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CHROMOSOMAL REARRANGEMENTS AND THE GENETICS OF REPRODUCTIVE BARRIERS IN *MIMULUS* (MONKEYFLOWERS)

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Chromosomal rearrangements may directly cause hybrid sterility and can facilitate speciation by preserving local adaptation in the face of gene flow. We used comparative linkage mapping with shared gene-based markers to identify potential chromosomal rearrangements between the sister monkeyflowers *Mimulus lewisii* and *Mimulus cardinalis*, which are textbook examples of ecological speciation. We then remapped quantitative trait loci (QTLs) for floral traits and flowering time (premating isolation) and hybrid sterility (postzygotic isolation). We identified three major regions of recombination suppression in the *M. lewisii* × *M. cardinalis* hybrid map compared to a relatively collinear *Mimulus parishii* × *M. lewisii* map, consistent with a reciprocal translocation and two inversions specific to *M. cardinalis*. These inferences were supported by targeted intraspecific mapping, which also implied a *M. lewisii*-specific reciprocal translocation causing chromosomal pseudo-linkage in both hybrid mapping populations. Floral QTLs mapped in this study, along with previously mapped adaptive QTLs, were clustered in putatively rearranged regions. All QTLs for male sterility, including two underdominant loci, mapped to regions of recombination suppression. We argue that chromosomal rearrangements may have played an important role in generating and consolidating barriers to gene flow as natural selection drove the dramatic ecological and morphological divergence of these species.

KEY WORDS: **Hybrid sterility, inversion, quantitative trait locus, speciation, translocation.**

Chromosomal rearrangements, particularly inversions and translocations, have a long history as correlates and potential promoters of speciation (Coyne and Orr 2004). Rearrangements may contribute to the evolution and maintenance of species barriers via two primary mechanisms. First, heterozygous rearrangements can directly cause hybrid sterility by disrupting meiotic pairing or causing the production of unfit recombinant gametes (Stebbins 1958; King 1987). Second, suppression of recombination in rearranged chromosomal regions may consolidate existing barriers and generally promote adaptive divergence and speciation in the face of gene flow (Noor et al. 2001; Rieseberg 2001; Ortiz-Barrientos et al. 2002; Navarro and Barton 2003; Feder et al. 2003a; Kirkpatrick and Barton 2006; Hoffmann and Rieseberg 2008; Feder and Nosil 2009; Faria and Navarro 2010; Feder et al. 2011; Nosil and Feder 2011; Feder et al. 2012). Recent empirical work supports rearrangement-assisted divergence-with-gene-flow models, as major quantitative trait loci for ecotypic differentiation map to inversions in diverse taxa (e.g., Hoffmann and Sgrò 2004; Lowry and Willis 2010; Joron et al. 2011; Jones et al. 2012). However, understanding the role of rearrangements in speciation requires investigation of both direct and indirect contributions to reproductive isolation, particularly in cases where the speciation process is near completion. Chromosomal rearrangements have historically been considered a major source of hybrid

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sterility, particularly in plants (Stebbins 1958), but fell from favor as a general mechanism of postzygotic reproductive isolation several decades ago. Because novel chromosomal arrangements must initially occur as heterozygotes, those with strongly underdominant effects on fertility (i.e., those that can cause significant sterility in heterokaryotypic hybrids) should be rapidly removed by natural selection. Thus, severe genetic drift or meiotic drive was deemed necessary for the fixation of such arrangements, making them theoretically implausible as species barriers (Walsh 1982). Furthermore, heterozygosity for rearranged regions has little or no effect on hybrid fertility when crossovers do not occur in the rearranged region, as is the case with some polymorphic inversions within *Drosophila* (Coyne et al. 1991, 1993). Finally, recent studies have shown that epistatic interactions among loci (Dobzhansky–Muller incompatibilities) are a major source of hybrid sterility, even in plants, focusing research on postzygotic reproductive isolation on the mapping of individual incompatibility genes (reviewed in Rieseberg and Blackman 2010; Presgraves 2010). However, the dismissal of rearrangements as important postzygotic barriers may be premature, as theory suggests that selection against local recombination (in addition to drive or drift) can promote the spread of even those rearrangements with underdominant effects on fertility (reviewed in Faria and Navarro 2010).

Suppression of recombination in hybrids, due either to reduced crossing over or to selective loss of recombinant gametes, may indirectly facilitate the evolution of species barriers via multiple scenarios. For example, if novel rearrangements that become fixed in allopatry contain multiple hybrid incompatibility genes, relatively large genomic regions may be resistant to introgression upon secondary contact (Noor et al. 2001; Rieseberg 2001). Such regions are also predicted to accumulate new incompatibility alleles, concentrating reproductive isolation loci in rearrangements (Navarro and Barton 2003). Kirkpatrick and Barton (2006) proposed an elegant extension of such models, which simultaneously explains both the spread of novel rearrangements and their association with species barriers. In their model, populations or incipient species along an ecological gradient are subject to divergent local selection but exchange migrants. Under such conditions, a new rearrangement that happens to capture two or more locally adapted alleles will have high fitness due to low recombination with immigrant chromosomes bearing maladaptive alleles. Feder et al. (2011) recently extended the divergence-with gene-flow model of chromosomal evolution to include "mixed geographic mode" speciation, in which rounds of allopatry and secondary contact facilitate associations between rearrangements and speciation genes over a greater range of values for migration and selection. Because many traits subject to local selection are also premating barriers, these models provide a general mechanism for rearrangements to contribute to speciation whenever divergence is not exclusively allopatric. Furthermore, because selection on captured loci can promote the fixation of even those rearrangements with underdominant effects in hybrids, it may also contribute directly to the evolution of postzygotic reproductive isolation (Kirkpatrick and Barton 2006).

Recent genetic investigations provide empirical support for the theoretical argument that rearrangements facilitate speciation when gene flow links populations under divergent ecological selection. Loci involved in reproductive isolation map to inversions in sympatric species of *Drosophila* (Noor et al. 2001) and sunflower (Kim and Rieseberg 1999) and in ecotypes of sticklebacks (Hohenlohe et al. 2011; Jones et al. 2012), monkeyflowers (Lowry and Willis 2010), *Heliconius* butterflies (Joron et al. 2011), and apple maggot flies (Feder et al. 2003a, b). In addition, sympatric species pairs in *Drosophila* (Noor et al. 2001) and *Anopheles* mosquitoes (Ayala and Coluzzi 2005) are distinguished by higher numbers of fixed inversions than allopatric pairs. These examples suggest that segregating inversion polymorphisms within species as well as those fixed between species are common contributors to adaptation and speciation. Translocations, which often have severe negative effects on heterozygote fitness (e.g., White 1969) may be more likely to cause postzygotic barriers but less likely to initially spread. However, they also have been shown to contribute to reduced gene flow in hybrid zones (e.g., Gimenez et al. 2013). Despite accumulating evidence that both inversions and translocations are important in speciation and ecotypic divergence, however, understanding the multiple contributions of rearrangements to the evolution of reproductive barriers remains a major challenge.

Here, we report the discovery and analysis of putative rearrangements differentiating the monkeyflowers *Mimulus cardinalis* and *Mimulus lewisii*, a classic model system for understanding the genetics and ecology of speciation (Hiesey et al. 1971; Bradshaw et al. 1995, 1998; Schemske and Bradshaw 1999; Bradshaw and Schemske 2003; Ramsey et al. 2003; Angert and Schemske 2005; Angert et al. 2008). These sister taxa are differentiated for elevation range and pollination syndrome, but are sympatric in the Sierra Nevada Mountains of California, form natural hybrids (P. Beardsley and A. Stathos, pers. obs.), and are highly cross-compatible in terms of F_1 production and viability (Heisey et al. 1971). Each species has relatively low survival and fecundity outside its home range and hybrids are selected against at sites within the parental ranges (Hiesey et al. 1971; Angert and Schemske 2005; Angert et al. 2008). A single Mendelian locus (*YUP*) controls yellow pigmentation in the corolla lobes of *M. cardinalis* (Hiesey et al. 1971; Bradshaw et al. 1995), and allele substitution at this locus alone is sufficient to change pollinator behavior and generate substantial reproductive isolation in sympatry (Schemske and Bradshaw 1999; Bradshaw and Schemske 2003). Genetic analyses have also

identified major QTLs underlying quantitative traits such as nectar volume and style length (Bradshaw et al. 1998), making this system a textbook example of speciation by major genes under strong ecological selection. Adaptive differentiation is a major cause of reproductive isolation, with limited overlap in elevational range and pollinator visitation accounting for a large proportion of the total isolation (Ramsey et al. 2003). However, the two species are also isolated by postzygotic barriers, which restrict introgression after the formation of natural F_1 hybrids and may also have been important contributors to reproductive isolation during divergence.

Sierran *M. lewisii* and *M. cardinalis* populations have generally been considered to have fully collinear genomes, as early cytogenetic studies reported no evidence of mispairing in meiosis (Hiesey et al. 1971). However, the genetic architecture of divergent traits in hybrids suggests that recombination may be restricted in some genomic regions. Specifically, Hiesey et al. (1971) noted remarkably strong correlations among traits associated with elevational adaptation and those involved in pollination syndrome in their F_2 hybrids. Similarly, major QTLs for floral traits with no possible shared molecular basis, such as style length and carotenoid concentration, tightly colocalize (Bradshaw et al. 1995, 1998). Tight linkage of major QTLs contributes to the rapid reconstitution of nearly pure species phenotypes when experimental hybrids are subject to natural selection in parental environments (Hiesey et al. 1971; Angert et al. 2008). Determining whether recombination suppression in hybrids (as expected in rearranged regions) contributes to these patterns, and whether it may play a role in the speciation process of these taxa, requires explicit comparison of *M. lewisii* × *M. cardinalis* hybrid maps with those constructed from crosses among relatively collinear taxa. In this study, we use shared gene-based markers to construct and compare linkage maps of *M. lewisii* × *M. cardinalis* and *M. lewisii* × *Mimulus parishii* hybrids and infer the position of putative rearrangements. Like *M. cardinalis*, the selfing species *M. parishii* is recently derived from a *M. lewisii*-like ancestor (Beardsley et al. 2003) and highly cross-compatible at the F_1 level.

Comparative genetic mapping is a valuable tool for the characterization of rearrangements associated with species differences. When informative shared markers are available, the simplest way to identify interspecific rearrangements is to construct separate intraspecific maps for each species, which will unambiguously reveal differences in marker order (Tanksley et al. 1992; Burke 2004). However, patterns of recombination in interspecific hybrid maps, especially when compared to relatively collinear maps, can also be used to identify inverted and translocated regions (e.g., Kaga et al. 2008; Lowry and Willis 2010). Maps constructed from heterokaryotypic crosses exhibit tight clustering of loci in inversions and linkage of markers from different chromosomes near translocation breakpoints (Livingstone et al. 2000), and the region of recombination suppression may extend well beyond the breakpoints (e.g. Stevison et al. 2011). Hybrid mapping has the advantage of directly estimating the effects of rearrangements on heterokaryotype recombination, which is key to their theoretical role in speciation, and allows the simultaneous mapping of loci affecting traits involved in species differentiation. Because both mapping crosses in our design share the same inbred *M. lewisii* parent, we can infer that any rearrangements detected by recombination suppression in one hybrid map relative to the other occurred along either the *M. parishii* or *M. cardinalis* branch, respectively. This allows comparison of the occurrence of rearrangements during the evolution of selfing (*M. parishii*) and during a well-characterized ecological speciation event (*M. cardinalis*). To confirm the location of rearrangements inferred from comparison of the two interspecific maps, we also analyzed patterns of intraspecific linkage at a small set of markers in putatively rearranged regions.

To investigate associations between rearrangements and loci involved in local adaptation and speciation, we also mapped QTLs for potential premating (floral traits) and postzygotic isolating barriers in the *M. lewisii* × *M. cardinalis* hybrids. Major floral QTLs have been well characterized in this cross (Bradshaw et al. 1995, 1998), so we focused on relocation of known major loci rather than a thorough analysis of genetic architecture. We also mapped and characterized QTLs for hybrid sterility (pollen inviability), which can be a direct by-product of chromosomal rearrangement (Stebbins 1958) and may also be selectively maintained in rearranged regions (Noor et al. 2001; Navarro and Barton 2003). F_1 hybrids are $> 50\%$ pollen sterile, but the underlying loci have not been previously mapped and characterized (Ramsey et al. 2003). For each type of species difference or barrier, we assessed whether QTLs mapped to regions of suppressed recombination (potential rearrangements) as is predicted by multiple models of speciation with gene flow.

Methods

STUDY SPECIES

The section Erythranthe of the genus *Mimulus*, including *M. lewisii*, *M. cardinalis*, and *M. parishii* (all taxa $2N = 16$), is found across Western North America. *Mimulus lewisii* and *M. cardinalis* are perennial rhizomatous herbs that both occur in riparian habitats, but segregate by elevation in their shared range. *Mimulus lewisii* occurs at relatively high elevations (generally $>$ 1200 m) and its broad-throated pink flowers are primarily bee pollinated. *Mimulus lewisii* consists of two races, a northern form found in the Cascade and Rocky Mountains and a southern form found in the Sierra Nevada Mountains of California. Although

the two *M. lewisii* races appear as sister taxa in the AFLP phylogeny of Erythranthe (Beardsley et al. 2003) and are morphologically similar, they are largely cross incompatible (Hiesey et al. 1971). In this study, we focus exclusively on the Sierran form of *M. lewisii*, which is parapatric with *M. cardinalis* and has been used in previous studies of the genetics of speciation. *Mimulus cardinalis* ranges from southern Oregon to Baja California and inland to Arizona and Nevada, occurring from sea level to 2400 m in elevation. *Mimulus cardinalis* displays classic floral adaptations for hummingbird pollination: red, long, tubular corollas with reflexed petals, exerted styles, and high nectar volume. Although largely parapatric, *M. lewisii* and *M. cardinalis* co-occur along rivers and streams at mid-elevations (1200–1500 m) in the Sierra Nevada Mountains of California (Angert and Schemske 2005) and hybrid swarms have been observed (P. Beardsley, pers. obs.). *Mimulus parishii* is an annual self-fertilizing herb found primarily in drier low elevation habitats such as ephemeral streams in sandy desert soils of southern California (Beardsley et al. 2003). The range of *M. parishii* is contiguous with that of *M. lewisii* in the foothills of the southern Sierra and it often co-occurs with *M. cardinalis* (P. Beardsley, pers. obs.). *Mimulus parishii* places unambiguously in section Erythranthe in phylogenetic analyses (Beardsley et al. 2003) and readily forms experimental hybrids with Sierran *M. lewisii* (this study).

PLANT MATERIALS AND LINKAGE MAPPING POPULATIONS

We took an inbred line-cross approach to mapping chromosomes and QTLs underlying species differences and barriers. The *M. lewisii* (LEW; 7th generation inbred line) and *M. cardinalis* (CARD; 10th generation inbred line) parents were derived from collections made at a sympatric site on the South Fork of the Tuolumne River, California, outside of Yosemite National Park, were generously supplied by H. D. Bradshaw, Jr., and were used in previous analyses of reproductive isolation (referred to as LF and CE, respectively; Schemske and Bradshaw 1999; Bradshaw and Schemske 2003; Ramsey et al. 2003). F_2 hybrids for linkage and QTL mapping were generated by selfing a single F_1 individual. The mapping population ($N = 192$) was grown in a randomized common garden with parents ($N = 50$–70) and reciprocal F_1 hybrids ($N = 50$ each) in a greenhouse at the University of Montana. Plants were grown in Sunshine #1 soil-free potting mix in 8 mm pots under supplemental light (16 h days) with day–night temperatures consistent with Sierran summer conditions, and were bottom-watered daily.

The *M. lewisii* × *M. parishii* F_2 mapping population shared the LEW parent. The *M. parishii* (PAR) parent was a third generation inbred line derived from a naturally inbred plant collected from Deep Creek near Palm Springs, California. Three PAR × LEW F_1 hybrids were selfed to produce a large F_2 hybrid population. This mapping population was grown under similar culture conditions in a greenhouse at Idaho State University, and a subset ($N = 192$) genotyped to maximize comparability with the LEW × CARD map.

GENOTYPING, LINKAGE MAPPING, AND TRANSMISSION RATIO DISTORTION ANALYSES IN F_2 POPULATIONS

We constructed LP (PAR × LEW F_2) and LC (LEW × CARD F_2) linkage maps using a set of exon-primed, intron-containing markers designed for broad utility across the genus *Mimulus* (MgSTS markers; $N = 855$ total). Briefly, the MgSTS primers were designed based on *Mimulus guttatus* cDNA libraries, with primers located in exon regions with high sequence identity to single copy genes in *Arabidopsis* (Bouck and Vision 2007). These markers have been used extensively for linkage and QTL mapping within the *M. guttatus* species complex (Fishman and Willis 2005, 2006; Fishman et al. 2008; Lowry et al. 2009; Lowry and Willis 2010). Most MgSTS primer sets amplify single loci, but a fraction of primer sets amplified multiple, presumably paralogous, loci with informative polymorphism (as noted by a and b suffixes; Fig. 1). Full sequence information on each MgSTS marker is available at www.mimulusevolution.org.

We used multiple rounds of marker testing, genotyping, and mapping to optimize map quality. Specifically, when it became clear that both the LP and LC maps had fewer linkage groups than expected from their chromosome numbers, we used segmental synteny between *M. guttatus* and *M. lewisii* maps (L. Fishman, J. H. Willis, C. Wu, and Y.W. Lee, unpubl. data) to target MgSTS markers from undersampled regions of the *M. guttatus* genome. In all, we screened > 500 MgSTS markers for amplification of LEW, PAR, and CARD genomic DNA using standard touchdown PCR protocols for these markers (see Fishman and Willis 2005). MgSTS markers with strong amplification in all three Erythranthe taxa were screened for amplicon length polymorphism scorable via capillary electrophoresis. Informative markers ($N = 180$ total) were assembled into multiplex sets for high-throughput genotyping of each mapping population. We extracted genomic DNA from fresh or silica-dried leaves using a CTAB–chloroform extraction protocol modified for high-throughput sample homogenization and DNA separation (Fishman and Willis 2005). Marker multiplexes were all amplified using the same standard touchdown PCR protocol. All forward primers were 5' fluorescent labeled for genotyping using automated capillary sequencers (ABI 3130 or ABI 3700 Genetic Analyzers; Applied Biosystems, Foster City, CA) with an in-lane size standard. Genotypes were scored automatically using Genemapper 3.2 software (Applied Biosystems), verified by eye and, where necessary, corrected by hand. Two markers with differential amplification in heterozygotes (one allele strong, one very

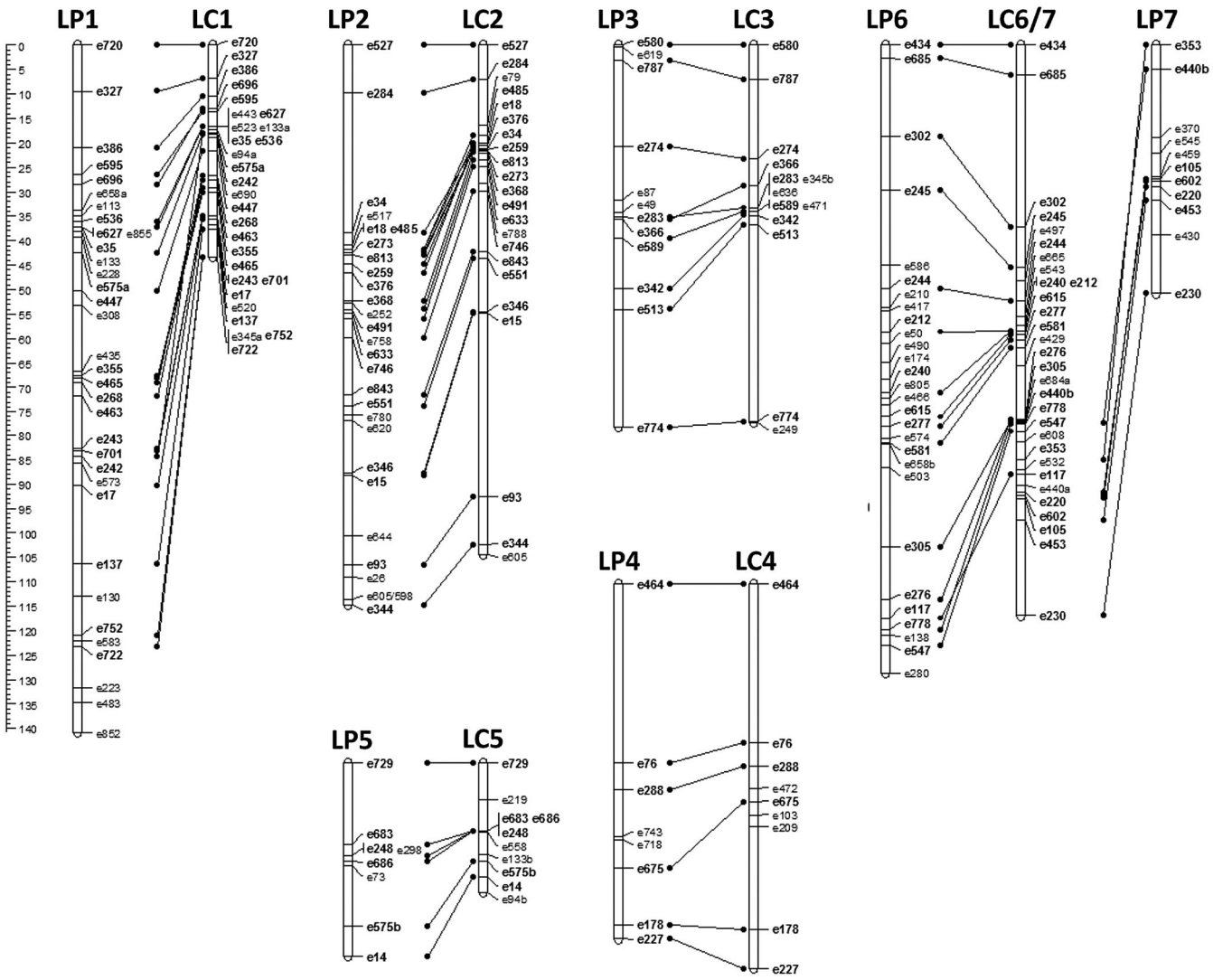

Figure 1. Comparative linkage maps of PAR × LEW (LP groups) and LEW × CARD (LC groups) F_2 hybrids. Distances (scale bar at left) are in cM Kosambi. MgSTS markers (right of bar) are shown with the prefix e- (for expressed sequence tag). Lines connect markers shared by the two maps (names in bold).

weak) in the LEW × CARD population were scored as dominant markers. We linkage mapped 114 and 130 informative markers in the LEW × CARD and PAR × LEW F_2 mapping populations, respectively.

Linkage mapping was performed in Joinmap 4.0 (Van Ooijen 2006), with smaller groups initially evaluated with an LOD threshold of 20 and groups joined at decreasing LOD thresholds where necessary. In the *M. lewisii* × *M. cardinalis* mapping (LC linkage groups), there were six primary groups plus five additional markers at LOD = 20, and the extra markers were all unambiguously assigned at LOD = 10. In the *M. lewisii* × *M. parishii* mapping (LP linkage groups), there were nine initial groups at LOD = 20, plus four additional markers. The two smallest groups joined larger groups at LOD = 15, and LP4 and LP5 merged at LOD = 17. The latter join was clearly due to shared extreme transmission ratio distortion (i.e., no *M. parishii* homozygotes at several markers on both groups), so we maintained LP4 and LP5 as separate groups. One marker (MgSTS.464) could not be assigned automatically without linking LP4 and LP5, but showed strongest linkage with markers on LP4 and so was manually added to this group for ordering. After initial grouping and ordering, two markers were removed from each map because they had "stress" values greater than 5, resulting in 128 and 112 markers on the final LP and LC maps, respectively.

Deviations from the expected Mendelian genotypic ratio (1:2:1) at each marker in both maps were assessed with χ^2-tests ($P < 0.05$, 2 df) in Joinmap. To characterize the pattern of transmission ratio distortion for investigation of postmating barriers between LEW and CARD, for LC map markers only we also examined whether allele ratios (LEW:CARD) and heterozygote

ratios (heterozygotes:homozygotes) fit the 1:1 expectation (χ^2, 1 df), using the Pearson χ^2 calculated in JMP 7.0.1 (SAS Institute 2007). If the first test was significant ($P < 0.05$), we characterized a marker as LEW or CARD excess as appropriate. If the latter was significant, we characterized it as HET excess (or deficit) as appropriate. All but 12 markers, distributed across all linkage groups, had sample sizes > 172 in the LEW × CARD mapping population. Missing genotypes were more common in the LP mapping population (median = 17%), but missing individuals were generally shared across markers.

VERIFICATION OF HYPOTHESIZED REARRANGEMENTS IN INTRASPECIFIC *M. CARDINALIS* CROSSES

Low levels of intraspecific MgSTS polymorphism prevented the construction of genome-wide *M. cardinalis* or *M. lewisii* maps, which would allow unambiguous confirmation of interspecific rearrangements. However, we verified the hypothesized *M. cardinalis* rearrangements involving *M. lewisii* × *M. cardinalis* linkage groups LC1 and LC2, and detected a potential *M. lewisii* rearrangement on LC/LP1, using a small number of markers polymorphic within *M. cardinalis*. We chose wild-derived *M. cardinalis* individuals heterozygous at multiple markers spanning regions of recombination suppression and crossed these to the LEW inbred line. The resulting heterospecific F_1 hybrids segregated for the alternative alleles carried by their *M. cardinalis* parent, allowing us to compare intraspecific recombination fractions to those for the same markers in the LC and LP mapping populations. Because it was difficult to obtain more than a few heterozygous markers per linkage group, we used a total of four F_1 populations each segregating for at least one pair of markers spanning a hypothesized rearrangement. We were unable to obtain a pair of informative *M. cardinalis* markers on LC6. However, this region (which contains the major anthocyanin locus ROI, and is thus readily identified across maps without shared markers) was examined in *M. lewisii* intraspecific hybrids in a previous study (Pince 2009).

MEASUREMENT OF TRAITS

We remapped major QTLs for several key floral traits in LEW × CARD, as it was not possible to match our linkage groups directly with previously published QTL maps based on anonymous RAPD and AFLP markers (Bradshaw et al. 1995, 1998). The quantitative floral traits (corolla tube length and style length) were measured on the first flower of each plant, and date of flowering was also recorded as a proxy for elevational adaptation (Hiesey et al. 1971). Corolla color segregated as distinct classes in the F_2 hybrids, so we categorized flowers as red (*M. cardinalis*-like), orange, dark pink, and light pink (*M. lewisii*-like). For mapping, we recoded color as two binary traits: corolla carotenoid presence (0 vs. 1) and anthocyanin intensity (0 vs. 1), with red = 1,1; orange = 1,0; dark pink = 0,1; and light pink = 0,0. A single locus (*YUP*; yellow upper epidermis) is known to control carotenoid deposition in the corolla and *M. cardinalis* (*yup*) homozygotes are either red flowered or orange flowered (Bradshaw et al. 1995, 1998). Anthocyanin (purple pigment) in the petals is also controlled by a major QTL (Bradshaw et al. 1995, 1998), so our coding captures the essentially discrete nature of both pigmentation traits. Male fertility (% pollen viability) was assessed by counting stained (viable) and unstained (inviable) pollen grains after lactophenol aniline blue staining, followed Fishman and Willis (2001) but with all four anthers of the first flower collected into 200 μL of stain solution.

QTL MAPPING

We used single marker regression implemented in WinQTL Cartographer 2.5 (Wang et al. 2007) to locate the *YUP* locus known to underlie the Mendelian petal carotenoid trait, as interval and composite interval mapping produced infinite likelihood ratio (LR) scores. To map QTLs for other floral traits, flowering time, and pollen viability, we used composite interval mapping (CIM) using Model 6 with up to five cofactors (obtained with forward–backward regression) and a window size of 10 cM. We used permutation (1000 runs) to set separate LR thresholds for QTL detection for each trait. Because our primary goal for floral traits was to locate known major QTLs mapped in two previous studies (Bradshaw et al. 1995, 1998), we did not search for epistatic interactions among these QTLs. To detect epistatic Dobzhansky–Muller interactions affecting pollen fertility, we implemented multiple interval mapping (MIM) using the output from the CIM analysis as a starting model. We then used standard least squares analysis of variance (ANOVA), implemented in the program JMP 7.0.1 (SAS Institute 2007) to refine QTL locations and directly assess the effects and interactions of individual sterility QTLs.

Results

COMPARATIVE LINKAGE MAPPING

Mimulus parishii × *M. lewisii* (LP; 128 markers) map spanned 550.4 cM Kosambi on seven linkage groups, whereas the *M. lewisii* × *M. cardinalis* (LC; 112 markers) map spanned 447.4 cM on six linkage groups (Fig. 1). The MgSTS markers mapped in this study span > 85% of the *M. guttatus* ($2n = 28$) linkage map (477 markers, ~1500 cM on 14 linkage groups) and include at least three markers from each *M. guttatus* chromosome (L. Fishman, J. H. Willis, C. Wu, and Y.W. Lee, unpubl. data). The LP and LC maps shared 92 MgSTS loci, including most terminal markers, so they could be unambiguously aligned over nearly their entire lengths (> 95% and > 99%, respectively).

The *M. lewisii* × *M. cardinalis* map exhibited three extensive regions of recombination suppression relative to the *M. lewisii* × *M. parishii* map: LC1 versus LP1, LC2 versus LP2, and LC6/7 versus LP6 and LP7 (Fig. 1). Both LC1 and LC2 were highly compressed, with marker-dense shared regions that were 37% (20 shared markers; 43.3 cM vs. 121.1 cM) and 47% (12 shared markers; 22.9 cM vs. 48.0 cM) as long as their LP counterparts, respectively. One additional region at the center of LC3 exhibited > 2-fold compression relative to the corresponding LP3 region (six shared markers; 13.5 vs. 33.2 cM), but as this difference depended heavily on the position of a single shared marker (MgSTS.274), we consider it only putatively compressed. The suppression of LC2 relative to LP2 is consistent with an *M. cardinalis*-specific inversion within the compressed region, and the similar suppression of LC1 relative to LP1 suggests a similar mechanism. However, as both LC1 and LP1 exhibit suppressed recombination relative to intraspecific *M. cardinalis* meioses, there may be both *M. lewisii*- and *M. cardinalis*-specific rearrangements involving this linkage group.

One LC group (LC6/7) exhibited novel tight linkage relative to the LP map, with completely unlinked markers from LP6 and LP7 tightly clustered in the center of a single continuous LC linkage group. Examination of F_2 genotypes from LC6/7 confirmed that this clustering reflected true linkage. For example, MgSTS.305 (on LP6) and MgSTS.220 (on LP7) had just 13 crossovers in 360 meioses in the LC map (∼3.5 cM), but were completely unlinked in the LP mapping population (χ^2; $P = 0.46$, $N = 290$ meioses). Transmission ratio distortion does not contribute to novel LC linkage; MgSTS.305 and MgSTS.220 show similar patterns of shared moderate distortion in the two mapping populations (62.4% and 66.4% CARD alleles in LC and 64.1% and 61.5% LEW alleles in the LP mapping population, respectively). Thus, the most likely scenario for the observed novel linkage and surrounding clustering of markers on LC6/7 is a reciprocal translocation. For example, assuming LEW has the LP order, a translocation in CARD that swapped the top of LP7 with the bottom of LP6 could lock together the two involved chromosomes as a single genetic unit during meiosis (Livingstone et al. 2000).

We detected no evidence of recombination suppression in the LP map relative to the LC map, indicating that few, if any, rearrangements uniquely distinguish *M. parishii* from *M. lewisii*. However, we failed to recover the expected eight linkage groups (all taxa are $2n = 16$) in either hybrid map, despite an explicit effort to sample MgSTS markers spanning the entire *M. guttatus* genome. Therefore, *M. lewisii* may have a unique reciprocal translocation locking together multiple chromosomes as a single linkage group in both maps. One tightly linked cluster of markers on LC/LP1, including MgSTS.355, MgSTS.463, MgSTS.465, and MgSTS.268 and corresponding to a contiguous segment of LG10 on the *M. guttatus* composite map (L. Fishman, J. H. Willis, C. Wu, and Y.W. Lee, unpubl. data), appeared to be the best candidate for such a scenario. Removing all six markers from *M. guttatus* LG10 (MgSTS.308–463; Fig. 1) from LP1 decreased its total length by 35 cM, consistent with the non-linearity of linkage relationships often seen in translocated regions (Livingstone et al. 2000; Farré et al. 2011).

To investigate the possibility of LEW-specific reciprocal translocation creating LC/LP1 from two chromosomes, and to verify the inferred *M. cardinalis*–specific rearrangements on LC2 and LC1, we examined recombination between marker pairs in informative *M. cardinalis* meioses (Table 1). For the suppressed region of LC/LP2, *M. cardinalis* recombination was equivalent to the LP map and significantly expanded relative to the LC map, consistent with our inference of an *M. cardinalis*-specific inversion. For LC/LP1, the pattern is more complex. In three independent crosses with *M. cardinalis* informative meioses, markers from the *M. guttatus* LG10 cluster (MgSTS.355, MgSTS.463) exhibited significantly increased recombination, becoming unlinked from flanking markers to which they were moderately (LP) or tightly (LC) linked in the interspecific crosses. Distances between other marker pairs also increased significantly relative to the LC map, matching the LP map. These findings are consistent with two rearrangements involving LP/LC1: a *M. lewisii*–specific reciprocal translocation (involving the LG10 markers) that creates as single linkage group from two chromosomes (thus explaining the shortage of linkage groups in both maps) and a *M. cardinalis*–specific rearrangement that further suppresses recombination only in the LC map. We present the interspecific maps in Figure 1, with indication of regions of putative rearrangement, because they represent the linkage relationships among markers in hybrids where speciation QTLs have been mapped, but emphasize that they do not correspond to the chromosomal arrangement in any of the parental species.

TRANSMISSION RATIO DISTORTION AND POSTMATING BARRIERS

Both maps exhibited substantial biologically based transmission ratio distortion (TRD). In the LP map, about half (54%; 70/129) of markers were distorted. LP1 and LP2 were relatively unaffected, with a few markers on LP1 showing a slight excess ($P < 0.05$) of heterozygotes. LP4 and LP5 exhibited extremely strong excesses of *M. lewisii* alleles, with LEW transmission ranging from 56% to 96% across LP4 and from 61% to 78% across LP5. Large portions of LP6 and LP7 exhibited similarly directional, but more moderate, TRD (maximum 61.5% and 65.5%, respectively). On each LC linkage group, at least a few (and often all) markers exhibited significant transmission ratio distortion (Fig. 2), with 92/112 (82%) of markers distorted at the $\alpha = 0.05$ level. Three linkage groups (LC1, LC3, LC6) exhibited excesses of *M. cardinalis* alleles at

Table 1. Recombination (*r*) between marker pairs spanning putative *Mimulus lewisii–Mimulus cardinalis* rearrangements in *M. cardinalis* (CC), as compared to LP and LC hybrid meioses. CC recombination values are shown in bold if they were not significantly different from the Mendelian expectation of independent assortment (that is, markers were unlinked) with χ^2-tests ($\alpha = 0.05$). χ^2-tests (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$) were used to evaluate whether *M. cardinalis* values of *r* were different than those predicted by recombination in each hybrid mapping population.

LP/LC group	Marker pair	Source pop.[1]	*N*	*r* CC	*r* LP	*r* LC	χ^2 vs. LP	χ^2 vs. LC
1	e355-e443	CUY	95	**0.45**	0.08	0.04	***	***
1	e463-e137	IND	74	0.39	0.28	0.09	***	***
1	e463-e536	IND	74	**0.41**	0.12	0.07	***	***
1	e536-e137	IND	96	0.21	0.17	0.02	N.S.	***
1	e355-e701	WAW	96	**0.42**	0.09	0.04	***	***
1	e355-e443	WAW	96	**0.43**	0.08	0.04	***	***
2	e527-e273	WAW	96	0.22	0.26	0.14	N.S.	*
2	e527-e746	RED	96	0.28	0.31	0.18	N.S.	**
2	e273-e746	RED	96	0.08	0.11	0.03	N.S.	**

[1]***M. cardinalis* source populations: CUY = Cuyamaca, San Diego Co., CA; IND = Indian Creek, Trinity Co., CA; RED = Redwood Creek, Fresno Co., CA; WAW = Wawona, Mariposa Co., CA.**

markers across their entire length. Transmission ratio distortion was moderate, with typical markers segregating 59:108:21 (60% CARD) on LC1, 82:91:16 on LC3 (67% CARD), and 75:100:13 (66% CARD) on LC6. Smaller regions on LC2 and LC4 also exhibited excess CARD transmission. To further investigate the pattern of transmission ratio distortion, we also genotyped markers ($N = 12$) targeted to distorted and compressed genomic region in a set of four reciprocal backcross hybrids ($n = 192$ each) using the same parental lines and grown simultaneously (Supplementary File S1). Patterns of distortion are complicated in the backcrosses, but suggest that loci underlying postmating barriers such as conspecific pollen precedence and Dobzhansky–Muller interactions affecting hybrid viability may map to regions with putative *M. cardinalis-M. lewisii* rearrangements (see Supplementary File S1, Fig. S1). However, the widespread suppression of recombination in the LC map prevents mechanistic dissection of the sources of TRD (see Fishman et al. 2008) throughout much of the hybrid genome.

FLORAL TRAITS—QTL MAPPING

As in previous studies, a few QTLs with major effects influenced each floral trait (Table S1; Fig. 2). In a few cases, it appeared (based on a > 2 LOD drop between QTL peaks) that there were multiple QTLs for a trait on a single linkage group. Given the difficulty in ordering markers in compressed/rearranged regions, it is likely that these drops represent signal quality variation across a single QTL rather than true multiple linked QTLs. In most cases, QTL effect sizes and dominances were consistent across the gaps; therefore, we identified a single QTL per linkage group, as in previous studies. With one exception, corolla length QTL on LC6, all floral QTLs had effects consistent with the differences between the parental lines. The carotenoid locus *YUP*, which was perfectly linked to marker MgSTS.472 on LC4, explained the presence of orange pigment (all red- and orange-flowered plants = *M. cardinalis* homozygotes). We detected two QTLs for anthocyanin (purple pigment) intensity, one coincident with *YUP* on LC4 and one on LC6. In combination with *YUP*, the latter explained all orange versus red flowers, and contributed to the intensity of pink color. That is, *yup*/*yup* individuals that were also *M. cardinalis* homozygotes at this QTL were all red, and all individuals that were *M. lewisii* homozygotes at the QTL were either orange or light pink. The anthocyanin QTL on LC4 appeared to act in a *M. lewisii*–recessive fashion, with heterozygotes resembling *M. cardinalis* homozygotes, but had a relatively small additive effect. We identified three QTLs for flowering time, which together account for ~85% of the >2-week difference in flowering (LEW earlier) between the parental lines grown under common greenhouse conditions.

HYBRID MALE STERILITY—QTL MAPPING AND SINGLE MARKER ANALYSES

Using CIM and MIM, we detected QTLs for pollen fertility on three linkage groups, LC1, LC2, and LC6/7, each of which also exhibited recombination suppression in the QTL region (Fig. 2). To refine these QTLs, we then examined pairwise interactions among the three QTLs using all markers within 10 cM of each initial peak (equivalent to a 2D scan). When interactions were explicitly included in the ANOVA model, the most strongly significant markers on LC1 and LC2 were at the CIM QTL peaks, but the LC6/7 QTL effect was strongest at MgSTS.305 rather than MgSTS.353. We then conducted ANOVA using representative markers on each linkage group (MgSTS.243, MgSTS.491,

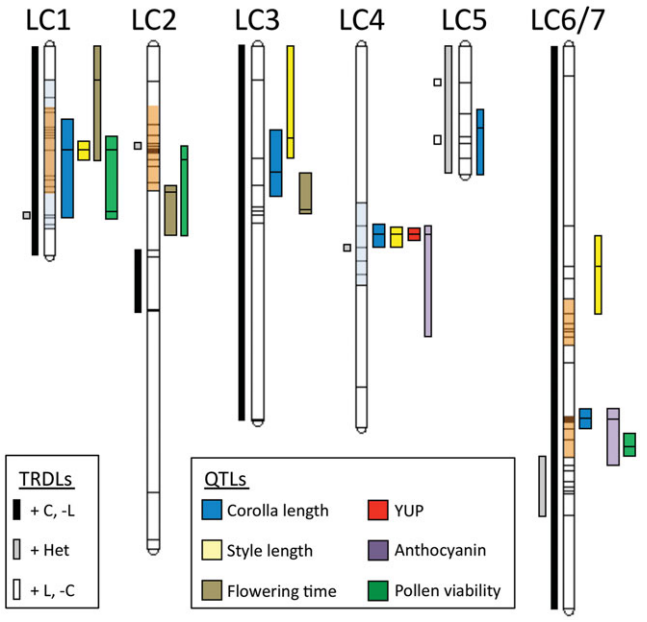

Figure 2. Quantitative trait loci (QTLs) and regions of significant transmission ratio distortion in the LEW × CARD F_2 map. Regions of each linkage group with putative rearrangements are broadly indicated by shading on the map (orange for *Mimulus cardinalis*-specific; blue for *Mimulus lewisii*-specific, including the LC4 inversion identified in Pince 2009). The two suppressed regions on LC6/7 are treated as a single putative reciprocal translocation in the text, but could represent two separate phenomena. For QTLs, each colored box represents a 1 LOD drop interval on either side of the QTL peak (center line). Two lines are shown for the location of the pollen viability QTL on LC1, as there were two equal peaks separated by a high ridge (likely due to nonlinearity of marker genotypes in this putatively rearranged region). The width of the box indicates whether a QTL was deemed major (> 20% of parental difference: wide) or minor (narrow). See Table S2 for details of QTL detection. Black, gray, and open bars to the left of the linkage groups indicate regions of transmission distortion with excess CARD alleles (+C, −L), heterozygote excess (+HET, −HOM), or excess LEW alleles (+L, −C), respectively. Tests for distortion are described in the text. See Supplementary File S1 and Figure S1 for more information on sources of transmission ratio distortion in *M. lewisii* × *M. cardinalis* hybrids.

MgSTS.305 for LC1, LC2, and L6/7, respectively) plus interactions. In the full model ($F_{18, 155} = 10.8$, $P < 0.001$), the QTLs on LC1 and LC6/7 were both highly significant ($P < 0.005$), but the LC2 QTL did not have a significant main effect ($P = 0.07$). However, the LC2 QTL interacted significantly with each of the others ($P < 0.05$ for both interaction effects). Individually, both the LC1 and LC6/7 pollen sterility QTLs exhibited low heterozygote fertility or underdominance (Fig. 3). For LC6/7, this effect was further enhanced by a strong interaction with the LC2 QTL, such that H/C genotypes were half as fertile as other heterozygotes at LC6. In contrast, the LC1–LC2 interaction appeared to be a recessive–recessive Dobzhansky–Muller incompatibility on top of the underdominant LC1 effect, with L/C individuals about half as fertile as either conspecific combination.

Discussion

Comparative linkage mapping revealed substantial suppression of recombination in hybrids between the monkeyflowers *Mimulus lewisii* and *M. cardinalis*, suggesting that multiple chromosomal rearrangements distinguish sympatric populations of these taxa. This finding has important implications for understanding the genetic architecture and evolutionary history of species differences and barriers in this model system. Major QTLs for both pre- and postmating species barriers were located in putatively rearranged genomic regions, including all three QTLs underlying hybrid male sterility. Two of the sterility QTLs had underdominant effects consistent with heterokaryotypes directly causing gamete dysfunction, but genic factors also contributed to F_2 sterility. Overall, our results fit models in which rearrangements are favored by selection during "divergence-with-gene-flow" and suggest a complex process of speciation.

IDENTIFICATION OF REARRANGEMENTS BETWEEN *M. LEWISII* AND *M. CARDINALIS*

The suppression of recombination we found in *M. lewisii* × *M. cardinalis* hybrids was not subtle. Despite an explicit effort to obtain markers spanning the entire *Mimulus* genome, we resolved six robust linkage groups representing the eight chromosomes of each parental species, and half of the groups exhibited marker clustering relative to the *M. parishii* × *M. lewisii* map. We infer that at least three major rearrangements—an LC2 inversion, an LC6/7 reciprocal translocation, and either an inversion or reciprocal translocation on LC1—or many smaller rearrangements, likely occurred in the *M. cardinalis* lineage to produce this pattern. In addition, the lack of one linkage group in both interspecific maps suggested an additional translocation specific to the *M. lewisii* lineage; this inference was supported by the observation that markers tightly linked in LC/LP1 became completely unlinked in *M. cardinalis* meioses (Table 1). The putative *M. cardinalis* rearrangements inferred on LC1 and LC2 were also supported by increased pairwise recombination values in *M. cardinalis* meioses (Table 1). The putative LC6/7 *M. cardinalis* reciprocal translocation identified in this study was also detected in a study comparing an LC hybrid map with an intraspecific *M. lewisii* map, as was an *M. lewisii*–specific major inversion containing the YUP region of LC4. If we add the LC4 inversion reported by Pince (2009) to the rearrangements identified in this study, a minimum of five major chromosomal rearrangements appear to distinguish these closely related sister taxa (locations roughly indicated in Fig. 2). This finding is consistent with previous evidence of marker

clustering and tightly linked major QTLs for developmentally unrelated traits in *M. lewisii* × *M. cardinalis* hybrids (Bradshaw et al. 1995, 1998). However, because Hiesey et al. (1971) reported no cytological evidence of chromosomal divergence between Sierran *M. lewisii* and *M. cardinalis*, this oligogenic genetic architecture has previously been interpreted strictly as evidence of rapid ecological speciation, with no reference to chromosomal divergence. Our finding of multiple rearrangements containing major QTLs for both pre- and postmating barriers likely distinguish *M. cardinalis* and *M. lewisii* suggests a more complex scenario in which both ecological selection and genetic events play important roles.

Before considering the evolutionary implications of rearrangements associated with species barriers, however, it is important to consider alternative sources of recombination suppression in our LC map. First, we can dismiss strictly technical sources for map differences, as the mapping populations share an inbred line parent, a set of highly informative, codominant gene-based markers chosen to represent the entire *Mimulus* genome, and identical analysis methods. Second, strong transmission ratio distortion can produce statistical associations between physically unlinked markers and may slightly decrease map distances, but is usually too weak to strong pseudo-linkage in an F_2 cross (Lorieux et al. 1995), as we observed in the LC cross described here. Third, it is theoretically possible that strong selection against particular recombinant genotypes could account for the observed patterns of recombination suppression. However, for those regions exhibiting both suppression and underdominant F_1 hybrid sterility (e.g., LC6/7 and LC1), we would need to invoke a very unusual symmetrical Dobzhansky–Muller haploid incompatibility killing both male and female gametes (many LC and CL recombinant gametes dead) to produce the same effects as, for example, a simple reciprocal translocation. For regions without F_1 infertility QTLs, we would need to invoke even more genetically complex sets of F_2 lethals or other incompatibilities to produce the observed >2-fold reduction in recombination. In addition, patterns of distortion and recombination across reciprocal backcross populations (described in Supplementary File S1) suggest that suppression of recombination is not dependent on the particular cross (as zygotic interactions would be) or associated with particular patterns of transmission ratio distortion. In the absence of cytogenetic evidence of rearrangements, however, it remains possible that other mechanisms explain the suppression of recombination in LC hybrids, particularly for regions (such as LC2) where recombination appears reduced but not completely eliminated. However, because rearrangements (including both translocations and inversions) are the most plausible explanation for the widespread suppression of recombination and fertility effects we observe in *M. lewisii* × *M. cardinalis* hybrids, we focus on them in the following discussion of the evolutionary causes and consequences of recombination suppression in interspecific hybrids.

The question remains of why Hiesey et al. (1971) did not detect visible cytogenetic evidence of rearrangements (i.e., inversion loops or chromosomal rings caused by translocations) in hybrids of Sierran *M. lewisii* and *M. cardinalis*. They readily identified major translocations between the Northern and Sierran races of *M. lewisii* (which are completely incompatible), as well as rearrangements distinguishing Arizona *M. cardinalis* from Sierran *M. cardinalis* and *M. lewisii*. This suggests either that the rearrangements inferred in this study were difficult to detect cytogenetically or that the *M. cardinalis* and *M. lewisii* accessions used in this and previous QTL studies are karyotypically distinct from some others in the Sierras. The chromosomal divergence between geographical races in both species makes polymorphism plausible; however, the sympatric site from which our lines were derived is very close to Hiesey et al.'s (1971) Yosemite area populations. Crosses using plants from widespread populations of *M. cardinalis* find that putatively rearranged regions remain equally suppressed regardless of the particular accession used (A. Stathos and L. Fishman, unpubl. data). However, additional crosses, as well as modern cytogenetic studies in the *M. lewisii* group, will be necessary to resolve this apparent contradiction and define any rearrangements karyotypically.

ASSOCIATION OF HYBRID STERILITY WITH PUTATIVE REARRANGEMENTS

Hybrid sterility QTLs were exclusively found in putatively rearranged regions (Fig. 2) and the two largest (on LC1 and LC6/7) were strongly underdominant. The third sterility QTL (on LC2) had little effect alone, but interacted epistatically with the other two QTLs (Fig. 3). Rearrangement-associated underdominance contrasts with the genetic basis of male sterility in hybrids of *Mimulus guttatus* and *M. nasutus*, which involves nuclear Dobzhansky–Muller incompatibility loci (Fishman and Willis 2001; Sweigart et al. 2006) or cyto-nuclear interactions (Fishman and Willis 2006; Barr and Fishman 2010), and affects only a small fraction of F_2 hybrids. In addition, segregating inversions within *M. guttatus* (Scoville et al. 2009; Lowry and Willis 2010) do not have underdominant effects on fitness. However, rearrangement-associated sterility in *Helianthus* (Rieseberg et al. 1995; Lai et al. 2005) and in sympatric pairs of *Drosophila* (Noor et al. 2001; Brown et al. 2004) is also underdominant. Whether rearrangements with underdominant effects are more likely to be involved in speciation, as opposed to being associated with intraspecific polymorphism or ecotypic differentiation, remains an open question.

Structural heterozygosity per se may explain the majority of LEW × CARD hybrid male sterility, as heterozygosity at the underdominant LC1 and LC6/7 QTLs together was sufficient to reduce F_2 pollen viability to F_1 levels (< 0.4: this study; Ramsey et al. 2003). Both of these QTLs occur in regions that appear to be reciprocally translocated (LC1 and LC6/7), but could also involve

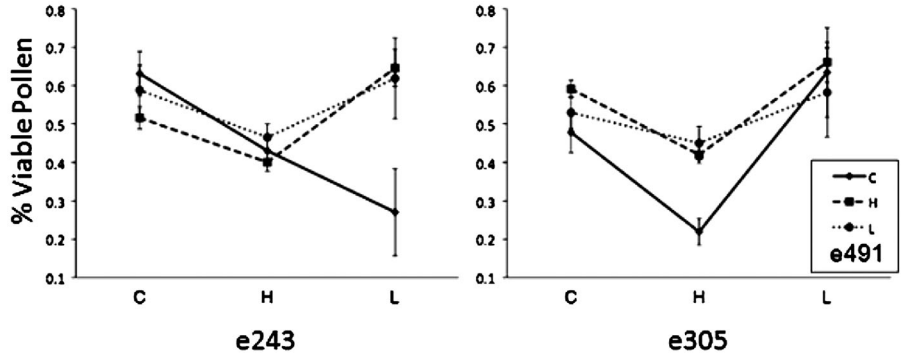

Figure 3. Least square means (± 1 SE) of percentage of pollen viability from analysis of variance, showing the underdominant effects of hybrid sterility quantitative trait loci (QTLs) on LC6 (MgSTS.305) and LC1 (MgSTS.243), and their interaction with the QTL on LC2 (MgSTS.491). e-prefix on marker names = MgSTS. Letters indicate genotypic categories: L = *Mimulus lewisii* homozygote at the marker, H = heterozygote, and C = *M. cardinalis* homozygote. See text for statistical analyses.

nested inversions or other factors that suppress recombination. Translocation heterozygotes commonly display semisterility due to the production of 50% unbalanced meiotic products (Stebbins 1958). As it is clear that genic factors on LC2 play a role in hybrid infertility, the LC1 and LC6/7 sterility QTLs could also contain genic factors that have been preferentially preserved in rearranged regions (Noor et al. 2001; Rieseberg 2001). Secondary contact between species divergent in both chromosome structure and genic incompatibilities will purge incompatibility alleles in collinear regions, but will preserve incompatibility alleles linked in repulsion within alternative rearrangements, causing apparent underdominance (Noor et al. 2001). Additional incompatibilities may accumulate in the rearranged regions (Navarro and Barton 2003). Thus, the underdominant LC1 and LC6/7 sterility QTLs, as well as the LC2 QTL, could represent multiple linked genic factors within a rearranged region.

The genetic basis of underdominant hybrid sterility (chromosomal vs. genic) is important: if the putative LC1 and LC6/7 translocations directly cause the sterility of hybrids, they should have been strongly disfavored when they first occurred in an ancestral karyotypic background, and strong drift or positive selection would be necessary to explain their presence in current populations (Walsh 1982). These possibilities can be distinguished with a classic test: artificial polyploidization of F_1 hybrids should eliminate sterility due to chromosomal rearrangements per se, as tetraploid hybrids can preferentially pair collinear homologues and thus avoid meiotic issues (Stebbins 1958). Preliminary analyses indicate that artificial polyploidy completely restores *M. lewisii* × *M. cardinalis* F_1 pollen fertility (A. Stathos and L. Fishman, unpubl. data), supporting the inference that rearrangements directly cause both the recombination suppression and underdominant pollen sterility of F_1 hybrids. Drift alone may explain the existence of such underdominant rearrangements differentiating *M. cardinalis* and *M. lewisii*, as both species can self-fertilize and may undergo frequent bottlenecks due to their ephemeral riparian habitat. However, drift should be strongest in highly selfing taxa, and we detected no recombination suppression unique to *M. parishii*, an exclusive selfer. Thus, selection for suppressed recombination (Kirkpatrick and Barton 2006; Feder et al. 2012) may have been important in the spread and establishment of multiple *M. cardinalis–M. lewisii* rearrangements, including those with underdominant effects on hybrid fertility.

ASSOCIATIONS OF PREMATING BARRIERS WITH PUTATIVE REARRANGEMENTS

Consistent with previous QTL maps, a small number of QTLs, often with major effects, were detected for each trait. Furthermore, QTLs for diverse traits associated with species differentiation often overlapped in their genomic location and were highly coincident with inferred rearrangements (Fig. 2). The concentration of species-diagnostic QTLs is almost certainly even stronger than this study alone shows, as several important traits that we did not measure are strongly genetically correlated with those we did measure (Hiesey et al. 1971; Bradshaw et al. 1995, 1998). For example, Bradshaw et al. (1995) mapped single major (20–60% PVE) QTLs for nectar volume and nectar concentration that were coincident with each other, explain important aspects of pollinator choice (Bradshaw and Schemske 2003), and were also coincident with major QTLs for elevational adaptation (H. D. Bradshaw, Jr., pers. comm.). These nectar and elevational adaptation QTLs are most closely associated with MgSTS.273 (Y. Yuan and H. D. Bradshaw, Jr., pers. comm.) in the putative inversion on LC2 that contains flowering time and genic hybrid sterility QTLs. Although we did not detect suppression of recombination on LC4, where major flower size (corolla and style length) QTLs colocalize with YUP, previous comparisons of an intraspecific *M. lewisii* map with LEW × CARD maps demonstrated that YUP is contained in a region of strongly suppressed hybrid recombination,

most likely due to a *M. lewisii*–specific inversion (Pince 2009). Thus, rearrangement may have played a role in the evolutionary dynamics of this classic Mendelian speciation gene (Bradshaw et al. 1995). Multiple major QTLs for premating isolation traits lie in each putatively rearranged region and multiple genes affecting the same trait may also be linked within rearrangements, potentially contributing to the large effect size of QTLs for some traits. These striking genetic associations place strong constraints on the phenotypic combinations and fitness of both natural and experimental hybrids, and their packaging in regions of strongly suppressed recombination is consistent with a history of divergent selection in the face of gene flow (Kirkpatrick and Barton 2006; Feder et al. 2011).

Conclusions

Mimulus lewisii and *M. cardinalis* are classic examples of speciation by a jump between distinct adaptive peaks. We suggest that this peak shift was achieved not solely by the fixation of a few single major genes that largely precluded further hybridization, as previously argued, but through interactions between divergent selection and ongoing gene flow in a heterogeneous environment. Chromosomal rearrangements (or possibly other mechanisms causing severe suppression of recombination) appear to have facilitated this process both by packaging adaptive genes together and by generating strong post-zygotic barriers. When the speciation process is near completion (as between *M. cardinalis* and *M. lewisii*), determining the underlying molecular and ecological mechanisms can be difficult from genome scans alone (e.g., Guerrero et al. 2011; Strasburg et al. 2011; Feder et al. 2012). Thus, in addition to enhancing our understanding of a textbook example of ecological speciation, this study sets the stage for integrative investigations of how recombination suppression, postzygotic barriers, and ecological selection interact to generate divergence genome-wide.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Table S1. Transmission ratio distortion for mapped LCF_2 markers.

Figure S1. Transmission ratio distortion at representative markers in F_2 hybrids and reciprocal backcross populations.