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A Simple Genetic Incompatibility Causes Hybrid Male Sterility in Mimulus

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ABSTRACT

Much evidence has shown that postzygotic reproductive isolation (hybrid inviability or sterility) evolves by the accumulation of interlocus incompatibilities between diverging populations. Although in theory only a single pair of incompatible loci is needed to isolate species, empirical work in Drosophila has revealed that hybrid fertility problems often are highly polygenic and complex. In this article we investigate the genetic basis of hybrid sterility between two closely related species of monkeyflower, *Mimulus guttatus* and *M. nasutus*. In striking contrast to Drosophila systems, we demonstrate that nearly complete hybrid male sterility in Mimulus results from a simple genetic incompatibility between a single pair of heterospecific loci. We have genetically mapped this sterility effect: the *M. guttatus* allele at the *hybrid male sterility 1* (*hms1*) locus acts dominantly in combination with recessive *M. nasutus* alleles at the *hybrid male sterility 2* (*hms2*) locus to cause nearly complete hybrid male sterility. In a preliminary screen to find additional small-effect male sterility factors, we identified one additional locus that also contributes to some of the variation in hybrid male fertility. Interestingly, *hms1* and *hms2* also cause a significant reduction in hybrid female fertility, suggesting that sex-specific hybrid defects might share a common genetic basis. This possibility is supported by our discovery that recombination is reduced dramatically in a cross involving a parent with the *hms1–hms2* incompatibility.

IN the classic model of allopatric speciation, a single species splits into two or more geographically isolated populations that thereafter diverge independently. Integral to the completion of this process is the evolution of reproductive isolation among nascent species, which is essential to prevent gene exchange upon secondary contact. Complete isolation may be caused by any combination of reproductive barriers, including hybrid inviability or sterility. Although Darwin and his contemporaries were well aware of the propensity for interspecific hybrids to be inviable or sterile, they were naïve of genetics and thus could not conceive how such inherently maladaptive traits might evolve. The key insight of the genic model of postzygotic isolation, proposed independently by Bateson (1909), Dobzhansky (1937), and Muller (1942) (commonly known as the Dobzhansky–Muller model), was that epistasis among two or more genes allows hybrid inviability or sterility to evolve without reducing the fitness of either ancestral lineage. In this model, alternate multilocus allele combinations evolve among geographically isolated populations, and inviability or sterility occurs only when novel incompatible genotypes come together in hybrids.

Soon after its conception, strong evidence for the Dobzhansky–Muller model of postzygotic isolation emerged from classical genetic demonstrations of hybrid incompatibilities in animals (*e. g.*, Phillips 1921; Bellamy 1922; Dobzhansky 1937; Cole and Hollander 1950) and plants (*e. g.*, Hollingshead 1930; Hutchinson 1932; Clausen *et al.* 1940, 1941; Babcock *et al.* 1942; Avers 1953). Recent years have seen resurgence in speciation research, accompanied by a directed effort to genetically map factors that contribute to hybrid incompatibilities (Hollocher and Wu 1996; True *et al.* 1996; Li *et al.* 1997; Harushima *et al.* 2001, 2002; Presgraves 2003; Tao *et al.* 2003b; Moyle and Graham 2005). A few studies have even identified the genes that cause hybrid inviability and sterility (Wittbrodt *et al.* 1989; Ting *et al.* 1998; Barbash *et al.* 2003; Presgraves *et al.* 2003). To date, much of our understanding of the genetics of postzygotic isolation is based on empirical studies of divergence between Drosophila species. Indeed, several patterns appear to characterize the genetic basis of hybrid incompatibility in Drosophila (reviewed in Coyne and Orr 2004): (1) hybrid incompatibility alleles are generally recessive (Presgraves 2003; Tao *et al.* 2003a,b; Tao and Hartl 2003), (2) hybrid male sterility is highly polygenic and complex (Davis and Wu 1996; Tao *et al.* 2003b), and (3) hybrid male sterility evolves more readily than female sterility or hybrid lethality (Hollocher and Wu 1996; True *et al.* 1996; Sawamura *et al.* 2000; Tao *et al.* 2003a).

There is some evidence that other biological groups do not adhere strictly to the patterns that characterize Drosophila systems. In plants, for example, dominant hybrid incompatibility alleles are not uncommon (*e.g.*,

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Hollingshead 1930; Stephens1946; Macnair and Christie 1983; Christie and Macnair 1984; Kubo and Yoshimura 2005; but see Moyle and Graham 2005). In addition, the genetic complexity that typifies Drosophila hybrid male sterility is not necessarily mirrored in other systems. Remarkably, hybrid sterility between varieties of cultivated rice, *Oryza sativa*, is often genetically simple (Oka 1974; Liu *et al.* 1997; Kubo and Yoshimura 2002; Kubo and Yoshimura 2005). It also is not clear that hybrid male sterility should always evolve more readily than female sterility or lethality. In Drosophila and other Dipteran systems, the greater abundance of hybrid male sterility factors relative to the number of hybrid female sterility or hybrid lethality factors usually has been attributed to the accelerated evolution of male traits via sexual selection or sexual conflict (Hollocher and Wu 1996; True *et al.* 1996; Presgraves and Orr 1998; Michalak and Noor 2003; Tao *et al.* 2003a; Tao and Hartl 2003). But what about evolutionary rates of incompatibility alleles in species that do not have genetic sex determination or separate sexes? In organisms that experience minimal sexual selection or sexual conflict, might male and female functions be equally vulnerable to genetic incompatibilities? General answers to such fundamental questions about species divergence await empirical studies in biologically diverse taxa.

Here we examine the genetic basis of hybrid incompatibility between two closely related species of yellow monkeyflower. *Mimulus guttatus* is a predominantly outcrossing plant species with showy, insect-pollinated flowers, and *M. nasutus* is a self-fertilizing species with small, often cleistogamous flowers. Natural populations of *M. guttatus* are abundant throughout western North America, occupying diverse ecological habitats. The distribution of *M. nasutus* overlaps broadly with that of *M. guttatus*, although its range is more restricted. The two species most often occur in allopatry, although sympatric populations are common in some geographic regions. Prezygotic barriers to interspecific crossing include species differences in floral morphology (Ritland and Ritland 1989; Dole 1992), flowering phenology (N. Martin, unpublished results), and pollen–pistil interactions (Kiang and Hamrick 1978; Diaz and Macnair 1999). Nevertheless, when populations of *M. guttatus* and *M. nasutus* occur in sympatry, hybrids frequently are observed (Vickery 1964, 1978; Kiang and Hamrick 1978; Ritland 1991; Fenster and Ritland 1992). Moreover, there is evidence for historical and ongoing introgression at nuclear loci in some areas of the shared range (Sweigart and Willis 2003.). Yet, because the two species maintain distinct phenotypes even in sympatric sites, genomewide interspecific gene flow seems unlikely. Indeed, postzygotic reproductive barriers are common, although their effects may vary among populations of *M. guttatus* and *M. nasutus* (Vickery 1978; Fishman and Willis 2001).

Previously we showed that hybrids from an interspecific cross between two allopatric populations of *M. nasutus* and *M. guttatus* suffer a marked reduction in male and female fertility relative to parental lines (Fishman and Willis 2001). Moreover, we observed a novel class of completely male-sterile individuals in the F_2 generation. This pattern is consistent with the segregation of Dobzhansky–Muller incompatibility factors that negatively affect male fertility. We hypothesized that the complete hybrid male sterility of some Mimulus F_2 hybrids might be governed by a relatively simple genetic incompatibility. At the time of our previous study, however, we had little power to test such a prediction because the number of available codominant genetic markers was insufficient to map the epistatic factors. We have now developed hundreds of codominant markers, affording us much greater genetic resolution.

In this article we examine the genetic basis of hybrid male sterility in Mimulus. Because even completely male-sterile hybrids are at least partially female fertile (see Fishman and Willis 2001 and results), they can be outcrossed using pollen from male-fertile lines. To achieve a broader understanding of the genetics of hybrid incompatibility, we have characterized the number, mode of action, and phenotypic effects of loci that cause male sterility in Mimulus hybrids. This investigation allows us to compare the genetic basis of hybrid incompatibility in Mimulus with that of other biologically distinct taxa, particularly well-studied Drosophila species.

MATERIALS AND METHODS

Mimulus lines and genetic crosses: To study the genetics of hybrid sterility we performed crosses between inbred lines of two closely related species of Mimulus, the predominantly outcrossing *M. guttatus* and the highly self-fertilizing *M. nasutus*. We intercrossed the same inbred parental lines that were used previously by Fishman and Willis (2001). The *M. guttatus* parental line (IM62), derived from the well-studied Iron Mountain population in the Oregon western Cascades, is highly inbred and was formed by more than six generations of selfing with single-seed descent. The *M. nasutus* parental line (SF5) originated from the Sherar's Falls population in central Oregon and has been maintained in the greenhouse for more than ten generations by autonomous self-fertilization. These two populations are allopatric, separated by ~120 km.

All plants were grown using similar conditions. Individual seeds were planted in 2.25-in. pots filled with soilless potting mix, watered, and stratified in a dark cold room (4°) for 1 week. Pots were then moved to a controlled environmental chamber with constant light and temperature (16°) for 1–2 weeks to promote germination. After germination, plants were moved to the Duke University greenhouses for subsequent growth. Greenhouse conditions included 16-hr days at 24° with supplemental high-pressure sodium lights and 8-hr nights at 16°.

Our first step toward a more detailed genetic characterization of Mimulus hybrid sterility was to generate backcross populations, along with parental, F_1, and F_2 hybrid lines. We formed F_1 hybrids by intercrossing *M. nasutus* (SF5, maternal parent) with *M. guttatus* (IM62, paternal parent), and then

self-fertilized a single F_1 to form the F_2 generation. In addition, we backcrossed F_1 hybrids to *M. guttatus* (BG_1) and *M. nasutus* (BN_1) using the parental lines as pollen donors. Because *M. nasutus* was used as the original maternal parent, all hybrid progeny contained *M. nasutus* cytoplasm. We grew the parental, F_1, F_2, BG_1, and BN_1 lines together in a common garden. To minimize environmental effects, plants were grown in a completely closed greenhouse unit in the Duke University Phytotron.

As part of an ongoing experiment to investigate genome-wide patterns of loci that contribute to species divergence, we have generated several hundred nearly isogenic lines (NILs) (see Fishman and Willis 2005). The NILs descend from replicate BN_1 [(SF5 × IM62) × SF5] plants derived from the same F_1 cross and replicate BG_1 [(IM62 × SF5) × IM62] plants derived from the same F_1 cross. Individuals from the BN_1 population (initially, $N > 500$) were backcrossed using pollen from the recurrent parent (*M. nasutus* SF5) and maintained by random single-seed descent to form a BN_4 population. Likewise, individuals from the BG_1 population (initially, $N > 500$) were backcrossed using pollen from the recurrent parent (*M. guttatus* IM62) and maintained by random single-seed descent to form a BG_4 population. Each independent, fourth-generation NIL has a unique set of heterozygous introgressions embedded in a genome that is expected to be 93.75% homozygous for parental alleles. We measured male fertility for BN_4 and BG_4 NILs that were grown in common garden experiments at the Duke University Research greenhouse.

To explore the genetic basis of Mimulus hybrid male sterility, we made several additional crosses, the details of which are provided in results.

Fertility assessments: The measure of male fertility used for this study was the proportion of viable pollen grains per flower. For each plant, we collected all anthers from the third and fourth flowers, suspended the pollen from each flower separately in 60 μl of aniline blue-lactophenol stain (Kearns and Inouye 1993), and visualized pollen grains using a compound microscope. To estimate pollen viability for each flower, we determined the proportion of viable (darkly stained) pollen grains in a sample of 100 that was haphazardly selected. Our estimate of male fertility was an average of the proportion of viable pollen grains measured for the third and fourth flowers. In crosses that segregated two discrete classes of completely male-fertile and -sterile progeny, self-fertilizing lines were simply examined for the presence or absence of swollen (*i.e.,* self-fertilized) fruits.

Our measure of female fertility for an individual was the number of seeds produced after hand pollination of the fifth flower with pollen from the recurrent parent, SF5. We used this highly fertile pollen source (see results and Figure 1) to ensure that differences in seed production were due to variation in ovule production or seed provisioning rather than variation in pollen quality. To prevent self-fertilization, we emasculated experimental flowers prior to hand pollination.

Molecular analyses: Genomic DNA was isolated from bud tissue using a modified hexadecyl trimethyl-ammonium bromide chloroform extraction protocol (Kelly and Willis 1998). Most of the markers used in this study were MgSTS markers, which are length polymorphisms in intronic regions of single-copy nuclear genes (Table 1) (Fishman and Willis 2005; Hall and Willis 2005), but we also used a few microsatellites and AFLPs (Fishman *et al.* 2001). All markers were amplified using standard conditions (90 sec at 94°, followed by 30 cycles of 40 sec at 92°, 1 min at 52°, and 40 sec at 72°). PCR reactions were performed using 10 ng of genomic DNA as template and were supplemented with 3mm $MgCl_2$. All marker genotyping was performed by sizing PCR-amplified DNA fragments with an incorporated 5′ fluorescent-labeled primer on ABI 3700 or 3100 automated capillary sequencers (Applied Biosystems, Foster City, CA). Marker genotypes were assigned automatically using the programs Genotyper or Genemapper (both from Applied Biosystems) and then verified by eye.

Genetic mapping and QTL analyses: We mapped several new codominant MgSTS markers to a preexisting *M. nasutus*–*M. guttatus* linkage map (Fishman *et al.* 2001) by genotyping a subset of the 2001 F_2 mapping population ($N = 288$). We constructed genetic linkage maps with MAP-MAKER 3.0 (Lander *et al.* 1987) using the same grouping and ordering parameters as in previous studies (Fishman *et al.* 2001). QTL analyses were performed using composite interval mapping in Windows QTL Cartographer V 2. Thresholds for QTL detection were set by permutation (experimentwise $P = 0.05$, $N = 500$ permutations).

RESULTS

Pattern of male sterility in early generation hybrids: To examine the genetic basis of Mimulus hybrid male sterility, we intercrossed *M. nasutus* and *M. guttatus* and compared pollen viabilities among F_1, F_2, BN_1, BG_1, and parental classes (Figure 1). Parental lines were almost completely male fertile (*M. guttatus*: mean = 0.949, SE = 0.005, $N = 56$; *M. nasutus*: mean = 0.961, SE = 0.004, $N = 98$) with only 3% of individuals displaying pollen viabilities <0.85. Pollen viability in the F_1 hybrids (mean = 0.509, SE = 0.014, $N = 96$) was reduced by 47% relative to the mid-parent value. By comparison, the F_2 hybrid class was more male fertile (mean = 0.672, SE = 0.013, $N = 388$) and thus had a less severe reduction in mean pollen viability (30% relative to the mid-parent value). Average male fertility was uniformly higher across all parental and hybrid classes than in our previous experiment (see Figure 1 in Fishman and Willis 2001), presumably as a consequence of minimizing environmental effects.

The F_2 hybrids included a novel class of individuals that produced few or no viable pollen grains (male fertility < 0.10 in ~6% of the F_2). Highly male-sterile individuals were even more common in the BN_1 population (male fertility < 0.10 in ~19% of the BN_1). In contrast, no highly male-sterile plants were observed among the BG_1 hybrids. The implications of these results are twofold. First, *M. guttatus* has one or more alleles with dominant male sterility effects in a predominantly *M. nasutus* genetic background. The fact that a substantial proportion of the BN_1 individuals were male sterile suggests that the number of loci with *M. guttatus* incompatibility alleles is small, perhaps one or two. Second, hybrid incompatibility alleles from *M. nasutus* must be homozygous to cause complete male sterility because of the lack of complete male sterility observed in the F_1 and BG_1 hybrids.

We predicted that self-fertilizing male-fertile BG_1 individuals should generate some highly sterile male progeny (*i.e.*, selfing should produce some individuals that are homozygous for *M. nasutus* incompatibility alleles). Indeed, when we selfed several of the fertile BG_1 progeny we discovered that ~50% of families contained

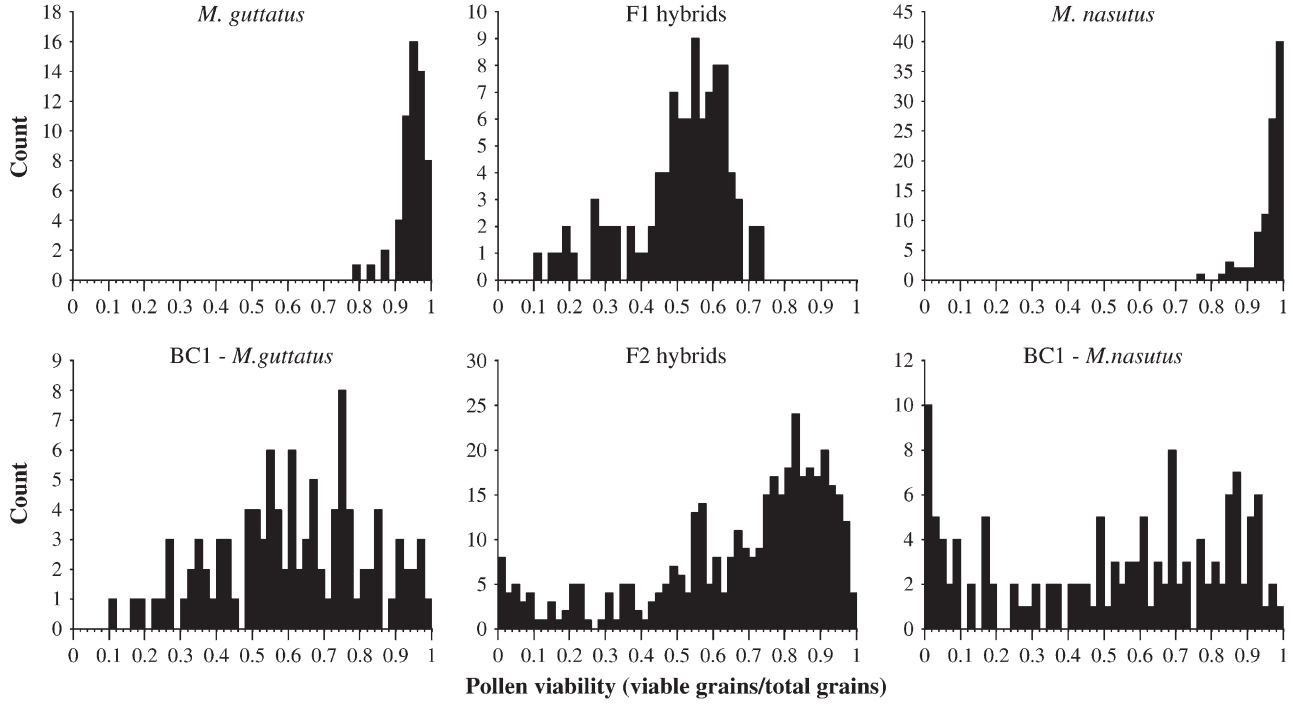

FIGURE 1.—Histograms of pollen viability (proportion viable pollen grains, averaged between two flowers per individual) in parental *M. guttatus* and *M. nasutus* lines ($N = 56$ and 98, respectively), F_1 hybrids ($N = 96$), F_2 hybrids ($N = 388$), *M. guttatus*-backcross (BG_1) lines ($N = 103$), and *M. nasutus*-backcross (BN_1) lines ($N = 133$).

individuals that were completely male sterile (10/19 families, $N = \sim 16$ per family; data not shown). This result suggests that dominant *M. guttatus* incompatibility alleles may confer complete male sterility in combination with a single additional locus that is homozygous recessive for *M. nasutus* alleles (*i.e.*, selfing uncovers recessive alleles in half of the BG_1 progeny).

Pattern of male sterility in nearly isogenic lines: To further characterize hybrid male sterility, we examined the phenotypes of NILs formed by four generations of backcrossing to *M. nasutus* (BN_4) and *M. guttatus* (BG_4). These NILs are expected to be heterozygous for introgressed heterospecific genomic regions. We reasoned that if the *M. guttatus* component of the hybrid incompatibility is caused by an allele from a single locus, then we might expect to recover some completely male-sterile BN_4 lines. Indeed, we discovered that 11 of the 184 BN_4 lines (6%) were completely male sterile. In contrast, we observed no completely male-sterile individuals in >200 BG_4 lines, providing further evidence that *M. nasutus* incompatibility alleles act recessively.

Genetic mapping of hybrid male sterility loci—*M. guttatus* component: Taken together, the crossing results suggest that complete male sterility in Mimulus hybrids might have a simple genetic basis. In fact, a two-locus dominant-recessive incompatibility appears most consistent with the phenotypic data. However, with these phenotypic results alone we cannot rule out the possibility that complex epistasis underlies a pattern of hybrid sterility that is only superficially simple.

To determine the number, location, and mode of action of Mimulus hybrid incompatibility loci, we attempted to genetically map the male sterility effects. Of course, the task of mapping individual sterility loci might be complicated by the complexity of the epistasis underlying the phenotype. Our first goal, then, was to "Mendelize" each sterility locus by generating experimental mapping populations that ideally would segregate alleles only at a single incompatibility locus against a uniform genetic background. This task proved straightforward for the dominant *M. guttatus* component, which could simply be introgressed into an *M. nasutus* genetic background by recurrent selection on the sterility phenotype and backcrossing. Using this approach we backcrossed a male-sterile individual from the BN_1 population to *M. nasutus* to form an introgression line that we refer to as RSB_1 (one generation of *r*ecurrent *s*election with *b*ackcrossing). Roughly 50% of the RSB_1 plants were highly male sterile (data not shown), a result that is consistent with a genetic model in which a single dominant incompatibility allele from *M. guttatus* causes sterility against a *M. nasutus* genetic background. We then performed an additional round of selection and backcrossing to generate an RSB_2 population, which continued to segregate 50% male steriles (data not shown).

If Mimulus hybrid male sterility requires a single dominant incompatibility allele from *M. guttatus*, we reasoned that male-sterile plants from the RSB_2 and BN_4 populations should be heterozygous for markers linked

TABLE 1

Names and primers for mapped *M. guttatus* sequence-tagged site (MgSTS) markers

Marker name	Forward primer (5′–3′)	Reverse primer (5′–3′)	Linkage group
MgSTS11	GCTCCAGATTTTCACCAAGC	ACATCCACCCTTCTGGTACG	13
MgSTS21	ACTTGTTTTCGCCAAGATGG	GTTGATGAAGAGGCAGCACA	6
MgSTS22	TGGGTGTTCAACCAAGTGAT	CCCATGCTTAAACATCATTTCA	6
MgSTS28	CGATTGTTGATGCAATCAGG	GCACGGCATACTGAAGACAA	6
MgSTS45	CTGCTGCTGCATCGAATAAA	GTCCAAATCCATCGATCCAC	13
MgSTS55	CCTCACTTCCAAGCTCCTCA	TGTGGACACAGTTTCAGCGTA	13
MgSTS58	TCAATCAGGGATGAGGAAGC	ATGGTGTCGTTTGCCTTGAT	6
MgSTS104	CCGAACCCTTACACAAGGAC	GGCACAACACAACCAAGATG	13
MgSTS105	CCCAAGCCACTTGTTGATTT	AGCCAGGACAACTTCAGTCAC	6
MgSTS120	CTTAACGGGTTCCCCAATTT	TTCCCCATATTCAGCTCCAA	6
MgSTS220	GCTCTCCACCAAAATCAACC	GCAAAGAAGGGGATGACTCC	6
MgSTS229	CCTTGTCTTCTGCCTTCACG	GGGAAATTCGATCTCACAGG	6
MgSTS314	GCGGTTTTGATTCAGATGC	TGTCCGATCATGTTTTACAAGG	6
MgSTS323	TGCAGAGAAGGAATTTCAAGG	CTCGCCTTGCTTGTTACTGC	6
MgSTS326	AGCATTGGCCATTATATCACC	GCGGACATTAACATCGAAGC	13
MgSTS388	GACAGTGGCTTCCTCTTGC	CAAGGATAAGCCTTCAATCG	10
MgSTS419	TCTGGCTGCACTTAAATATTGG	TGATGCTAGATAATCCTCTCTCG	13
MgSTS426	TGATAAACATTGCCGTTTCG	GTGCTGTGATGGATCTTTCG	6
MgSTS430	CATTGCCTTGGAGTCTCG	CCGATTAAAACCTTCTAGTGATGG	6
MgSTS456	TCCACCTCTTCAGCATCTCC	AAGTTGCTTTTGAAGTTGATGG	6
MgSTS459	CGTCTCGTTGTAGCAGTTTCC	GCGATGAAAAGCATGTTGC	6
MgSTS467	GAAACCTCCGTTTTCAGTAAGG	TGATACCCTTGAATGCATCG	6
MgSTS504a, b	CTCGAAGGTGGTGATTCAGG	GACGATGGCATCAAAGATCC	6
MgSTS508	CTGGCCACATCTCTCTTTCC	GTTGAGAACAACGAGCATGG	6
MgSTS529	GGAAGAATGTTGGGCTTGG	GTGCAGCAGAAGCAAGTACC	6
MgSTS542a	CAGTTTGAGGCCAAGAGAGC	GTGCAGGAGTTGGAGTTGG	6
MgSTS545	GGCCAATTGTTATCATCTTCC	CGCATCGTCTCCGATTCC	6
MgSTS577	GCTCATGGACTTTCAGATCTCC	TCCTTTGACCTGTGCTTTCG	13
MgSTS599	AGTAACGGGGCATGAATCG	TGTTCCCAAAGAAGTCATGG	13
MgSTS601	TCTTGGAATGAAAGCAATCG	TATTGGCTGTACACGGAACG	13
MgSTS606	TTCGTGGCAAGAGAATTAACC	GGGGAAGTGAAGTGGATGG	6
MgSTS622	GAGCAGGGGAATTGTTTGG	GCGATACGATAAGCCAGTGC	13

to the putative incompatibility locus, whereas fertile individuals should be homozygous for the *M. nasutus* allele. Moreover, genomic regions that are unlinked to the putative incompatibility locus usually should be homozygous for parental alleles (on average, the RSB_2 and BN_4 individuals are expected to be homozygous for *M. nasutus* alleles at 87.5 and 93.75% of their genomes, respectively). To identify regions of heterozygosity associated with hybrid male sterility, we performed bulked segregant analysis using the RSB_2 and BN_4 populations. We created six sets of bulked segregants, each one containing pooled DNA from several individuals to use as template in a single PCR reaction. From the RSB_2 population, we formed four sets of pooled DNA from sterile individuals, each set containing a pool of eight unique male-sterile segregants. Also from the RSB_2 population we formed one set of pooled DNA from eight fertile segregants. From the BN_4 population, we created one set of pooled DNA from three male-sterile individuals (lines 139, 164, and 204). Next, we genotyped the six sets of bulked segregants for 348 polymorphic MgSTS markers and identified several markers that appeared associated with hybrid male sterility (*i.e.*, markers that were heterozygous in one or more sets of bulked sterile segregants but homozygous for *M. nasutus* alleles in fertile segregants). Finally, to confirm that these marker–sterility associations were real, we individually genotyped eight male steriles and four male fertiles from the RSB_2 population and three male steriles from the BN_4 population. Using this approach, we detected 22 MgSTS markers that were heterozygous in most of the male steriles but homozygous in most of the male-fertile individuals (Table 1).

To identify the genomic locations of these putative sterility-associated MgSTS markers, we genotyped 288 individuals from the 2001 F_2 mapping population (Fishman *et al.* 2001). We discovered that all 22 of these sterility-associated markers map to a single linkage group (LG6) of the framework map (Figure 2, and see Fishman *et al.* 2001). In addition, by genotyping individuals from the RSB_2 population for all 22 LG6 markers, we determined that the introgression containing the incompatibility locus was confined to a relatively small

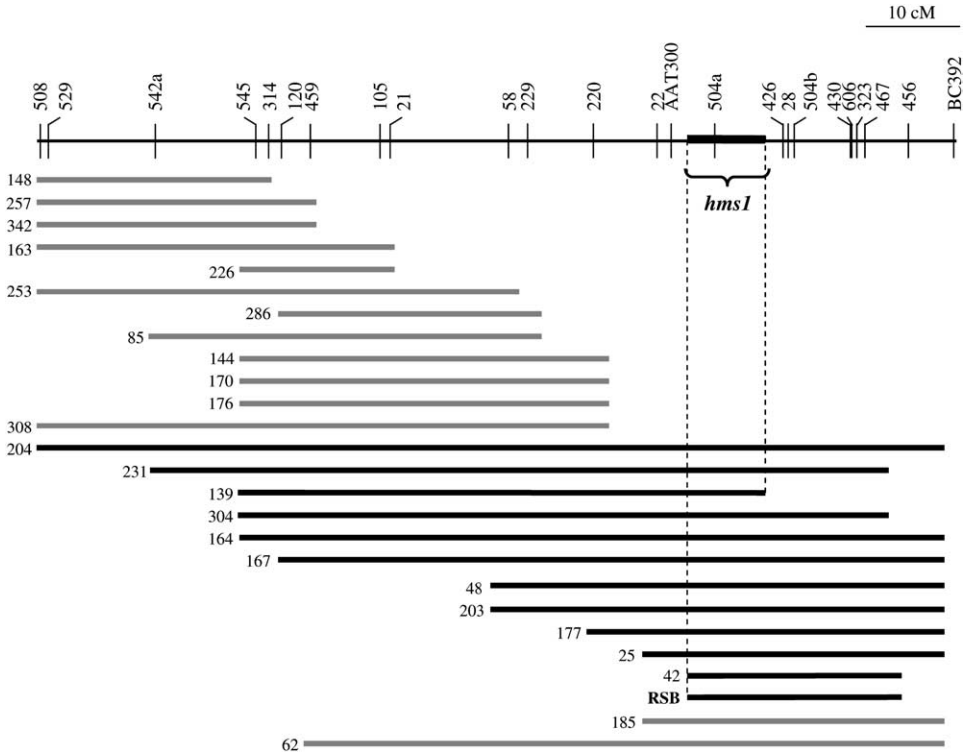

FIGURE 2.—Genetic dissection of the effect of LG6 on Mimulus hybrid male sterility using molecular markers (indicated along the top). Horizontal bars represent regions of heterozygosity for BN_4 lines (numbered bars) and for individuals from the RSB_2 mapping population. Shaded bars indicate male-fertile lines and solid bars indicate male-sterile lines. Complete hybrid male sterility maps to a locus of roughly 12 cM between AAT300 and MgSTS426. We refer to this locus as *hybrid male sterility 1* (*hms1*).

region (*i.e.*, male steriles were only heterozygous for a subset of these markers; Figure 2).

Next, we genotyped individuals from the BN_4 mapping population and found that all 11 male steriles contained regions of heterozygosity on LG6 (Figure 2). We also found 14 male-fertile BN_4 individuals with heterozygous introgressions on LG6; most of these segments mapped to one half of the linkage group. Only two of the male-fertile BN_4 individuals (62 and 185) were heterozygous for regions that overlapped with the RSB_2 introgression. Because hybrid male sterility results from interlocus epistasis, we hypothesized that the male fertility of these two individuals might be due to their having retained additional introgressions, such that they were heterozygous for an interacting incompatibility locus (see below). With these two male fertile lines excluded, the genotypes of the remaining BN_4 individuals—particularly lines 42 and 139—indicated that a hybrid male-sterility locus occurs in the interval between markers AAT300 and MgSTS426. This region corresponds to 12 cM based on the F_2 genetic map. We refer to the mapped locus as *hybrid male sterility 1* (*hms1*).

To define the *hms1* locus more precisely, we selected a male sterile individual from the RSB_2 population and backcrossed it to the recurrent parent to form a large RSB_3 mapping population. Individuals from this RSB_3 population segregated in two discrete phenotypic classes: male fertile and male sterile (0.548:0.452, $N = 2968$). Like the recurrent parent, individuals from the RSB_3 population were highly self-fertilizing and thus male-sterile plants were identified easily as those that lacked swollen (*i.e.*, self-fertilized) fruits (Figure 3). To ensure that the presence of swollen fruits was an accurate indicator of male fertility, we measured pollen viabilities for a subset of the RSB_3 population ($N = 35$). Indeed, plants with swollen fruits were highly male fertile (mean = 0.972, SD = 0.024) and plants with unfertilized fruits were male sterile (mean = 0.009, SD = 0.010).

Our expectation was that the RSB_3 population would contain a sufficient number of informative recombinants to fine-map the *hms1* locus (given sufficient

FIGURE 3.—Male-sterile (left) and -fertile (right) segregants from the Mimulus RSB_3 mapping population. Arrows indicate fruits at similar stages of development. Male-fertile plants are highly self-fertilizing and produce swollen fruits. In contrast, the ovules of male-sterile plants remain unfertilized and fruits do not swell.

TABLE 2

Genetic mapping of the *hms1* locus

RSB_3 individual[a]	504a[b]	*hms1*[c]	426[b]	28[b]	504b[b]	606[b]	323[b]	430[b]
426	N	H	H	H	H	H	H	H
463	N	H	H	H	H	H	H	H
1761	N	H	—	H	H	—	—	H
1998	N	H	—	H	H	H	—	H
2380	N	H	H	H	H	—	H	H
2684	N	H	—	H	H	—	—	H
107	H	N	—	—	N	—	N	N
1263	N	N	H	H	H	H	H	H
2519	N	N	N	N	N	H	H	H
889	H	H	—	H	H	N	—	N
1243	H	H	H	H	H	N	N	N
No. recombinants[d]	7		1	1	1	4	3	4
N[e]	1927		614	2909	1818	1455	811	2405

[a] Individuals are the recombinant progeny (between MgSTS markers 504a and 430) from the RSB_3 mapping population ($N = 2968$).

[b] Genotypes of LG6 MgSTS markers linked to *hms1*. Markers were ordered according to linkage relationships based on the F_2 mapping population. H, heterozygote; N, *M. nasutus* homozygote. Missing data are indicated by —.

[c] Inferred genotypes at *hms1*. H, male sterile; N, male fertile.

[d] Number of recombination events between MgSTS marker and hybrid sterility *hms1*.

[e] Total number of genotyped individuals.

marker density). Initially we found what appeared to be extremely tight linkage between marker MgSTS28 and *hms1*. Indeed, we only observed a single recombinant in 2909 progeny (Table 2), which implies a distance of 0.034 cM between the two loci. But as we continued to genotype the RSB_3 population for additional LG6 markers, we discovered that most of them were very tightly linked to *hms1*. In fact, several genetic markers mapped to within 1 cM of *hms1*, and some markers were completely linked to one another (Table 2). This result was completely unexpected, as the recombination distances between genetic markers estimated in this advanced generation backcross were at least an order of magnitude less than those estimated in the 2001 F_2 population (Figure 2). For example, the genetic distance between markers 504a and 504b was only 0.46 cM in the RSB_3 population (Table 2), whereas it was 8 cM in the 2001 F_2 mapping population (see Figure 2). Moreover, the greater levels of recombination among LG6 markers in the F_2 population do not represent an idiosyncratic result unique to the 2001 F_2 data set. Indeed, estimates of distances between LG6 markers based on the F_2 population that was generated for the current study (see above) are very similar to those based on the 2001 F_2 (data not shown). This unexpected discrepancy between genetic maps depending on the mapping population used suggests a dramatic suppression of recombination on LG6 in the RSB_3 population, but not in F_2 populations. Despite low recombination, the large RSB_3 mapping population allowed us to resolve the location of *hms1* to between markers MgSTS504a and MgSTS426, a region that spans 7.2 cM in the F_2 mapping population.

Genetic mapping of hybrid male sterility loci—*M. nasutus* component: Our next step was to determine whether the *M. nasutus* component of hybrid male sterility also maps to a single locus. We designed a crossing scheme to generate a mapping population that was (1) genetically uniform for the dominant *M. guttatus* allele at the *hms1* locus and (2) segregating for incompatible *M. nasutus* alleles at other loci. First we backcrossed a male-sterile individual from the RSB_3 line to *M. guttatus*. Because this plant contained a single copy of the *M. guttatus hms1* allele in a predominantly *M. nasutus* genome, we were able to generate progeny homozygous for the *M. guttatus* allele at the *hms1* locus and heterozygous at any interacting incompatibility loci. Next, by genotyping at flanking markers MgSTS504a and MgSTS504b, we selected an *hms1 M. guttatus* homozygote and backcrossed it to *M. nasutus*. This cross allowed us to form a large mapping population ($N = 940$) that was uniformly heterozygous at the *hms1* locus, but segregating at any interacting loci. We refer to this mapping population as BN_1 + *hms1*.

To determine whether hybrid male sterility is associated with *M. nasutus* alleles at a particular genetic locus, we performed bulked segregant analysis. We formed six distinct sets of pooled DNA (four male-sterile individuals each) from the BN_1 + *hms1* mapping population. Because the crossing results (see above) suggested that *M. nasutus* incompatibility alleles act recessively, our expectation was that male sterile individuals should be homozygous for genetic markers linked to the *M. nasutus* incompatibility locus. In contrast, genetic markers that are unlinked to a sterility-causing locus should have a 50% chance of being heterozygous and a 50% chance of being homozygous for *M. nasutus* alleles. We genotyped the six sets of bulked male sterile segregants for 348 polymorphic MgSTS markers and identified several markers that appeared associated with hybrid male sterility (*i.e.*, markers that were homozygous in at least one set of bulked sterile segregants).

Our bulked segregant analysis provided evidence that a single major locus interacts with *hms1* to cause complete male sterility in Mimulus hybrids. We found five MgSTS markers that were associated with hybrid male sterility (Table 1). By genotyping these markers in the 2001 F_2 mapping population, we determined that all five were located on linkage group 13. Next, we genotyped the LG13 markers in the BN_1 + *hms1* mapping population. Because male sterility was not a discrete trait in this population (see below) we used a QTL mapping approach to localize the sterility locus (Figure 4a). The hybrid male-sterility phenotype mapped to an interval of roughly 8 cM between the flanking markers MgSTS104 and MgSTS599. We refer to this second incompatibility

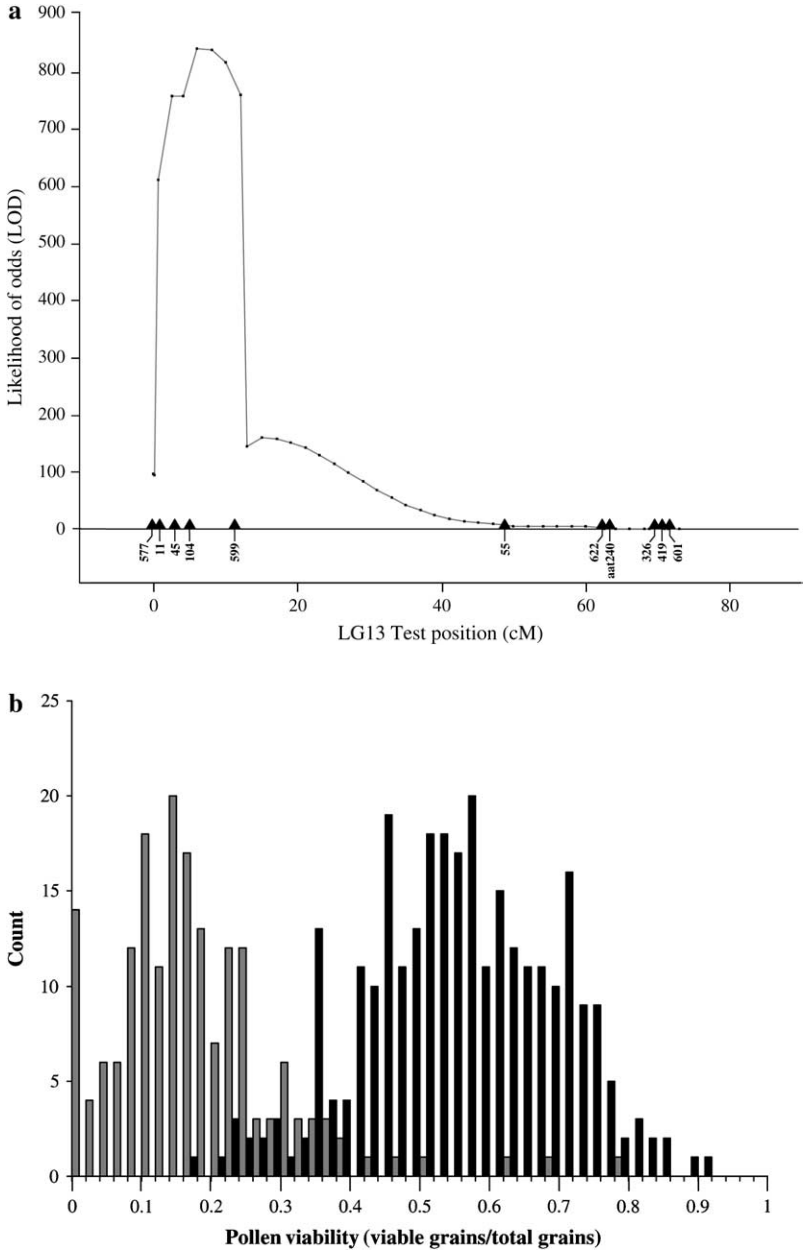

FIGURE 4.—Genetic dissection of the effect of LG13 on Mimulus hybrid male sterility. (a) Likelihood ratio (LR) test statistic profile from composite interval mapping of male sterility in the BN_1+*hms1* mapping population. The positions of molecular markers are indicated along the bottom. Hybrid male-sterility effects were mapped to an interval of roughly 8 cM between MgSTS104 and MgSTS599, which we refer to as *hybrid male sterility 2* (*hms2*). (b) Histogram of pollen viability (proportion viable pollen grains, averaged between two flowers per individual) in the BN_1 + *hms1* mapping population, grouped by *hms2* genotype. Individuals are uniformly heterozygous for *hms1* but are segregating at *hms2*. Shaded bars indicate individuals that are homozygous for the *M. nasutus* allele at *hms2*. Solid bars represent individuals that are heterozygous for the *M. nasutus* allele at *hms2*. Genotypes at *hms2* were inferred by genotyping the flanking markers MgSTS104 and MgSTS599. All recombinants between the flanking markers were excluded (with the exception of double crossovers, which could not be detected).

locus as *hybrid male sterility 2* (*hms2*). The phenotypic effect of the *hms2* locus is dramatic and highly significant [additive effect = −40.63, likelihood ratio (LR) = 841.05 *vs.* an LR threshold of 8.12]. A second broad peak centered at position 16 cM (Figure 4a) also was detected, but is likely to be a statistical artifact of the low information content in the 30 cM gap between MgSTS599 and the next closest marker, MgSTS55. However, it is possible that an additional locus on LG13 has a moderate effect on the hybrid male-sterility phenotype. Future efforts to increase marker density in this region should allow us to distinguish between these possibilities. As expected, the two anomalous male-fertile *hms1* heterozygotes from the BN_4 population (lines 62 and 185) also were heterozygous at the *hms2* locus.

Phenotypic effects of hybrid male sterility loci: Once we had genetically mapped *hms1* and *hms2*, we wanted to directly determine the contribution of each incompatibility locus to the overall pattern of male sterility among *M. nasutus*-*M. guttatus* hybrids. We observed that introgressing a heterozygous segment that contains the *M. guttatus hms1* allele into a *M. nasutus* background has profound phenotypic effects: an individual from the RSB_3 population that is heterozygous at *hms1* produces ~1% viable pollen, whereas an individual with two *M. nasutus* alleles at *hms1* is just as fertile as the recurrent parent. We reasoned that if the genetic incompatibility that causes complete hybrid male sterility only involves *hms1* and *hms2*, we should observe these two phenotypic classes irrespective of genetic background. However, it

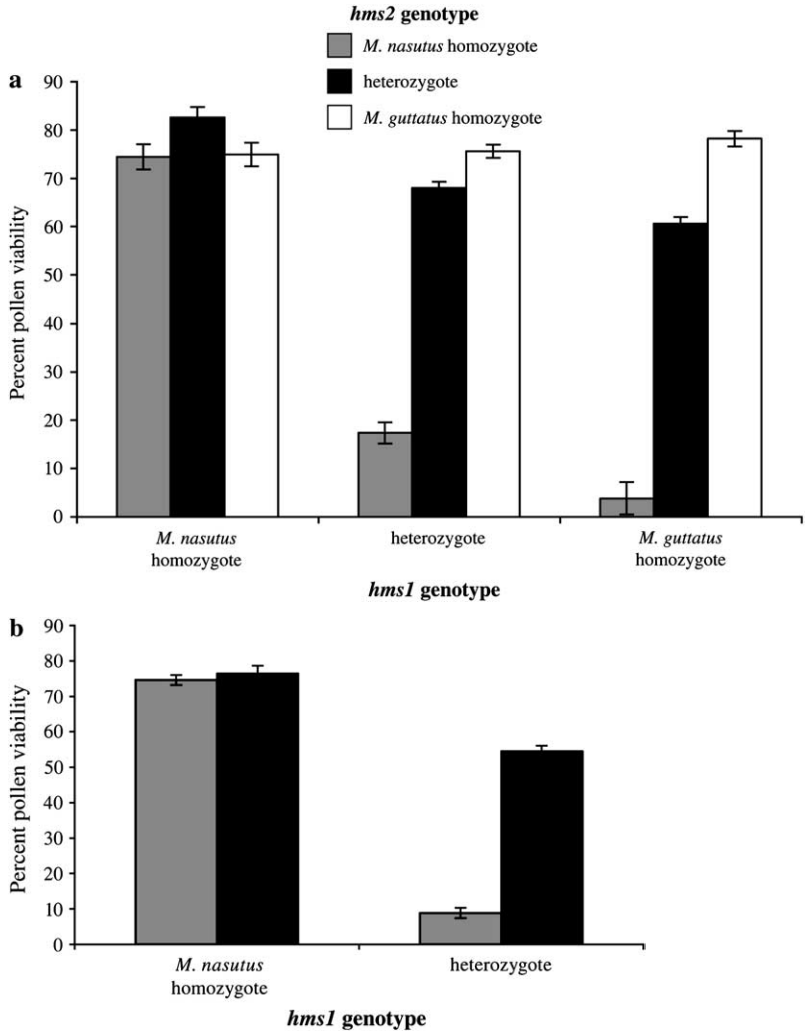

FIGURE 5.—Least square means of pollen viability vary among *hms1*–*hms2* genotypes in (a) 2005 F_2 hybrids ($N = 272$) and (b) *M. nasutus*-backcross (BN_1) lines ($N = 106$). Bars indicate standard errors.

appears that hybrid male fertility falls into discrete classes only when the dominant *M. guttatus* allele at *hms1* is against a nearly isogenic *M. nasutus* genetic background. When instead the *M. guttatus hms1* allele is present in a heterospecific genetic background, hybrid male sterility is not always complete. For instance, we found continuous phenotypic variation in the progeny from the cross to map *hms2* (BN_1 + *hms1* mapping population), in which a single copy of the *M. guttatus hms1* allele was held constant against a genetic background that was essentially equivalent to a first-generation backcross to *M. nasutus*. To visualize the effect of *hms2* on male fertility in the BN_1 + *hms1* population, we first inferred *hms2* genotypes (*i.e.*, we genotyped flanking markers MgSTS104 and MgSTS599, and excluded any individuals with crossovers between the two markers). Indeed, the two segregating *hms2* genotypes (*M. nasutus* homozygotes and heterozygotes) define distinct but partially overlapping and variable phenotypic classes (Figure 4b).

Next, we directly measured the contribution of each incompatibility locus to hybrid male sterility in the F_2 and BN_1 populations. To infer *hms1* and *hms2* genotypes in these populations, we genotyped *hms1* flanking markers (MgSTS504a, MgSTS504b) and *hms2* flanking markers (MgSTS104, MgSTS599), and excluded any individuals with crossovers between the two markers. Pollen viability was significantly affected by *hms1* (ANOVA: F_2, $F = 34.178$, $P < 0.0001$; BN_1, $F = 165.097$, $P < 0.0001$), *hms2* (F_2, $F = 70.820$, $P < 0.0001$; BN_1, $F = 48.265$, $P < 0.0001$), and by the genetic interaction between the two loci (F_2, $F = 15.742$, $P < 0.0001$; BN_1, $F = 41.412$, $P < 0.0001$). As expected, hybrid male sterility was most severe in classes that contained one or two copies of the *M. guttatus* allele at *hms1* and two copies of the *M. nasutus* allele at *hms2* (Figures 5a, b). Interestingly, the *M. guttatus* allele at *hms1* does not appear to be completely dominant: given homozygosity for *M. nasutus* alleles at *hms2*, only those F_2 hybrids with two copies of the *M. guttatus* allele at *hms1* were highly sterile (mean = 0.038, SE = 0.067), whereas individuals heterozygous for *hms1* were partially male fertile (mean = 0.174, SE = 0.044). This result contrasts sharply with that of the RSB_3 and BN_4 populations, in which *hms1* heterozygotes are completely male sterile. It appears, then, that while *hms1* and *hms2* are the major factors that

cause hybrid male sterility, additional loci also are involved. This fact is further substantiated by the genetic basis of partial male sterility in the F_1 hybrids. The average pollen viability of the F_1 hybrids (mean = 0.509, SE = 0.014) was much lower than that of double heterozygotes (for *hms1* and *hms2*) from the F_2 population (mean = 0.680, SE = 0.025), indicating that additional segregating loci contribute to variation in hybrid male fertility.

Preliminary screen for additional small-effect hybrid male sterility loci: Because the *hms1*–*hms2* incompatibility causes complete hybrid male sterility when against a nearly isogenic *M. nasutus* background (*e.g.*, RSB_3 population) but is less penetrant against a heterospecific genetic background (*e.g.*, BN_1 + *hms1*, F_2 populations), additional *M. nasutus* factors with relatively small effects must be required for complete male sterility to occur. As a first step toward identifying these putative incompatibility loci, we performed ANOVA tests for three-way epistasis between *hms1*, *hms2*, and each of 125 unlinked MgSTS markers recently mapped in the 2001 F_2 population (L. Fishman, unpublished results). Using this approach we identified a three-way interaction between *hms1*, *hms2*, and the genetic marker MgSTS388, which is located on LG10 ($F = 2.088$, $P = 0.039$) (Table 1). Among individuals of the highly sterile F_2 genotypic class (*i.e.*, two copies of the *M. guttatus* allele at *hms1* and two copies of the *M. nasutus* allele at *hms2*), MgSTS388 genotype has no effect on male fertility. In contrast, among individuals of the partially fertile F_2 genotypic class (*i.e.*, heterozygous at *hms1* and homozygous for *M. nasutus* alleles at *hms2*), MgSTS388 does contribute to variation in hybrid male sterility. Indeed, in this genotypic class average pollen viabilities for individuals with one or two copies of the *M. nasutus* allele at MgSTS388 (mean = 12.11, SE = 6.64 and mean = 18.44, SE = 6.64, respectively) are much lower than in individuals with two copies of the *M. guttatus* allele at MgSTS388 (mean = 47.5, SE = 17.57).

Effects of hybrid male sterility loci on female fertility: Finally, we asked whether the hybrid male sterility loci *hms1* and *hms2* also contribute to hybrid female sterility. In our 2001 experiment we noted that completely male-sterile F_2 hybrids produced significantly fewer seeds than the remainder of the F_2 population, suggesting that male and female sterility might share a common genetic basis (Fishman and Willis 2001). Once it became possible to directly determine genotypes at *hms1* and *hms2*, we were able to examine explicitly the effects of these loci on female fertility in the 2001 F_2. Indeed, supplemental seed set in the 2001 F_2 was significantly affected by genotype at *hms1* (ANOVA: $F = 7.668$, $P = 0.0007$) and marginally significantly affected by *hms2* ($F = 2.882$, $P = 0.060$; Figure 6a). Unfortunately, we had little power to test whether epistasis between *hms1* and *hms2* also contributes to the variation in female fertility because only a small number of individuals ($N = 7$) could be assigned definitively to the relevant genotypes. The direction of the effects of *hms1* and *hms2* on female fertility was similar to what we saw for male fertility: the most female-sterile classes contained one or two copies of the *M. guttatus* allele at *hms1* and two copies of the *M. nasutus* allele at *hms2*. We also measured female fertility in a subset of individuals from the RSB_3 mapping population. Male-sterile and -fertile plants from this population are full sibs and should differ only in terms of their genotype for the introgression containing the *hms1* locus. Strikingly, female fertility was reduced by 76% in completely male-sterile plants (mean = 72.18, SE = 10.31, $N = 28$) relative to their male-fertile sibs (mean = 310.82, SE = 11.63, $N = 22$; Figure 6b).

DISCUSSION

Although it has been nearly 70 years since the Dobzhansky–Muller model of postzygotic reproductive isolation became widely known, we still have few detailed genetic studies of hybrid incompatibility factors, and the vast majority of those that exist are from a single genus, Drosophila. This lack of taxonomic breadth has made it difficult to generalize about the nature of genes that underlie reproductive incompatibility between species. Knowledge of the genetic basis of hybrid incompatibilities is essential if we are to understand the evolutionary dynamics of postzygotic isolation and, ultimately, the process of species divergence. In this report, we have demonstrated that a single pair of incompatible loci causes nearly complete male sterility in hybrids between *M. guttatus* and *M. nasutus*. The incompatibility allele at *hms1* is dominant with respect to hybrid male sterility and the incompatibility allele at *hms2* is recessive. We have also shown that the incompatible genotypes at *hms1* and *hms2* severely reduce female fertility, leaving open the possibility that Mimulus hybrid male and female sterility are caused by the same genes. Our findings show that the genetic basis of hybrid incompatibility differs substantially between Mimulus and Drosophila systems, perhaps a consequence of their biological differences and distinct evolutionary histories.

In Drosophila species, the genetic basis of hybrid male sterility is highly polygenic and complex (*e.g.*, Perez and Wu 1995; Davis and Wu 1996; Orr and Irving 2001; Tao *et al.* 2003a,b; Sawamura *et al.* 2004). Moreover, the number of factors that cause hybrid male sterility between Drosophila species often greatly exceeds the number of factors that cause hybrid inviability or female sterility (True *et al.* 1996; Sawamura *et al.* 2000; Tao *et al.* 2003a). For example, when Tao *et al.* (2003a) introgressed segments of the *Drosophila mauritiana* third chromosome into a *D. simulans* genetic background, they found that many introgressions caused male sterility, but none caused hybrid lethality or female

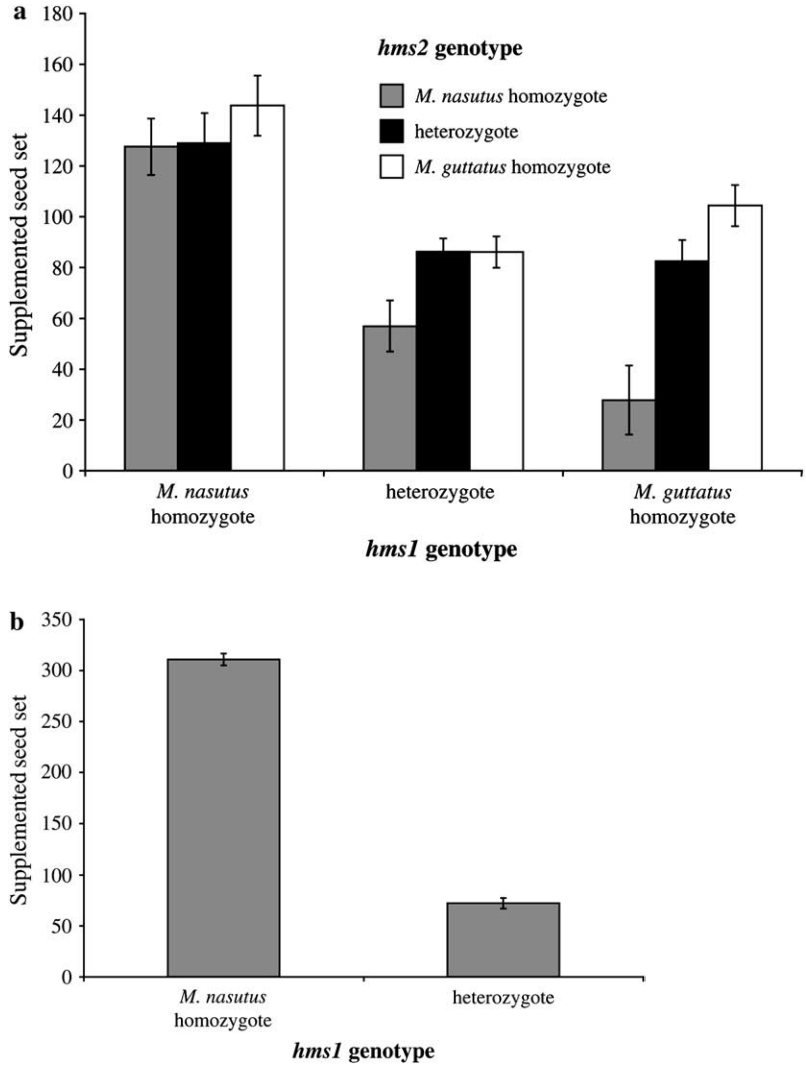

FIGURE 6.—Least square means of supplemental seed set vary among *hms1*–*hms2* genotypes in (a) 2001 F_2 hybrids ($N = 288$) and (b) RSB_3 individuals ($N = 50$). Bars indicate standard errors.

sterility. From the same set of experiments, TAO *et al.* (2003a,b) estimated that ~60 minor-effect genes contribute to hybrid male sterility between *D. mauritiana* and *D. simulans*, and that an average of four genes together are required for complete male sterility. Of course, a large number of hybrid incompatibility loci may simply reflect the age of the species pair; many genetic changes might have accumulated after reproductive isolation initially evolved. In addition, an elevated number of hybrid male sterility factors might be a consequence of faster male evolution, driven by sexual selection, sexual conflict, or an inherent sensitivity of spermatogenesis (WU and DAVIS 1993). However, it is not apparent that either of these factors—species divergence time or faster male evolution—should influence the genetic complexity of hybrid male sterility or the strength of individual effects of incompatibility loci. Accordingly, the genetic basis of hybrid male sterility between a younger pair of taxa, the USA and Bogota subspecies of *D. pseudoobscura*, involves a more modest number of incompatibility factors, but it is still polygenic and complex (ORR and IRVING 2001).

In striking contrast to Drosophila species, we have shown a simple genetic basis for hybrid male sterility between *M. guttatus* and *M. nasutus*. In the simplest version of the Dobzhansky–Muller model, postzygotic reproductive isolation evolves due to a genetic incompatibility between a single pair of heterospecific factors. However, as MULLER (1942) himself discussed, hybrid incompatibilities might very well involve more than two genes. In fact, theory predicts that complexity may facilitate the evolution of hybrid incompatibilities by allowing ancestral species to circumvent deleterious genotypic combinations (ORR 1995). Hybrids between subspecies of *D. pseudoobscura* suffer no reductions in male fertility unless they carry incompatibility alleles at a minimum of four loci (ORR and IRVING 2001). Likewise, the *D. mauritiana* Odysseus-H (OdsH) gene causes complete hybrid male sterility only when introgressed into *D. simulans* along with an additional, distal region of the X chromosome (PEREZ and WU 1995). In contrast, our results show that a single, heterozygous introgression of the *M. guttatus hms1* into a *M. nasutus* genetic background (*i.e.*, the RSB_3 line) results in

complete male sterility. Most likely, a homozygous introgression of the *M. nasutus hms2* allele into a *M. guttatus* genetic background would have a similarly large effect: F_2 individuals that are homozygous for *M. guttatus* alleles at *hms1* and homozygous for *M. nasutus* alleles at *hms2* are completely male sterile. Of course, it is always possible that further genetic dissection of *hms1* and *hms2* will reveal that more than one gene underlies each locus (*e.g.*, see Davis and Wu 1996).

The genetic basis of hybrid sterility in Mimulus is consistent with that observed in other plant species. For example, hybrid sterility between different varieties of the cultivated rice, *O. sativa*, is often genetically simple (Oka 1974; Liu *et al.* 1997; Kubo and Yoshimura 2002, 2005, but also see Li *et al.* 1997). In this system, Kubo and Yoshimura (2002) have genetically mapped both partners of a two-locus incompatibility with major effects on hybrid viability and fertility. Recently, the same authors mapped a different three-locus genetic incompatibility that exclusively affects hybrid female fertility (Kubo and Yoshimura 2005). The genetic basis of hybrid male sterility between species of Oryza also appears simple: a major sterility factor from *O. glaberrimo* causes male gamete abortion against an *O. sativa* genetic background (Sano 1990). Likewise, only a moderate number of incompatibility factors contribute to hybrid sterility between Lycopersicon species (Moyle and Graham 2005).

Interestingly, hybrid lethality often has been shown to have a simple genetic basis in plants and animals. In fact, two different two-locus incompatibility systems cause lethality in hybrids between populations of *M. guttatus* (Macnair and Christie 1983; Christie and Macnair 1984). Moreover, classic experiments demonstrated that simple genetic incompatibilities underlie hybrid inviability between Crepis species and cause the "Corky" syndrome of Gossypium species hybrids (Hollingshead 1930; Stephens1946). Similarly, in *Tigriopus californicus*, enzymatic activity of two interacting proteins, cytochrome *c* oxidase and cytochrome *c*, is reduced when they come from different populations (Rawson and Burton 2002), which might cause hybrid fitness problems (Willett and Burton 2001). In Xiphophorus, a simple two-locus incompatibility causes malignant tumor formation in species hybrids (Wittbrodt *et al.* 1989). Even in Drosophila, incompatibility effects occasionally map to a single locus (*e.g.*, Barbash *et al.* 2003; Presgraves 2003; Presgraves *et al.* 2003). It is worth noting, however, that in none of these Drosophila cases is the interacting partner known. Although several studies have identified loci that cause complete postzygotic isolation when combined with a heterospecific chromosome or genome, none have mapped the individual incompatible loci from both species. Depending on how many interacting loci are discovered, epistasis might be considerably more complex than presently thought.

We have shown that the *M. guttatus* allele at *hms1* acts dominantly to cause male sterility when introgressed into a heterospecific background. This result is interesting in light of much evidence from Drosophila and other groups that genes causing postzygotic isolation are on average partially recessive (*e.g.*, Hollocher and Wu 1996; True *et al.* 1996; Presgraves 2003; Slotman *et al.* 2004; Moyle and Graham 2005), as predicted by the dominance theory. Nevertheless, individual incompatibility loci certainly differ in dominance. Both hybrid lethality systems in *M. guttatus* involve dominant incompatibility alleles (Macnair and Christie 1983; Christie and Macnair 1984), as does hybrid lethality in Gossypium (Stephens1946). In addition, the *D. melanogaster* allele at the X-linked *Hybrid male rescue* (*Hmr*) locus interacts with dominant partner loci from *D. simulans* to cause lethality in some F_1 hybrids (Hutter *et al.* 1990). Likewise, the *Tumor* locus acts dominantly to cause melanoma formation in Xiphophorus hybrids that lack dominant suppressor alleles at the *R* locus (Schartl 1995). Furthermore, the frequency of F_1 hybrid problems observed across diverse taxa (*e.g.*, Coyne and Orr 1989; Sasa *et al.* 1998; Presgraves 2002; Moyle *et al.* 2004) implies that dominant incompatibility alleles are not uncommon.

In addition to causing nearly complete hybrid male sterility, *hms1* and *hms2* incompatibility alleles also dramatically reduce hybrid female fertility. In contrast, the genes causing male sterility in Drosophila hybrids typically do not affect female fertility (Hollocher and Wu 1996; True *et al.* 1996; Tao *et al.* 2003a). Mimulus species are hermaphroditic and flowers are perfect (contain both male and female parts). In higher plants, the initial stages of gametogenesis include the differentiation of archisporial cells and the initiation of meiosis. These two processes are similar for both male and female gametophytes, and are controlled by some of the same genes (Wilson and Yang 2004). In Arabidopsis, several meiotic mutants have been isolated that cause both male and female sterility (see Caryl *et al.* 2003). Interestingly, a study of hybrid incompatibilities between Lycopersicon species identified several QTL that affect both male and female fertility, which could be due to pleiotropic effects of individual Dobzhansky–Muller interactions (Moyle and Graham 2005). Furthermore, a two-locus genetic incompatibility causes male and female sterility between the Indica and Japonica varieties of *O. sativa* (Kubo and Yoshimura 2005).

In this study we discovered a dramatic suppression of recombination in the RSB_3 population relative to that in the F_2 populations. Recall that to generate the RSB_3 population, we backcrossed a male-sterile RSB_2 individual heterozygous for *hms1* to homozygous *M. nasutus*. In this cross, all informative meiotic recombination events occur in the RSB_2 parent, which has the highly sterile *hms1–hms2* genotype. In contrast, to generate the F_2 mapping population we self-fertilized an F_1 hybrid, which

lacks the *hms1–hms2* incompatibility. Because of this correspondence between the presence of the *hms1–hms2* incompatibility in the informative parent of a cross and the occurrence of low recombination, it is possible that the *hms1–hms2* incompatibility directly causes a general reduction in meiotic recombination frequency. Interestingly, suppression of recombination also was discovered proximate to a hybrid male sterility locus in *O. sativa*, although in this case the effect might have been due to the presence of a linked inversion (Sano 1990). It is particularly intriguing to note that induced mutations that cause meiotic defects also can reduce rates of recombination. Indeed, several Arabidopsis fertility mutants exhibit dramatic reductions in recombination frequency (Couteau *et al.* 1999; Gallego *et al.* 2001; Grelon *et al.* 2001). It should be straightforward to determine whether the *hms1–hms2* hybrid incompatibility causes a general suppression of recombination by performing testcrosses with F_2 hybrids that carry incompatible *vs.* parental alleles at *hms1* and *hms2* and that also are segregating for unlinked chromosomal segments throughout the genome. Of course, we also would like to investigate whether there is any cytological evidence for meiotic defects in male-sterile hybrids. Alternatively, it is possible that differential rates of recombination in Mimulus hybrid populations are not caused by the *hms1–hms2* incompatibility, but instead are due to differences in genetic background (*e.g.*, analogous to interchromosomal effects seen in some Drosophila crosses, see Lucchesi 1976).

Interestingly, several Drosophila studies have discovered that hybrid male sterility loci occasionally map to regions that also show segregation distortion in hybrids (Hauschteck-Jungen 1990; Tao *et al.* 2001; Orr and Irving 2005). Both *hms1* and *hms2* show significant segregation distortion (*hms1*: heterozygotes to *M. nasutus* homozygotes in the RSB_3 population, expected = 0.5:0.5, observed = 0.45:0.55, $N = 2968$, $\chi^2 = 27.18$, d.f. =1, $P < 0.001$; *hms2*: heterozygotes to *M. nasutus* homozygote in the BN_1 + *hms1* population, expected = 0.5:0.5, observed = 0.62:0.38, $N = 478$, $\chi^2 = 27.18$, d.f. = 1, $P < 0.001$). Recently, a strong meiotic drive locus was found in *M. nasutus–M. guttatus* hybrids (generated by crossing the same inbred parental lines used in this study), but this locus maps to a different linkage group than either *hms1* or *hms2* (Fishman and Willis 2005).

We are now poised to begin fine-scale genetic mapping and, ultimately, positional cloning of Mimulus hybrid incompatibility loci, *hms1* and *hms2* (which will require the use of a population that is not limited by the number of recombinants, *i.e.*, an F_2 population). Elucidation of the molecular genetic basis of *hms1* and *hms2* will allow basic questions to be asked about their normal functions within pure species, such as whether they interact with one another at a molecular level, are in the same genetic pathway, and/or represent recently duplicated genes. Of course, we also would like to determine whether the incompatibility is the result of evolutionary divergence in coding or *cis*-regulatory regions. Furthermore, knowledge of which genes cause Mimulus hybrid sterility will allow molecular population genetic tests of the role of selection and population structure in shaping the pattern of sequence variation at these loci. A striking pattern to emerge from Drosophila is that natural selection can promote the evolution of postzygotic reproductive isolation: positive selection appears to have driven rapid sequence divergence in the cloned hybrid incompatibility genes *Ods*, *Hmr*, and *Nup96* (Ting *et al.* 1998; Barbash *et al.* 2003; Presgraves *et al.* 2003). Alternatively, fixation of hybrid incompatibility alleles that are slightly deleterious within species is expected to occur more readily in small populations (Nei *et al.* 1983), which may be particularly relevant for substructured or partially inbred species like Mimulus. It is certainly possible that the evolutionary dynamics of hybrid incompatibility will vary between biologically diverse taxa.

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