each parental accession (SL and SP), and the grand mean phenotypic value of all 71 NILs (NIL), are indicated with arrows. (A) Corolla diameter (CD). (B) Stigma exsertion (SE). (C) Flower shape (FSH). (D) Rachis length (RL).
SE QTL generally conferred substantial increases or decreases in SE when quantified as a percentage of the SL parental SE (Δ% in Table 1), although these values correspond to between −1.5 mm and +0.98 mm differences in stigma length from the SL parental average.

**Flower Shape**

I detected 4 QTL for FSH, all of which were positive and therefore consistent with SH alleles conferring the broader corolla surface observed in the wild species (Figure 3). Each QTL conferred only a modest increase in the trait value for FSH, above the SL average (Table 1).

**Rachis (Inflorescence) Length**

I detected 2 QTL for RL (Figure 3) that conferred an average increase in length of 2.5–2.8 cm in comparison to the SL parental average, corresponding to moderate to large trait value increases (Table 1). Neither QTL corresponded to the 3 QTL previously described in the earlier BC1 population; as with CD, one of these (rl1.1 in Bernacchi and Tanksley 1997) could not be detected because the relevant region on chromosome 1 was not represented in the NIL mapping population.

**Pollen and Seed Sterility**

The previous analysis of hybrid incompatibility in this mapping population (Moyle and Graham 2005) revealed 3 QTL for PN, 8 QTL for PV, and 9 QTL for SSS. Because SSS QTL could be the result of pleiotropic effects of pollen sterility (i.e., lines with poor pollen fertility fail to set substantial selfed seed), analyses were also performed on seed set data after the effect of pollen fertility was removed statistically. This analysis revealed 6 SSS QTL with significant fertility effects independent of pollen sterility (Moyle and Graham 2005). These PN, PV, and independent SSS QTL are also shown in Figure 3. Each hybrid incompatibility QTL was found to have moderate to large effects on fertility, although no individual locus conferred complete sterility (Moyle and Graham 2005).

**Comparisons between Floral Morphology and Hybrid Incompatibility QTL**

**Correlations among Traits**

Pairwise correlation analyses using mean values for each of the 71 NILs indicated few significant correlations between traits (Table 2). SE was modestly negatively correlated with FSH, and positively correlated with pollen fertility. These weak or absent correlations between floral traits are consistent with a classical genetic analysis of trait correlations in BC populations between *S. pimpinellifolium* (a self-compatible wild *Lycopersicon* species, possibly the progenitor of SL) and both SH and *S. pennellii* (self-incompatible wild *Lycopersicons*) that also suggested little evidence for frequent genetic linkage between flower size, shape, and SE (Rick 1982). In addition, as previously shown, pollen fertility was also positively correlated with SSS (Moyle and Graham 2005), likely because poor pollen fertility directly affected (i.e., reduced) successful selfing rates in some NILs.

---

**Table 1.** QTL associated with floral traits in NILs between SL and SH. Direction of effect describes an increase (+) or decrease (−) in the mean phenotype at each locus, compared with the SL parent. Mean phenotype is calculated from all NILs (i.e., “No. of NILs observed”) showing the QTL phenotype at each genomic location. Additive effect is calculated as (SH/SH-SL/SL)/2 and Δ% describes the percent phenotypic change from the SL parent.

<table>
<thead>
<tr>
<th>Trait</th>
<th>QTL</th>
<th>Direction of effect</th>
<th>Mean phenotype</th>
<th>Additive effect</th>
<th>Δ%</th>
<th>No. of NILs observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD</td>
<td>cd1.1</td>
<td>+</td>
<td>3.634</td>
<td>0.375</td>
<td>26.00</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>cd4.1</td>
<td>+</td>
<td>3.478</td>
<td>0.297</td>
<td>20.59</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>cd4.2</td>
<td>+</td>
<td>3.477</td>
<td>0.297</td>
<td>20.57</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>cd10.1</td>
<td>+</td>
<td>3.420</td>
<td>0.268</td>
<td>18.59</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>cd11.1</td>
<td>+</td>
<td>3.601</td>
<td>0.358</td>
<td>24.86</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>cd11.2</td>
<td>+</td>
<td>3.412</td>
<td>0.264</td>
<td>18.32</td>
<td>2</td>
</tr>
<tr>
<td>SE</td>
<td>se2.1</td>
<td>+</td>
<td>0.014</td>
<td>0.041</td>
<td>120.6</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>se4.1</td>
<td>−</td>
<td>−0.194</td>
<td>−0.063</td>
<td>−185.9</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>se5.1</td>
<td>+</td>
<td>0.029</td>
<td>0.049</td>
<td>143.1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>se6.1</td>
<td>−</td>
<td>−0.217</td>
<td>−0.075</td>
<td>−220.2</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>se11.1</td>
<td>+</td>
<td>0.017</td>
<td>0.043</td>
<td>125.5</td>
<td>1</td>
</tr>
<tr>
<td>FSH</td>
<td>fsh1.1</td>
<td>+</td>
<td>1.148</td>
<td>0.0503</td>
<td>9.593</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>fsh1.2</td>
<td>+</td>
<td>1.196</td>
<td>0.0742</td>
<td>14.161</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>fsh5.1</td>
<td>+</td>
<td>1.142</td>
<td>0.0472</td>
<td>9.003</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>fsh6.1</td>
<td>+</td>
<td>1.143</td>
<td>0.0477</td>
<td>9.110</td>
<td>3</td>
</tr>
<tr>
<td>RL</td>
<td>rl1.1</td>
<td>+</td>
<td>6.644</td>
<td>1.222</td>
<td>58.20</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>rl9.1</td>
<td>+</td>
<td>7.078</td>
<td>1.439</td>
<td>68.52</td>
<td>1</td>
</tr>
</tbody>
</table>
Genomic Co-Localization among QTL and between QTL and Centromeres

Similar to trait correlations, tests of genomic co-localization among detected QTLs found few significant associations (Table 3). SE and FSH QTL were significantly associated, as were RL and PF QTL. One to 2 significant results are expected by chance from 21 tests of association. Similarly, in no case was there a significant association detected between QTL for individual traits, and centromeric chromosomal regions (data not shown); each of the 7 floral and hybrid sterility traits had one QTL associated with a known centromere, except SSS that was never associated with centromeres (Figure 3).
the chromosomes. Moyle and Graham 2005). The locations of the self-incompatibility (for the trait. SSS QTL locations are only those that were found to be significant independently of pollen fertility effects (see text; indicated by symbols to the right of each chromosome, marked at the center of the chromosomal region showing a significant effect.

Table 2. Correlations between trait values from 71 NILs between SL and SH. CD, corolla diameter; SE, stigma exsertion; FSH, flower shape; RL, inflorescence length; PN, total pollen count per flower; PF, proportion fertile pollen per flower (arc sine square root transformed); SSS, self-seed set. Data for PN, PF, and SSS are from Moyle and Graham (2005)

<table>
<thead>
<tr>
<th></th>
<th>CD</th>
<th>SE</th>
<th>FSH</th>
<th>RL</th>
<th>PN</th>
<th>PF</th>
</tr>
</thead>
<tbody>
<tr>
<td>SE</td>
<td>-0.015</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FSH</td>
<td>0.060</td>
<td>-0.249*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RL</td>
<td>0.173</td>
<td>0.023</td>
<td>0.020</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PN</td>
<td>0.174</td>
<td>0.039</td>
<td>-0.108</td>
<td>0.076</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PF</td>
<td>0.092</td>
<td>0.266*</td>
<td>-0.104</td>
<td>-0.184</td>
<td>0.008</td>
<td></td>
</tr>
<tr>
<td>SSS</td>
<td>-0.070</td>
<td>-0.006</td>
<td>-0.058</td>
<td>-0.049</td>
<td>-0.137</td>
<td>0.359**</td>
</tr>
</tbody>
</table>

* P < 0.05; ** P < 0.01.

Discussion

Three substantive results emerge from these analyses. First, the genetic basis of floral differentiation appears to be somewhat less complex than the genetic basis of postzygotic hybrid sterility, although these differences are very modest. Second, there is little evidence that traits for floral differentiation are causally or mechanistically associated with hybrid sterility traits in this species cross. Third, in comparison to floral morphology QTL, hybrid sterility QTL do not appear to be preferentially associated with centromeric regions, on the basis of the analysis performed here. Each of these findings is discussed in turn.

Relative Complexity of Floral QTL and QTL for Hybrid Sterility

The number of QTL that underlie individual traits involved in floral differentiation between SL and SH are generally fewer than those detected for hybrid sterility, however, these differences are small. Between 2 and 6 QTL were found to underlie individual floral traits, whereas 3–8 QTL underlie different aspects of hybrid sterility. For both classes of traits, the individual sizes of QTL are relatively modest, though hybrid sterility QTL tends to have somewhat larger individual effects than most of the floral QTL (SE is an exception to this, although this trait might also be subject to larger measurement error). In principle, the power to detect QTL for these different classes of traits is identical in these studies, as they were measured in the same mapping population. On the basis of this analysis, then, there is little compelling evidence that potential prezygotic isolation via changes in floral morphology is substantially less complex, and thus more readily “evolvable,” than postzygotic isolating traits.

At least 2 caveats must be considered when interpreting the significance of this finding. First, unlike the assessment of individual effects of hybrid sterility QTL, the current analysis is unable to directly evaluate the individual importance of any single floral morphology QTL in reducing gene flow between species. It might be that only one or few of the detected QTL are sufficient to substantially reduce prezygotic gene flow; if so, the total number of QTL underlying these traits is not the important factor to consider, but rather their individual influence on reducing between-species gene flow. For example, floral changes (including reductions in flower size and increased stigma insertion) that increase the propensity for self-pollination might be more important for patterns of interspecific gene flow, than changes in floral morphology that could influence pollinator preference or efficiency. Mating system shifts are frequent across angiosperms, and are potentially commonly associated with increased prezygotic barriers between diverging lineages (e.g., Fishman and Wyatt 1999). The relative importance of these effects remains to be evaluated in this system in the future, and will require a direct assessment of the degree to which modest to large changes in Lycopersicon floral morphology have direct effects on gene flow via changed pollinator behavior and/or efficacy, or via shifts in mating system that indirectly prevent interspecific gene flow.

Second, one aspect of complexity that cannot be examined here is the prevalence of epistatic interactions between detected QTL (i.e., NILs contain one or few introgressed regions on an isogenic genetic background, therefore interactions between introgressed regions cannot be systematically evaluated). If there are systematic differences in between-QTL epistasis in these 2 classes of traits, these differences might influence the ease of evolution of different isolating stages. For example, hybrid sterility loci necessarily involve pairwise or greater epistatic interactions between genes from divergent lineages (according to the Dobzhansky–Muller

Figure 3. QTL for floral morphology traits, and for pollen and seed fertility traits (from Moyle and Graham 2005), with their location on the SL × SH linkage map. Chromosomal regions shaded with gray stripes on the linkage map indicate areas of the genome fixed for SL alleles (i.e., SH genomic regions not represented in the NIL population). The shaded bars to the right of the chromosomes show the individual analyses for the following traits: CD, corolla diameter; SE, stigma exsertion; FSH, flower shape (see text); RL, rachis length. Also shown are previously reported QTL locations for: PN, total pollen count per flower; PF, proportion fertile pollen per flower; SSS, self-(homozygous) seed set. Levels of percentage difference (Δ%) from the SL control parent for 10 > Δ > 20, 20 > Δ > 50 and Δ > 50 are indicated by the intensity of shading (see figure key). Putative floral QTL are indicated by symbols to the right of each chromosome, marked at the center of the chromosomal region showing a significant effect for the trait. SSS QTL locations are only those that were found to be significant independently of pollen fertility effects (see text; Moyle and Graham 2005). The locations of the self-incompatibility (S) locus and self-pruning (sp) locus are indicated to the left of the chromosomes.
model; Coyne and Orr 2004). Floral changes can potentially involve straightforward allelic changes in one of the 2 diverging lineages. A complete assessment of the relative complexity of these classes of traits therefore requires an analysis of differences in the prevalence of epistasis contributing to each class of traits.

In the interim, however, the findings generated here can be compared with equivalent data from other systems. Unfortunately, there are few prior studies that directly compare the genetic basis of different classes of isolating traits. The most comprehensive set of analyses to date has compared QTL underlying hybrid male sterility (postzygotic isolation), and pigmentation differences and mating discrimination (prezygotic isolation), between the fruit fly species Drosophila santomea and Drosophila yakuba (Moehring et al. 2006a, 2006b). In one study (Moehring et al. 2006a), no-choice mating tests between backcross hybrids and both parental species were used to map QTL associated with frequency of mating, and time to mating, among species. A total of 6 QTL were detected for mating discrimination, each of relatively modest effect ($R^2$ between 0.020 and 0.091); different QTL were detected for male versus female mate discrimination. An earlier study of abdominal pigmentation differences between species, that might be associated with species barriers, mapped 4 QTL with moderate to large effects (Carbone et al. 2005). In contrast, an analysis of male fertility (measured both as sperm and spermatid presence/absence and as a qualitative estimate based on sperm presence and motility) revealed a total of 23 QTL when considering both reciprocal crossing directions (Moehring et al. 2006b); each QTL had small to large effects on sterility ($R^2$ between $\sim0.017$ and 0.730). Although there might have been different power to detect QTL in these studies, these results suggest that the genetic basis of prezygotic isolating traits is less complex than postzygotic hybrid sterility in this species cross. In addition, significant pairwise epistasis was detected for hybrid sterility QTL but not for mate discrimination (although epistatic interactions were detected for abdominal pigmentation differentiation).

For prezygotic isolating traits, similar patterns have been detected in other Drosophila species crosses (see Moehring et al. 2004 for references). For example, between 5 and 8 QTL of moderate to large effects were associated with male and female mate discrimination between Drosophila mauritiana and Drosophila simulans (Moehring et al. 2004). In contrast, the genetic basis of hybrid male sterility in Drosophila is typically highly polygenic, and frequently involves complex epistatic interactions (Coyne and Orr 2004). Together, these studies suggest that in Drosophila, prezygotic isolating traits might typically be less genetically complex than postzygotic isolating traits. These differences between classes of traits are certainly larger than those suggested here for Lycopersicon, most likely because the genetic complexity of postzygotic hybrid sterility differs between Lycopersicon and Drosophila. Unlike in Drosophila, Lycopersicon hybrid sterility appears to be based on fewer QTL, and there is more parity between the number of loci for male (i.e., pollen) sterility and sterility at other stages (e.g., seed sterility) (Moyle and Graham 2005; Moyle LC, Nakazato T, unpublished data). This difference might contribute to the only modest differences in complexity observed between putative pre- and postzygotic QTL reported here.

<table>
<thead>
<tr>
<th>Trait group</th>
<th>Trait 1</th>
<th>No. of QTL</th>
<th>Trait 2</th>
<th>No. of QTL</th>
<th>No. of co-localized QTL</th>
<th>Significance (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Floral traits</td>
<td>CD</td>
<td>6</td>
<td>SE</td>
<td>5</td>
<td>2</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>CD</td>
<td>6</td>
<td>FSH</td>
<td>4</td>
<td>1</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>CD</td>
<td>6</td>
<td>RL</td>
<td>2</td>
<td>0</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>5</td>
<td>FSH</td>
<td>4</td>
<td>3</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>5</td>
<td>RL</td>
<td>2</td>
<td>0</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>FSH</td>
<td>4</td>
<td>RL</td>
<td>2</td>
<td>0</td>
<td>ns</td>
</tr>
<tr>
<td>Floral and sterility</td>
<td>CD</td>
<td>6</td>
<td>PN</td>
<td>3</td>
<td>0</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>CD</td>
<td>6</td>
<td>PF</td>
<td>8</td>
<td>2</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>CD</td>
<td>6</td>
<td>SSS</td>
<td>6</td>
<td>1</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>5</td>
<td>PN</td>
<td>3</td>
<td>0</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>5</td>
<td>PF</td>
<td>8</td>
<td>0</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>5</td>
<td>SSS</td>
<td>6</td>
<td>0</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>FSH</td>
<td>4</td>
<td>PN</td>
<td>3</td>
<td>0</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>FSH</td>
<td>4</td>
<td>PF</td>
<td>8</td>
<td>1</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>FSH</td>
<td>4</td>
<td>SSS</td>
<td>6</td>
<td>0</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>RL</td>
<td>2</td>
<td>PN</td>
<td>3</td>
<td>0</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>RL</td>
<td>2</td>
<td>PF</td>
<td>8</td>
<td>2</td>
<td>0.044</td>
</tr>
<tr>
<td></td>
<td>RL</td>
<td>2</td>
<td>SSS</td>
<td>6</td>
<td>0</td>
<td>ns</td>
</tr>
<tr>
<td>Sterility traits</td>
<td>PN</td>
<td>3</td>
<td>PF</td>
<td>8</td>
<td>2</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>PN</td>
<td>3</td>
<td>SSS</td>
<td>6</td>
<td>0</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>PF</td>
<td>8</td>
<td>SSS</td>
<td>6</td>
<td>1</td>
<td>ns*</td>
</tr>
</tbody>
</table>

ns, not significant.

* Significance of co-localization when only SSS QTL that are statistically independent of pollen fertility (see text) are considered. When all 9 detected SSS QTL (Moyle and Graham 2005) are considered, co-localization significance is $P = 0.073$. 

Table 3. Significance of QTL co-localization among all floral and sterility traits
The data available for other species groups are less comprehensive, making direct comparisons more difficult. One exception is Kim and Rieseberg’s (1999) analysis of differences between the sunflower species *Helianthus annuus* and *Helianthus debilis*. This study detected an average of 3.3 QTL (range 2–6) for each of 5 floral trait differences and 2 QTL for pollen sterility, suggesting that the complexity of postzygotic and floral traits was roughly equivalent in this species cross. Interestingly, using a different methodology, Rieseberg et al. (1999) detected at least 14 chromosomal regions associated with pollen sterility between *Helianthus petiolaris* and *H. annuus*, a result that suggests that hybrid sterility could also be quite complex in other sunflower species crosses. In both cases, both pollen fertility QTL and several floral QTL are associated with extensive chromosomal rearrangements that differentiate these species, making it particularly difficult to determine how many loci are likely to occur in these chromosomal regions. Most other analyses that are currently available quantify the QTL responsible for prezygotic or postzygotic isolating traits only. For example, the elegant studies of pollinator-mediated isolation between *Mimulus lewisi* and *Mimulus cardinalis* indicate that an allelic substitution at a single flower color QTL can produce a substantial shift in pollinator visitation (Bradshaw and Schemske 2003, and references therein). This case suggests that prezygotic isolating traits that influence gene flow via pollinators can have a very simple genetic basis. Nonetheless, differentiation between *Mimulus guttatus* and *Mimulus nasutus* at several different floral traits appears to be based on many (an average of 13) QTL of small effect (Fishman et al. 2002), indicating that species floral differentiation need not always be simple, even among closely related species in a single genus. In many of these and other groups, including *Lycopersicon*, more detailed studies that simultaneously analyze both potential pre- and postzygotic isolating traits, will be valuable in determining whether general differences emerge in the complexity of traits acting at these alternative isolating stages.

**Relationship between Floral Differentiation and Postzygotic Hybrid Incompatibility**

The co-localization of QTL for different traits can suggest an underlying causal connection between them, although fine-scale mapping and eventual identification of the underlying loci is essential to verify this inference. For example, co-localization of QTL for species morphological differences and for hybrid incompatibility could indicate that selection for morphological trait differentiation inadvertently led to fixation of changes causing hybrid dysfunction, consistent with the Dobzhansky–Muller model of the evolution of hybrid inviability and sterility. Among SL and SH, however, there is no evidence for a systematic association between species floral differentiation and loci for hybrid sterility, on the basis of either trait correlations or QTL co-localization. On the basis of these results, therefore, there is little evidence to indicate a mechanistic or causal connection between changes in these different classes of potential isolating barriers. Such a relationship has been inferred in other studies. For example, in the *D. santomea* and *D. yakuba* species pair discussed above, 2 (of 4) abdominal pigmentation QTL co-localized with 2 (of total 23) hybrid male sterility QTL (Mochring et al. 2006a). The authors hypothesize that this co-localization suggests that selection for species pigmentation differentiation might have been responsible for fixing genes involved postzygotic isolation, although the authors do not formally evaluate whether this degree of QTL overlap is greater than would be expected by chance.

Although the comparative mapping presented here does not support a systematic connection between different traits, individual chromosomal locations where multiple QTL cluster still suggest interesting regions for future exploration. In particular, QTL for 2 hybrid incompatibility and 3 QTL for floral morphology are all clustered in one region of chromosome 1 in this species cross (Figure 3). This chromosomal region lies below the location of the self-incompatibility (3) locus in *Lycopersicon*, although the SH 3 locus itself was not included in the analyzed mapping population (see Methods; Figure 3). This broad chromosomal region has previously been identified as harboring multiple loci involved in genetic and morphological mechanisms of reproduction in this and other *Lycopersicon* species crosses (e.g., deVicente and Tanksley 1993; Bernacchi and Tanksley 1997). In multiple studies in other plant systems, loci associated with floral heteromorphism and genetic self-incompatibility have regularly been co-localized, presumably due to the advantage of maintaining linkage disequilibrium between specific floral morphs and classes of self-incompatibility alleles (de Nettancourt 2001). However, the chromosomal association between the S locus and loci for both floral morphology and hybrid sterility has not previously been emphasized, and merits further genetic exploration via fine mapping of this region in this cross. Regardless of the possible mechanistic associations at this specific chromosomal location, however, it is interesting to note that intrinsic postzygotic reproductive isolation loci are not globally associated with floral changes (particularly in flower size and SE) that might have occurred during a shift from outcrossing to selfing. As such, these data also provide potential insight into the genetic architecture of mating system shifts, and their association (or lack thereof) with postzygotic barriers between species.

**Evidence for Specific Evolutionary Models of Hybrid Sterility**

The preferential association between reproductive isolating traits (or some subset of these traits) and genomic structural features (e.g., inversions and/or centromeres) can imply that specific modes of the evolution of reproductive isolation are more or less plausible (see Introduction). In *Lycopersicon*, species are not known to be separated by large-scale chromosomal rearrangements, therefore models of reproductive isolation based on large-scale chromosomal differentiation (e.g., Noor et al. 2001; Rieseberg 2001) are unlikely to generally apply in this group. In contrast, the involvement of segregation distorters and/or meiotic drive in the evolution of hybrid sterility (Henikoff et al. 2001) can be assessed in this
species cross by evaluating whether QTL for hybrid sterility are preferentially associated with centromeric regions. On the basis of the data analyzed here, there is no evidence that postzygotic hybrid sterility is preferentially associated with centromeres, in comparison to centromere associations with QTL for species floral differentiation. As such, there is little evidence that meiotic drive dynamics are generally responsible for the fixation of hybrid sterility QTL detected between these *Lycopersicon* species.

It is worth noting, however, that only 9 of the possible 12 centromeric regions from SH were represented in the NIL mapping population used to evaluate QTL for pre- and postzygotic isolation (see Figure 3). One of these regions (on chromosome 1) was intentionally selected against during development of NILs because it is adjacent to the self-incompatibility locus from SH (Bernacchi et al. 1998a), and therefore prevents self-fertilization in carrier lines. Of the other 2 missing regions, the SH centromere region on chromosome 8 was introgressed in heterozygous form but could not be made homozygous during the generation of NILs (Monforte and Tanksley 2000); this strongly suggests that this region contains a locus (or loci) that acts partially or fully recessively to generate gametic incompatibility or hybrid lethality. The second SH centromeric region (on chromosome 2) was lost earlier in the development of the NIL population, and potentially harbors one or more dominant incompatibility factors that were unable to be introgressed into the SL genetic background.

The analysis of hybrid sterility in Moyle and Graham (2005) could not address whether incompatibility factors were, or were not, contained within these unrepresented genomic regions, so they were not included in the analyses evaluating QTL/centromere associations presented here. However, if these additional 2 centromeric regions on chromosomes 2 and 8 are also assumed to contain hybrid sterility (or inviability) loci, then 3 of 11 centromeric regions have an associated hybrid incompatibility phenotype between these 2 species. In comparison, 2 centromeric regions are associated with one or more floral traits. Previous analyses in other systems have presented convincing cases that individual hybrid sterility loci are likely associated with meiotic drivers (see Coyne and Orr 2004). It remains to be seen whether these individual genomic locations also suggest a role for active drive processes in the evolution of postzygotic reproductive isolation in this *Lycopersicon* species pair.

**Acknowledgments**

I am grateful to E. Graham for her substantial contributions to the SL/SH mapping experiment, to M. Hahn, L. Rieseberg, and one anonymous reviewer for comments that improved earlier versions of the paper, and to L. Rieseberg for the invitation to speak at the July 2006 American Genetic Association (AGA) “Genetics of Speciation” symposium in Vancouver, BC. R. Chetelat and the TGRC (UC Davis) kindly provided seed stocks of the introgression lines. This research was supported by the Center for Population Biology, UC Davis, the Indiana University Department of Biology, and the National Science Foundation (Division of Environmental Biology grant 0532097). This paper is based on a presentation given at the 2006 Annual Meeting of the AGA, “Genetics of Speciation,” University of British Columbia, Vancouver, Canada, July 21–24, 2006.

**References**


Paterson AH. 2002. What has QTL mapping taught us about plant domes-


Corresponding Editor: Loren Rieseberg