Evolution, 60(7), 2006, pp. 000–000

A CYTONUCLEAR INCOMPATIBILITY CAUSES ANTHER STERILITY IN *MIMULUS* HYBRIDS

LILA FISHMAN[1] AND JOHN H. WILLIS[2]
[1]*Division of Biological Sciences, University of Montana, Missoula, Montana 59812*
E-mail: lila.fishman@mso.umt.edu
[2]*Department of Biology, Duke University, Durham, North Carolina 27708*
E-mail: jwillis@duke.edu

Abstract.—Multilocus interactions (also known as Dobzhansky-Muller incompatibilities) are thought to be the major source of hybrid inviability and sterility. Because cytoplasmic and nuclear genomes have conflicting evolutionary interests and are often highly coevolved, cytonuclear incompatibilities may be among the first to develop in incipient species. Here, we report the discovery of cytoplasm-dependent anther sterility in hybrids between closely related *Mimulus* species, outcrossing *M. guttatus* and selfing *M. nasutus*. A novel pollenless anther phenotype was observed in F_2 hybrids with the *M. guttatus* cytoplasm (F_{2G}) but not in the reciprocal F_{2N} hybrids, F_1 hybrids or parental genotypes. The pattern of phenotypic segregation in the F_{2G} hybrids and two backcross populations fit a Mendelian single-locus recessive model, allowing us to map the underlying nuclear locus to a small region on LG7 of the *Mimulus* linkage map. Anther sterility was associated with a 20% reduction in flower size in backcross hybrids and we mapped a major cytoplasm-dependent corolla width QTL with its peak at the anther sterility locus. We argue that the cytonuclear anther sterility seen in hybrids reflects the presence of a cryptic cytoplasmic male sterility (CMS) and restorer system within the hermaphroditic *M. guttatus* population and therefore name the anther sterility locus *restorer-of-male-fertility* (RMF). The genetic mapping of RMF is a first step toward testing hypotheses about the molecular basis, individual fitness consequences, and ecological context of CMS and restoration in a system without stable CMS-restorer polymorphism (i.e., gynodioecy). The discovery of cryptic CMS in a hermaphroditic wildflower further suggests that selfish cytoplasmic evolution may play an important, but often undetected, role in shaping patterns of hybrid incompatibility and interspecific introgression in plants.

Key words.—CMS, cytoplasmic male sterility, hybrid incompatibility, *Mimulus*, QTL mapping, postzygotic reproductive isolation.

Received December 21, 2005. Accepted May 4, 2006.

Hybrids between divergent populations or species are frequently sterile or inviable; indeed, hybrid incompatibilities are often an important component of the reproductive isolation that defines species. Early evolutionists, including Darwin, struggled to explain low hybrid fitness in the context of natural selection alone. How can a trait as inherently maladaptive as the production of dead or sterile offspring evolve? The Dobzhansky-Muller model (independently developed by Bateson [1909], Dobzhansky [1937], and Muller [1942]), which posits that hybrid sterility and inviability result from negative epistatic interactions among loci, provides a genetic solution to this long-standing problem. The key feature of the DM model is its generality: because derived incompatible alleles need not reduce fitness in their ancestral genetic background, hybrid breakdown may evolve via ordinary natural selection or drift. However, the DM model also encompasses epistatic interactions between genotypes that do have a history of within-species fitness costs and strong countervailing selection, such as meiotic drive loci and their suppressors (e.g., Frank 1991; Hurst and Pomiankowski 1991; Henikoff et al. 2001). Recent work in *Drosophila* has implicated sex ratio distortion and suppression in the evolution of hybrid male sterility (Tao et al. 2003; Orr and Irving 2005), but it is not yet clear how often selfish evolution plays a role in the development of hybrid incompatibility. Dobzhansky-Muller interactions appear to be an important source of hybrid incompatibility in many systems (for review, see Orr 2005) but, with the exception of a few well-studied systems (Wittbrodt et al. 1989; Presgraves et al. 2003; Sun et al. 2004), the function and evolutionary history of the genes involved are largely unknown (Orr and Presgraves 2000).

Theoretical and empirical exploration of Dobzhansky-Muller models has primarily focused on epistatic interactions among nuclear loci (e.g., Orr 1995; Turelli and Orr 2000; Gavrilets 2003) but incompatibility between alleles at nuclear and cytoplasmic genes may also contribute to hybrid breakdown (Levin 2003). Epistasis between nuclear and cytoplasmic genomes is pervasive at the molecular level, as most genes encoded in cytoplasmic genomes are modified by the products of nuclear genes and essential components of the chloroplast and mitochondrion are encoded in the nuclear genome (Rand et al. 2004). Abundant indirect evidence for cytonuclear incompatibility in interspecific hybrids comes from differences in the fitness of reciprocal hybrids (e.g., Edmands and Burton 1999; Tiffin et al. 2001). Incompatibility between mitochondrial and nuclear genotypes has been directly implicated in hybrid inviability in interpopulation crosses of the copepod *Tigriopus californicus* (Willett and Burton 2001) and in male sterility of some interspecific *Drosophila* hybrids (Sackton et al. 2003). Similarly, the substitution of cytoplasmic genomes from one species or strain into a foreign nuclear background frequently results in male sterility in crop plants (Kaul 1988, Hanson and Bentolila 2004). These lines of evidence suggest that any investigation of the magnitude and nature of hybrid incompatibilities consider cytonuclear interactions as possible contributors to the phenomenon.

The wildflowers of the *Mimulus guttatus* species complex provide an ideal system for examining the evolution and genetics of hybrid incompatibilities, including cytonuclear incompatibilities. This research focuses on two members of the

complex, the geographically widespread, outcrossing species *Mimulus guttatus* and its close relative, the highly selfing *M. nasutus*. These taxa are divergent in mating system, floral morphology, and edaphic ecology, but largely cross-fertile (Vickery 1978). Hybrids are often observed in areas of sympathy (e.g., Vickery 1978; Ritland 1991; Martin 2004). Thus, loci involved in the reduced fitness of their hybrids may be representative of processes and genes important early in genomic divergence. Previous investigations found that epistatic interactions contributed to male and female sterility in hybrids between *M. nasutus* and *M. guttatus* (Fishman and Willis 2001) and we have fine-mapped one pair of nuclear Dobzhansky-Muller loci causing nearly complete pollen inviability and partial female infertility in a fraction of F_2 genotypes (Sweigart et al. 2006). However, because these earlier analyses were conducted only on hybrids carrying the cytoplasmic genomes of the *M. nasutus* parent, we could not assess the contribution of cytonuclear interactions to total hybrid breakdown. Given the high frequency of asymmetric crossing barriers in it is important to also explore the nature of hybrid incompatibilities in the reciprocal hybrids.

In this study, we first test for cytonuclear incompatibility in *Mimulus* hybrids by comparing the nature and magnitude of hybrid male sterility in reciprocal F_2 populations. We observe Mendelian segregation of a novel sterile anther morphology only in hybrids with the *M. guttatus* cytoplasmic background, and then use genetic mapping in additional experimental crosses to confirm that a single nuclear locus contributes to this major cytonuclear incompatibility. The data suggest the presence, within the *M. guttatus* parent, of both an organelle genotype causing cytoplasmic male sterility (CMS) and a single nuclear locus with a dominant allele that restores fertility. We then use quantitative trait loci (QTL) mapping approaches to test for pleiotropic effects of cytonuclear anther sterility on corolla morphology. We discuss the cryptic cytoplasmic male sterility revealed in *M. guttatus* × *M. nasutus* hybrids in the context of selfish genome evolution, the development of hybrid incompatibility, and the potential for postzygotic reproductive isolation.

Materials and Methods

Study System

The yellow monkey flowers of the *Mimulus guttatus* species complex are a morphologically diverse but largely interfertile group of wildflowers with their center of diversity in Western North America (Vickery 1978). *Mimulus guttatus* (2n = 28), the most common species in the complex, has large, insect-pollinated flowers and is predominantly outcrossing (Willis 1993). Routine selfing appears to have evolved several times within the group. The most widespread of the selfing taxa, *Mimulus nasutus* Greene (2n = 28) produces reduced, often cleistogamous, flowers and is highly self-fertilizing. Where *M. guttatus* and *M. nasutus* co-occur, potential premating barriers to hybridization include differences in microhabitat and flowering time (Martin 2004), as well as differences in floral morphology (Ritland and Ritland 1989), pollen production (Fenster and Carr 1997) and pollen tube growth (Diaz and Macnair 1999) associated with their divergent mating systems. At one sympatric site in California, the two species were more than 99% isolated by such prezygotic barriers (Martin 2004). Despite these strong barriers to mating, hybrids are often observed at low frequency at sympatric sites and there is evidence of recent local introgression at nuclear loci (Sweigart and Willis 2003).

Discovery and Characterization of Cytonuclear Male Sterility in Hybrids

Crosses.—Previous analyses of the genetics of floral divergence and sterility of hybrids between *M. guttatus* and *M. nasutus* focused on an F_2 mapping population derived by selfing an F_1 hybrid between inbred lines from allopatric populations of *M. guttatus* (IM62; Iron Mountain, OR) and *M. nasutus* (SF5; Sherar's Falls, OR) (Fishman and Willis 2001; Fishman et al. 2001, 2002). Because the SF line was used as the seed parent of initial F_1 cross, all hybrids in those studies had the *M. nasutus* cytoplasmic background. We will refer to those hybrids as F_{1N} and F_{2N} here. In the current study, we investigate hybrid sterility differentially expressed in the *M. guttatus* cytoplasmic background. We first created an F_1 hybrid (F_{1G}) using the *M. guttatus* line as the seed parent, then formed an F_{2G} hybrid population by selfing a single F_{1G} individual. For the initial screen for cytoplasm-specific sterility, we grew F_{2G} and F_{2N} hybrids (n = 120 each) as well as F_{1N} and F_{2N} hybrids and parental lines (n = 30 each) in a randomized common garden in the Duke University Department of Biology Greenhouse using culture conditions similar to previous studies.

To genetically characterize and map the nuclear factor(s) involved in cytonuclear anther sterility, we generated a backcross population by a crossing a single F_{1G} (ovule parent) to the SF *M. nasutus* line (pollen parent). Because the expression of the novel anther sterility phenotype was clearly dependent on the *M. guttatus* cytoplasmic background, we refer to this population as Cytoplasmic Sterile Backcross 1 (CSB_1). All CSB_1 plants carry the *M. guttatus* cytoplasm, but the population should segregate 1:1 for heterozygotes: *M. nasutus* homozygotes at nuclear loci.

To confirm the single locus nature and position of the nuclear component of cytonuclear anther sterility, we generated two advanced backcross populations descended from the CSB_1 mapping population. These independent populations (N = 30–40 each) were made by selecting two fertile CSB_1 individuals (CSB_1-23, CSB_1-29), which should be heterozygous at any nuclear loci involved in anther sterility, and fertilizing them with *M. nasutus* pollen. This selection and backcross process was repeated for two more generations to form two independently-derived cytoplasmic sterile backcross 4 (CSB_4) populations that carry the *M. guttatus* cytoplasm, are *M. nasutus* homozygotes at 93.75% of Mendelian loci, and still segregate at nuclear restorers of cytoplasm-dependent sterility. The CSB_1 population and later generations were grown under greenhouse culture conditions similar to the initial common garden experiment.

Phenotypic analyses.—For the initial assay of male fertility in the common garden experiment, we collected all four anthers from the first flower on each plant on the day that it opened. Anthers were placed in 30 μl of aniline blue/lactophenol solution, which stains starch-containing (fertile) pol-

TABLE 1. Primer sequences for MgSTS markers mapped in this study.

MgSTS marker	F-primer	R-primer
34	5′-ACGAACGAGCAAATCGAAAT	5′-CTCCTGGACGACAATGACCT
251	5′-TCACAAATCGAGAGGAAGTGG	5′-AAAGCAGCAGCAAATGAACC
369	5′-CGATCTTAAGGAAAAATTTCAGC	5′-AGAGAAGGTGCCATGTACGC
376	5′-TACAAAACGCTTCGTGATCG	5′-CAGTGATTCCCCAATAAAGAGG
512	5′-TGGTGCATTCATAGACATTGG	5′-GTCCTCTCTCACGGTCATGG
574	5′-TTTTGGAGGAGTTGTGTTAGC	5′-GAAATCCCAAGAACCACAGC

len grains dark blue. The anthers were macerated, the solution was vortexed and the numbers darkly stained, and unstained grains were counted in a subsample of the solution with a haemocytometer. Total flower pollen counts were obtained by multiplying the number of pollen grains counted in a known volume of solution (v = 0.2–2.7 μl) by 30/v. When it became apparent that substantial fraction of the F_{2G} plants produced no pollen and had deformed anthers (see RESULTS), we collected whole anthers of two additional flowers from each F_{2G} plant directly into drops of aniline blue/lactophenol dye on a microscope slide and squashed them with a cover slip for classification of anther morphology. All screening of the CSB_1 and later generations involved whole anthers directly squashed in aniline blue.

In the CSB_1 population only, we measured corolla width (maximum horizontal distance across corolla lobes) using an engineering ruler.

Markers and genotyping.—The MgSTS markers used in this study were developed from a library of *M. guttatus* expressed gene sequences and amplify length-polymorphic regions (generally including introns) between conserved exon sequences. The primers for MgSTS markers mapped in this study are given in Table 1. Information on the other markers (amplified fragment length polymorphism; AFLPs and microsatellites) shown in Figure 3 can be found in Fishman et al. (2001).

Genomic DNA for genotyping was extracted from leaf tissue using a standard CTAB/chloroform protocol modified for use in 96-well format. The MgSTS markers were amplified using standard touchdown PCR conditions (annealing temperatures decremented from 62° to 52°C for the first 10 cycles, then an additional 30 cycles at 52°C), usually with 3–6 distinguishable markers multiplexed in a single reaction. Marker genotyping was performed by sizing PCR-amplified DNA fragments with an incorporated 5′ fluorescent-labeled primer on an ABI 3700 or ABI 3100 automated capillary sequencer (Applied Biosystems, Foster City, CA). Marker genotypes were assigned automatically using the programs Genotyper or Genemapper (Applied Biosystems) then verified by eye.

Mapping analyses.—We first used a bulk segregant analysis of the CSB_1 population to screen a large number of MgSTS markers and identify those potentially linked to nuclear loci involved in anther sterility. For this screen, we constructed six template pools, each consisting of DNA from three sterile CSB_1 individuals. The sterile pools were genotyped at approximately 300 MgSTS markers previously identified as polymorphic between the parental lines. Markers with three or more homozygous pools were identified as candidates. Even a single heterozygote in the pooled template will produce a ''heterozygous'' genotype for the pool, so this is an efficient method to screen for markers linked to a recessive allele. Because completely unlinked loci should be 50% homozygous, whereas anther sterile individuals must be *M. nasutus* homozygotes at the target locus, this criterion has a false positive rate of <2% for unlinked Mendelian markers. Candidate markers identified via the bulk segregant screens were then genotyped in all CBS_1 individuals and tested for association with anther sterility with χ^2 tests for independence.

To test the hypothesis that a single nuclear locus was involved in the cytonuclear anther sterility, we first assigned markers significantly associated with sterility in the CSB_1 to framework linkage groups by genotyping and mapping them in a subset (N = 287) of individuals from the original F_{2N} population (Fishman et al. 2001). We then genotyped all other markers known to be on that linkage group(s) (L. Fishman, unpubl. data) in the CSB_1 and ordered all markers relative to the cytoplasm-dependent anther sterility locus. Because anther sterility appeared to be a discrete character following a simple Mendelian segregation pattern, we mapped it relative to linked markers by coding the phenotypes as genotypes (steriles = *M. nasutus* homozygotes, fertiles = heterozygotes) rather than using a QTL mapping approach. The linkage mapping analyses were conducted in the program Mapmaker (Lander et al. 1987) using parameters similar to previous *Mimulus* studies (Fishman et al. 2001).

For QTL analyses of corolla width in the CSB_1 generation, we implemented composite interval mapping (CIM) on individual linkage groups in the program WinQTLCart (Wang et al. 2005), using permutation (N = 500) to set an experiment-wide significance threshold of 0.05 for QTL detection. For comparison of QTL effects in different cytoplasmic backgrounds, we conducted re-analyses of the original F_{2N} population using a subset (N = 287) of individuals from previous mapping studies (Fishman et al. 2001, 2002).

RESULTS

Phenotypic and Mendelian Characterization of Cytonuclear Anther Sterility

In the initial common garden experiment, mean pollen viability did not differ significantly between reciprocal hybrid populations (data not shown), but we observed a striking difference in distribution of individual pollen counts between the reciprocal F_2 hybrid classes (Fig. 1). In the F_{2G} hybrids alone, a substantial proportion of individuals had nearzero pollen counts. Upon closer inspection, the anthers of plants producing no pollen displayed a unique ''arrowhead'' shape

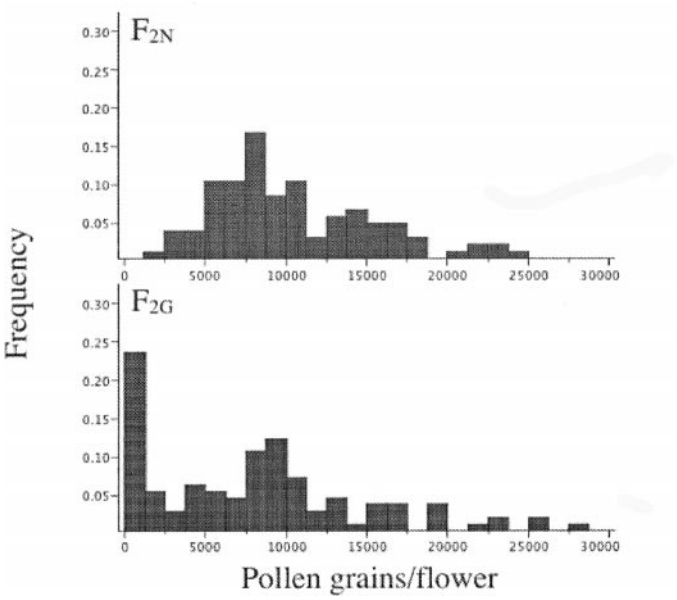

FIG. 1. Frequency distributions of pollen number per flower in reciprocal *Mimulus* F_2 hybrids grown in the common garden experiment.

caused by incomplete development of the anther sacs (Fig. 2a). This phenotype is quite distinct from the appearance of both fertile anthers (Fig. 2b) and the pollen-sterile anthers previously characterized in the F_{2N} population, which have fully developed anther sacs and often quite large numbers of (sterile) pollen grains (Fishman and Willis 2001; Sweigart et al. 2006). In the F_{2G} population, sterile anthers were readily identifiable without magnification or staining due to their shape and translucence, as well as their lack of pollen. Using these visual criteria, we scored anther sterility in a larger set of F_{2G} plants and found that approximately one-quarter (23%, 59/254) had completely sterile anthers. With one or two exceptions, all examined flowers of a plant exhibited the same anther phenotype.

The appearance of sterile deformed anthers in F_{2G} hybrids, but not in the parental, F_1 or F_{2N} populations, strongly suggests that *M. nasutus* carries nuclear allele(s) that fail to restore sterility caused by the *M. guttatus* cytoplasmic background. Furthermore, the lack of anther sterility in the F_{1G} and the segregation pattern in the F_{2G} is consistent with a recessive allele at a single Mendelian locus. To test this genetic inference, we examined the pattern of anther sterility in the CSB_1 backcross population. All CSB_1 plants carry the *M. guttatus* cytoplasm, but the population should segregate 1:1 for heterozygotes: *M. nasutus* homozygotes at Mendelian nuclear loci. Thus, under a single-locus recessive model of cytoplasm-dependent anther sterility, the 50% of plants homozygous for *M. nasutus* alleles at the nuclear locus would be sterile. In the CSB_1, 45% (81/180) of scored individuals displayed anther sterility, which is not significantly different from the Mendelian expectation ($\chi^2 = 1.8$, $P > 0.10$).

During the screening of the CSB_1 generation for anther sterility, we noticed that sterile plants appeared to have consistently smaller flowers than fertile plants. Measures of corolla width confirmed a strong statistical association between sterility and flower size. On average, the corollas of fertile

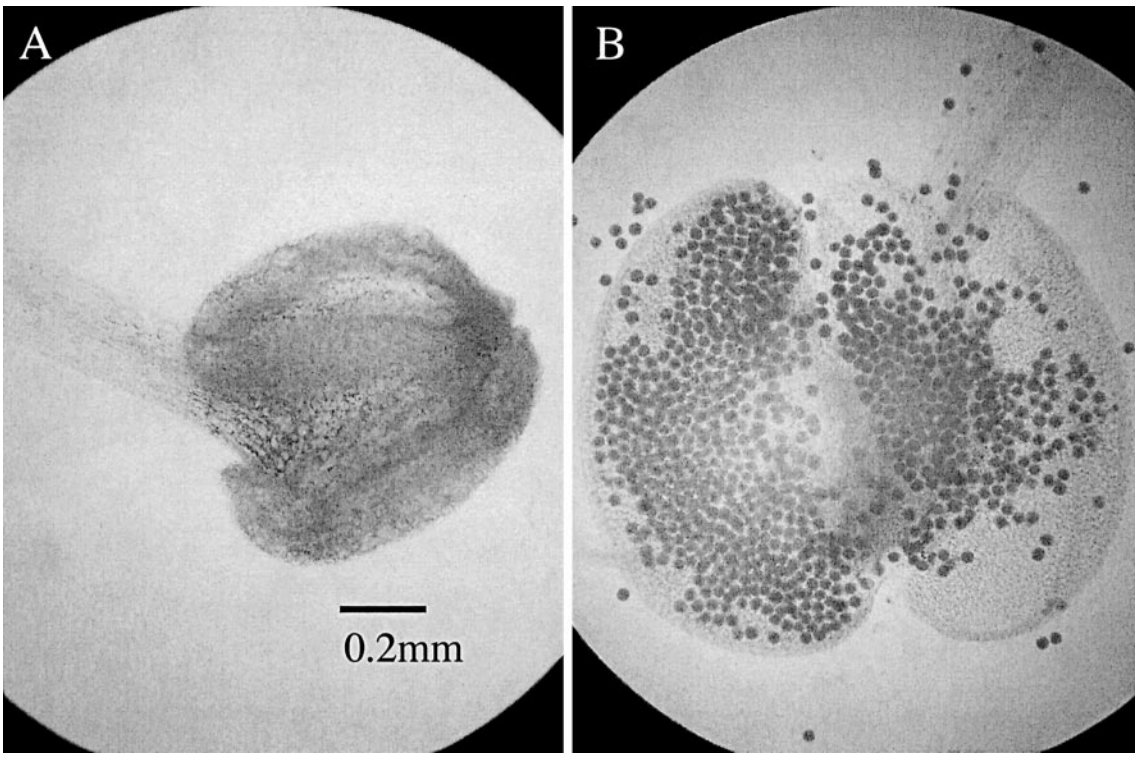

FIG. 2. Anthers of *Mimulus* hybrids illustrating (A) cytoplasm-dependent anther sterility and (B) full male fertility. Fertile pollen grains are darkly stained with lactophenol-aniline blue.

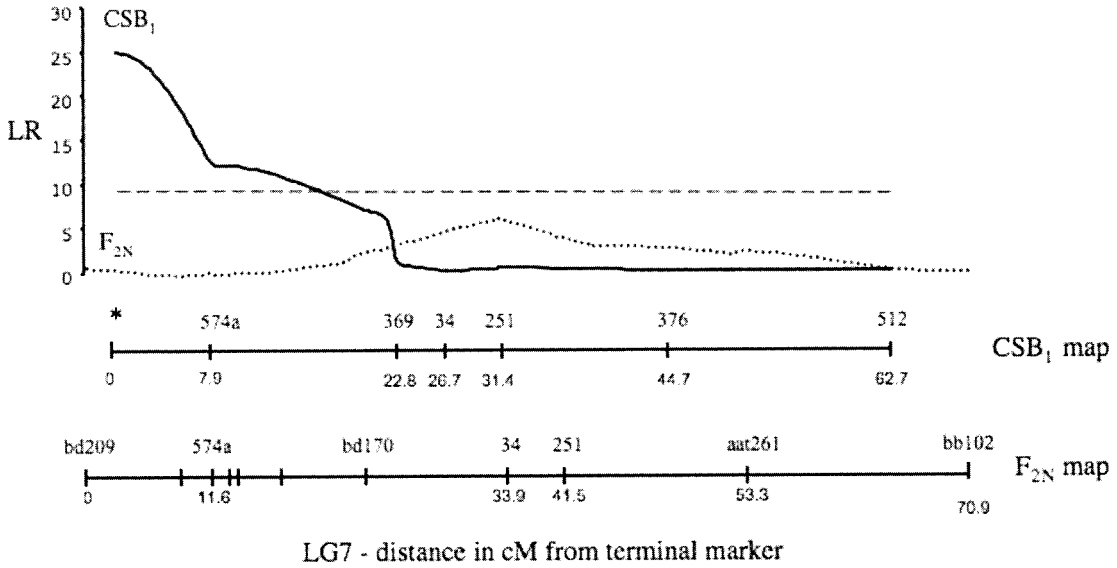

FIG. 3. Linkage and QTL maps of LG7 in the CSB_1 and F_{2N} mapping populations. The asterisk on the CSB_1 linkage map marks the location of the cytoplasm-dependent anther sterility locus as placed by linkage analysis. Marker names are given above the line on linkage maps with cumulative genetic distance below. Marker numbers without prefixes are MgSTS markers, aat261 is a microsatellite, and others shown are dominant AFLPs. The names of some unshared AFLPs on the on the F_{2N} map are not shown for clarity. Likelihood ratio (LR) traces are shown for separate corolla width QTL scans of the CSB_1 (—) and F_{2N} (. . .) populations. The experiment-wise LR significance thresholds ($\alpha = 0.05$; 9.1 and 9.6, respectively) were very similar in the two QTL scans and are indicated by a single dashed line (—).

plants were 20% wider than those of their sterile siblings ($mean_{fertile} = 23.33$ mm, $mean_{sterile} = 19.04$ mm, $F = 34.9$, $P < 0.0001$, $N = 180$). We did not measure other floral dimensions, but also noticed that CMS flowers often had distinctly exserted stigmas, which is not normally observed in either parental species. This suggests shortening of the corolla or lengthening of the gynoecium in addition to the reduction in flower width.

Mapping of the Nuclear Component of Cytonuclear Anther Sterility

Three markers identified in the initial bulk segregant screen (MgSTS-34, MgSTS-251 and MgSTS-574a) showed strong associations with anther fertility in the CSB_1 population (χ^2 tests for independence, all $P < 0.0001$). We located these markers on the *M. nasutus* × *M. guttatus* framework map (Fishman et al. 2001) by genotyping them in the F_{2N} mapping population ($N = 287$) and ordering them relative to previously mapped markers. Because all three markers mapped to a single linkage group (LG7), this demonstrates that a single major nuclear locus interacts with cytoplasmic genotype to cause hybrid anther sterility.

To map cytoplasm-dependent anther sterility within LG7, we genotyped the CSB_1 population at all codominant markers known to be on that linkage group from this and other mapping studies (L. Fishman, unpubl. data). Markers (including anther sterility coded as a marker genotype) were ordered in Mapmaker using standard settings and the Kosambi mapping function. This analysis placed the cytoplasm-dependent anther sterility locus between MgSTS-574a and the end of the LG7, at a distance of ~8 cM from the nearest marker mapped in the CSB_1 population (Fig 3).

The nature and position of the major nuclear locus involved in cytonuclear anther sterility was confirmed by analysis of the two independent CSB_4 populations descended from the CSB_1 mapping population. These populations carry the *M. guttatus* cytoplasm and are *M. nasutus* homozygotes at 93.75% of Mendelian loci, but were selected to maintain heterozygosity at nuclear loci with dominant M. guttatus alleles that restore fertility. As expected if cytonuclear anther sterility depends on a single locus, approximately 50% of each independent family was anther sterile (CSB_4-23 = 21/42, CSB_4-29 = 14/33). Of the MgSTS markers mapped to LG7 in the CSB_1 generation (see Fig. 3), only the four markers mapped to within 32cM of cytoplasm-dependent anther sterility locus were still segregating in these populations.

Mapping of Corolla Width QTL Relative to Anther Sterility

To further investigate the strong association between flower size and anther sterility observed in the CSB_1 generation, we implemented composite interval mapping (CIM) in the program WinQTLCart, using the LG7 linkage map including anther sterility as a marker locus. A QTL scan of LG7 located a highly significant corolla width QTL with its peak at the anther sterility locus (Fig. 3).

There are three possible sources of the strong corolla width QTL signal on LG7: (1) a pleiotropic effect of cytoplasm-dependent anther sterility itself, (2) a cytoplasm-dependent floral QTL linked to the locus involved in anther sterility, or (3) a cytoplasm-independent floral QTL linked to the anther sterility locus. To distinguish the two cytoplasm-dependent hypotheses from the third, we conducted a QTL scan of LG7 (including genotypes at linked MgSTS markers) in the 2001 F_{2N} mapping population. In contrast to the CSB_1 results, no corolla width QTL on LG7 was detected in the F_{2N} population (highest LR = 5.8, threshold = 9.6). To verify our power

for QTL detection in the F_{2N}, we also rescanned LG10, which contained the largest floral size QTL in our original F_{2N} analysis (Fishman et al. 2002). The effects of this QTL on flower size in the CSB_1 are similar in magnitude to those of cytoplasm-dependent anther sterility (i.e., *M. nasutus* homozygotes at a marker within 1 cM of the QTL peak are 24% smaller than heterozygotes, data not shown), so the LG7 and LG10 QTLs would be equally detectable in the *M. nasutus* cytoplasmic background if both present. The major corolla width QTL on LG10 was again highly significant (LR = 30.6, threshold = 9.6, map not shown) indicating no lack of power for QTL detection in the reanalyzed F_{2N} population. Together, these data suggest that the major flower size QTL associated with anther sterility in the CSB_1 population is due to either pleiotropic effects on floral development or the action of a linked gene with cytoplasm-specific effects.

Discussion

Negative interactions among genes, known as Dobzhansky-Muller incompatibilities, are thought to be the major source of sterility and inviability in interspecific hybrids (e.g., Orr 2005). Dobzhansky-Muller incompatibilities are generally viewed as interactions among nuclear loci, but epistasis between nuclear and cytoplasmic genotypes may also contribute to hybrid breakdown. Here, we demonstrated that cytoplasmic background has discrete effects on male fertility in hybrids between two closely related wildflowers, outcrosser *Mimulus guttatus* and selfer *M. nasutus*. In a common garden experiment with reciprocal F_1 and F_2 hybrids, we observed a novel anther sterile phenotype exclusively in the F_{2G} population carrying the *M. guttatus* cytoplasm. The sterile plants, which had stunted anthers and produced no pollen grains, comprised approximately one-fourth of the F_{2G} population, suggesting segregation at a single nuclear locus with cytoplasm-specific effects on anther development. Anther sterility segregated 50:50 in a backcross population (CSB_1) with *M. guttatus* cytoplasm and *M. nasutus* as the recurrent parent, suggesting that two (recessive) *M. nasutus* alleles at a single locus were necessary for the expression of anther sterility. We then confirmed this inference by mapping anther sterility to within 8 cM of a marker at one end of LG7 of the hybrid linkage map. Advanced generation backcrosses demonstrated that a single dominant *M. guttatus* allele at this single locus *alone* was sufficient to restore anther fertility to carriers of the sterile *M. guttatus* cytoplasm. Accordingly, we name the nuclear locus contributing to the cytonuclear incompatibility *Restorer-of-male-fertility* (*RMF*), with dominant restorer allele *Rmf* in *M. guttatus* (IM62 line) and nonrestorer allele *rmf* in *M. nasutus* (SF line).

Evolutionary Origins of Cytonuclear Anther Sterility

We can imagine two general scenarios for the evolution of cytoplasm-dependent hybrid male sterility. The first scenario does not limit the mechanisms leading to fixation of the interacting components. Imagine an ancestral population with the cytoplasmic-RMF genotypic combination C *Rmf*/*Rmf*, which is fertile. One lineage (leading to the *M. guttatus* accession used in this study) fixes a new cytoplasmic genotype C′ and remains fully fertile (C′ *Rmf*/*Rmf*). Meanwhile, an allopatric lineage leading to our *M. nasutus* parent fixes a mutant nuclear allele *rmf*, also remaining fertile (C *rmf*/*rmf*). (This scenario could also involve the serial fixation, in Iron Mountain *M. guttatus* but not *M. nasutus*, of the *Rmf* allele and the *C′* cytoplasm, which would have the same dynamics.) Under this scenario, a novel *M. guttatus* element (C′) and a uniquely *M. nasutus* element (*rmf*) are both necessary to cause anther sterility; only when the novel genotypic combination C′ *rmf*/*rmf* segregates out in interspecific hybrids is male fertility lost. Consequently, the evolution of the incompatibility need not have involved strong selection to overcome the individual fitness costs of complete male sterility.

A second scenario arrives at the same genotypic outcomes via a different path. In this case, the ancestral population is C *rmf*/*rmf*. The lineage leading to our *M. guttatus* parent first acquires a novel cytoplasmic genotype (C′) that causes complete male sterility in the *rmf*/*rmf* background, and subsequently fixes a dominant nuclear restorer allele *Rmf* that returns the population to a uniformly hermaphroditic state (C′ *Rmf*/*Rmf*). Meanwhile, the allopatric lineage leading to our *M. nasutus* parent maintains the ancestral genotype. Under this model, hybridization with *M. nasutus* recreates a cytoplasmically male-sterile genotype (C′ *rmf*/*rmf*) historically present in the *M. guttatus* population. Thus, this scenario requires the *M. guttatus* population have passed through the potentially deep fitness valley of high frequencies of anther sterility.

The first scenario, which entails no fitness costs and could result from drift or ordinary natural selection, cannot be ruled out based on the data presented here. However, despite the individual fitness costs associated with the complete loss of male function, the second scenario is also possible (and even likely) when there is strong natural selection for a male-sterile cytoplasm. In fact, the fixation of cytoplasmic mutations causing male sterility appears to be theoretically favored under broad conditions (Lewis 1941; Charlesworth 1981), and the spread of such mutations is a classic example of selfish evolution (e.g., Zeh and Zeh 2005). Because organellar genomes are typically maternally inherited, they accrue fitness only through successful ovules (seeds), whereas autosomal nuclear genes benefit through both ovule and pollen success. Thus, a cytoplasmic male sterility (CMS) mutation can spread rapidly, despite decreasing total individual fitness, if CMS individuals have even slightly higher fitness through female function than hermaphrodites (Lewis 1941; Charlesworth 1981). In nature, this criterion of increased female fitness may be readily met by increased seed number due to reallocation of resources otherwise devoted to pollen production (Charlesworth and Charlesworth 1978) or, in self-compatible species, by increased seed quality due to avoidance of inbreeding depression by obligately outcrossing females (Charlesworth 1981). Therefore, in self-compatible species a CMS mutation will generally spread until CMS (female) individuals reach a frequency at which pollen limitation eliminates its transmission advantage through seeds (Lloyd 1974). However, as the selfish spread of the CMS mutant drives the population toward a female-biased sex ratio, nuclear mutations that restore male fertility become favored by selection for allocation to the minority sex (Frank 1989). Under re-

strictive theoretical conditions, the joint evolutionary dynamics of CMS mutations and nuclear restorer alleles can result in a stable restorer-CMS polymorphism (see Saur Jacobs and Wade [2003] and Bailey et al. [2003] for recent syntheses). Stable polymorphisms of hermaphrodites and females (male-steriles) result in gynodioecy, a mating system observed in ~5–10% of plant species (Darwin 1877). However, an alternative outcome appears far more theoretically likely: the CMS cytotype (which has an advantage over other cytotypes) and the restorer allele (which has an advantage over alternative alleles in the presence of CMS) jointly go to fixation, returning the population to a fixed hermaphroditic state. Of course, the selfish dynamics of CMS are limited to predominantly outcrossing species, such as *M. guttatus*, as selfers like *M. nasutus* would suffer both male and female fitness costs of male sterility.

In addition to the theory predicting spread and subsequent restoration of CMS under broad conditions (Frank 1989), there is evidence that many hermaphroditic plants harbor both male sterile cytoplasms and nuclear restorer alleles. In cultivated plants, breeders have long used interspecific or interstrain hybridization to generate male-sterile lines (Kaul 1988). Mismatch of restorer-CMS genotypes has been implicated in the male sterility of many such crosses (Laser and Lersten 1972; Kaul 1988) and detailed molecular investigations of a few systems reveal common genetic features (reviewed in Hanson and Bentolila 2004). In all known cases, CMS results from a structural rearrangement of the mitochondrial genome that produces a novel chimeric open reading frame involving an essential respiratory gene. It is thought that the chimeric gene product is either directly toxic to pollen or has reduced function that compromises the high energy demands of pollen development. Restoration of fertility generally involves post-transcriptional processing of the chimeric CMS transcripts or resultant proteins (Schnable and Wise 1998).

Until recently, study of the molecular mechanisms of cryptic CMS has focused exclusively on crop plants and few CMS restorer loci have been genetically mapped in natural populations. Conversely, empirical studies of CMS in wild species have focused almost entirely on the dynamics of restorer polymorphism and gynodioecy, rather than the causes or consequences of restored CMS in hermaphroditic species (but see Dudle et al. [2001] and Barr [2004] for cases of fixed CMS in hermaphroditic populations of gynodioecious species). However, it is generally accepted that gynodioecy and the cryptic CMS revealed in crop hybrids share an evolutionary basis (e.g., Charlesworth 2002; Budar et al. 2003; Hanson and Bentolila 2004). The morphological effects, inheritance pattern, and population genetic context (see below) of the cytoplasm-dependent hybrid incompatibility identified in this study are similarly consistent with the exposure of cryptic CMS within the Iron Mountain population of *M. guttatus*. Because the cryptic CMS scenario has interesting evolutionary implications, we explore it in more detail in the next sections.

Nature of Cryptic CMS within the Iron Mountain M. guttatus Population

If the cytonuclear incompatibility observed in our interspecific hybrids does reflect a history of selfish CMS and restoration within the Iron Mountain *M. guttatus* population, it provides novel insight into the nature of cytoplasmic male sterility in wild plants. Only a single inbred line of *M. guttatus* was used in this study, but it is likely that both the CMS cytoplasm and the dominant *Rmf* allele are fixed or near fixation in the Iron Mountain *M. guttatus* population. There is no evidence of gender polymorphism in this population, and gynodioecy has never been recorded in any population of *Mimulus guttatus* or, to our knowledge, in any member the genus *Mimulus*. A field survey of floral variation at the Iron Mountain site, including measures of stigma-anther separation, noted only 2/409 (0.5%) plants with deformed anthers (L. Fishman, unpubl. data). These few deformities could be caused by unrestored CMS, but could also reflect other genetic or environmental effects. Because the Iron Mountain population is primarily outcrossing (outcrossing rates 0.76–0.90; Willis 1993), however, we would expect it to be difficult to detect recessive nonrestorer alleles segregating at very low frequency in the field. Plants producing no pollen have been observed upon serial inbreeding of individuals from this population (Willis 1999), and homozygosity of rare nonrestorer alleles is one possible source of such inbreeding depression of male fertility.

In this system, the *Rmf* allele carried by the *M. guttatus* parent appears completely dominant to the nonrestoring *M. nasutus* allele. Although it is not necessarily the case that the *M. nasutus* allele assayed here is similar in expression to the ancestral *M. guttatus* nonrestoring allele, the observed dominance relationship is consistent with theoretical expectations about the spread of restorer alleles. In general, model of the dynamics of CMS/restoration suggest that completely dominant restorers fix more readily than those with incomplete dominance (e.g., Lewis 1941). Interestingly, most of the cryptic CMS-restorer systems that have been genetically characterized have single dominant restorers (e.g., *Petunia* and radish; Hanson and Bentolila 2004), but gynodioecious species frequently exhibit quite complex inheritance of CMS-restoration as well as multiple CMS types within populations (e.g., Koelewijn and Van Damme 1995; Charlesworth and Laporte 1998; Taylor et al. 2001). This may in part reflect a detection bias, but the simple inheritance pattern found in this study lends additional support to the idea that the outcome of CMS invasion may be in part contingent upon the dominance of available restorer mutants.

In addition to its qualitative effects on anther morphology and pollen production, the CMS revealed in our interspecific hybrids was strongly associated with quantitative changes in floral morphology. In the CSB_1, anther-sterile flowers were, on average, 20% smaller than fertile flowers and a significant QTL for corolla width was located at RMF (Fig. 3). This genomic region had no effect on corolla width in a parallel QTL analysis of our original F_{2N} population, so the reduced flowers appear to be either a direct pleiotropic effect of expressed CMS or the effect of a linked QTL with cytoplasm-specific expression. In some wellcharacterized examples of cryptic CMS, anther-sterile flowers may appear otherwise normal (e.g., *Petunia*, sunflower), but in other cases (e.g., wheat, carrot, radish, *Nicotiana*), other flower parts are also affected (Hanson and Bentolila 2004). Early disruption of pollen/anther development (perhaps through programmed

cell death of tapetal tissue; Schnable and Wise 1998) may have cascading effects on other aspects of floral morphology.

Finer-scale mapping will be necessary to determine whether the corolla size effects associated with anther sterility are due to pleiotropy of RMF or a linked locus, but either outcome would be interesting. If the flower size QTL is not a pleiotropic effect of RMF but a separate linked locus, our results raise the interesting possibility of cytoplasm-specific expression of floral variation independent of CMS. On the other hand, if the reduced corolla display we observe is a pleiotropic consequence of anther-sterility, it could complicate the invasion dynamics of CMS variants by decreasing attractiveness to pollinators. Although female fitness is not necessarily correlated with on display size, there is evidence in *M. guttatus* that bees preferentially visit relatively large flowers (Martin 2003) and that seed set in the Iron Mountain population is pollenlimited (L. Fishman and J. Willis, unpubl. data). Pollen limitation of female fitness may be an important contributor to the relative fitness of hermaphrodites and females in gynodioecious populations (e.g., McCauley 1997), and pleiotropic reductions in flower size could increase the severity of this cost to obligately outcrossing females during the initial spread of CMS. Additional female fitness advantages to CMS (in ovule production, for example) might be necessary to compensate for this reduction. Mapping the RMF locus allows us to genetically isolate the effects of cytonuclear incompatibility from other (unlinked) hybrid incompatibility loci in this system, which affect both male and female function (Sweigart et al. 2006). Thus, future experiments can directly assess the female fitness consequences of cytoplasm-dependent anther sterility, an essential test of whether or not increased transmission via female fitness (i.e., selfish evolution) is a plausible explanation for the existence of the Iron Mountain *M. guttatus* cytoplasmic type.

Can Cytonuclear Incompatibility Contribute to Postzygotic Barriers between Species?

Our results suggest that CMS may play an important role in generating patterns of hybrid sterility, adding support to the idea that sexual or genomic conflict can drive the evolution of hybrid breakdown (e.g., Frank 1991; Hurst and Pomiankowski 1991). However, not all hybrid incompatibilities necessarily generate long-term barriers to introgression between incipient species. Can cryptic CMS, which clearly has great potential to cause hybrid male sterility, also act as a postzygotic barrier to introgression between naturally hybridizing populations or species?

Because the initial fitness consequences can be quite severe (in this system ~50% of backcross individuals were completely male sterile), hybrid male sterility caused by exposed CMS may initially contribute to postzygotic reproductive isolation and affect the population dynamics of hybrid zones. It has been argued, for example, that introgression of a novel CMS type and resultant male sterility could contribute to population demographic instability or extinction in small populations (Barr 2004). Although no Dobzhansky-Muller interaction that does not cause complete F_1 hybrid inviability or sterility can act as a species barrier across the entire genome, such hybrid incompatibilities may also cause important barriers to introgression in local genomic regions. The significance of cytonuclear incompatibility as such a postzygotic barrier will depend on the evolutionary history of the interaction (selfish cytoplasmic evolution and restoration vs. a neutral or adaptive evolution), on the population genetics of the hybridizing species, and on the locus (nuclear or cytoplasmic) under consideration.

Like nuclear-nuclear Dobzhansky-Muller incompatibilities, cytonuclear incompatibilities should generate asymmetric barriers to introgression of the specific genomic regions involved. The parental lines used in this study are derived from allopatric populations (so we cannot directly test the incidence and consequence of cytonuclear hybrid sterility in the field), but there would no constraint on the introgression of the *M. nasutus* cytoplasm into the *M. guttatus* nuclear background or on the introgression of the *M. guttatus* restorer allele into an *M. nasutus* cytoplasmic background in a hybrid zone between these genotypes. The opposite introgressions would be constrained and the potential strength of each barrier reflects the specific biology of these species. In our system, data from sympatric sites suggests that natural F_1 hybrids primarily form via pollen transfer from *M. guttatus* to *M. nasutus* (i.e., are F_{1N} hybrids; Martin 2004). Thus, if a hybridizing *M. guttatus* population contained a cryptic CMS-restorer system, backcrossing to *M. guttatus* by the F_{1N} as a pollen parent and further interbreeding or selfing would be necessary to form the anther-sterile genotypic combination. Thus, a cytonuclear incompatibility would play a relatively minor role in the dynamics of hybridization over the short term and be a fairly weak barrier to introgression. In such a situation, the introgressed *M. nasutus rmf* allele would essentially act as a deleterious recessive allele causing inbreeding depression of male fertility in *M. guttatus*-like advanced generation hybrids, and would be maintained at a low frequency dependent on the rate of hybridization.

In contrast to the nuclear locus, introgression of the Iron Mountain *M. guttatus* cytoplasm into *M. nasutus* would be highly constrained. Regardless of its evolutionary history, this cytoplasmic genotype causes male sterility against a homozygous (*rmf*/*rmf*) *M. nasutus* nuclear background. In a primarily selfing species such as *M. nasutus*, male sterility also directly reduces seed set, making the fitness costs of introgression particularly high. This tight coupling between male and female fertility in *M. nasutus* should act as a complete barrier to the introgression of a male-sterile cytoplasm from *M. guttatus*.

The previous discussion of barriers and introgression focuses on the dynamics of gene flow between selfers and outcrossers, as would occur in our *Mimulus* system. However, cytonuclear mismatches may be one of the earliest forms of hybrid incompatibility to evolve between plant populations in general (Levin 2003). In particular, allopatric populations of outcrossing species may readily fix alternative CMS-restorer systems or, as we argue for *Mimulus*, one population may fix a CMS-restorer whereas another fixes neither. Because the spread of cytoplasmic genotypes among populations is dependent on dispersal via seeds, which are often considerably more restricted than pollen dispersal (e.g., McCauley and Taylor 1997), the scale of such variation in cytonuclear epistasis may be quite local. Thus, cryptic CMS

may be a common source of both asymmetric (Tiffin et al. 2001) and non-asymmetric hybrid incompatibility upon secondary contact.

In pairs of outcrossing taxa, the longer-term consequences of cytonuclear incompatibility for introgression will fundamentally depend on its evolutionary history within populations. If a cytonuclear incompatibility has arisen as a byproduct of neutral or adaptive divergence, the short-term and long-term dynamics in outcrossers should resemble those described above for hybridizing *M. nasutus* and *M. guttatus* (except that the sterile genotypic combination would be formed more frequently). However, if the incompatibility is due to CMS that arose via selfish cytoplasmic evolution in one or both taxa, the long-term dynamics might be quite different. The same features that contribute to the rapid evolution of cryptic CMS also constrain its potential as long-term stable postzygotic barrier to introgression. In many natural populations, the postzygotic cost of male sterility may not be sufficient to prevent the introgression of a foreign CMS (with a female fitness advantage) into a hybridizing non-CMS or noncomplementary CMS population. The long-term dynamics will depend, as with the spread of a novel CMS mutant, on the magnitude of the female fitness advantage of the CMS cytotype relative to alternative cytotypes. However, because the restorer allele is already present at high frequency in hybrids, a likely outcome may be the rapid introgression of the matched nuclear restorer allele along with the CMS cytotype. Thus, hybrid male sterility caused by exposure of selfish CMS may actually accelerate introgression in secondarily hybridizing populations/species, as has been suggested for other selfish elements (e.g., Hurst and Schilthuizen 1998).

Further work will be necessary to determine whether the cytonuclear incompatibility expressed as anther sterility in our *Mimulus* hybrids arose through selfish cytoplasmic evolution or as a byproduct of ordinary natural selection or drift. In either case, our results suggest that epistasis between male-sterilizing cytoplasmic genomes and nuclear loci that restore or maintain male function may occur within entirely hermaphroditic plant populations as well as gynodioecious species. The simple genetics of the heterospecific incompatibility described here will facilitate controlled estimation of the female fitness costs and benefits of cytoplasmic male sterility, and may allow detailed investigation of the molecular mechanisms and potential costs of male fertility restoration in a system without CMS-restorer polymorphism. Understanding the evolutionary history of both elements promises to shed further light on the role of selfish evolution in the development of hybrid incompatibilities and on the nature of cytonuclear coevolution within species.

Acknowledgments

We thank N. Martin for allowing us to nest the reciprocal F_2 phenotyping within his common garden experiment and for sharing his unpublished results; D. Pedersen for her expert help with genotyping; A. Case, M. Hall, Y. W. Lee, N. Martin, L. Moyle, A. Sweigart, and other past and present members of the Willis lab for stimulating discussions of this research; and the greenhouse staff at Duke University and E. Peters for plant care. We are grateful to J. Kohn and two anonymous reviewers for their comments on earlier versions of this manuscript. This research was supported by National Science Foundation grants DEB-0075704, DEB-0316786, BIO-0328326, and DBI-0321329.

Literature Cited

Bailey, M. F., L. F. Delph, and C. M. Lively. 2003. Modeling gynodioecy: novel scenarios for maintaining polymorphism. Am. Nat. 161:762–766.

Barr, C. M. 2004. Hybridization and regional sex ratios in *Nemophila menziesii*. J. Evol. Biol. 17:786–794.

Bateson, W. 1909. Heredity and variation in modern lights. Pp. 85–101 *in* A. C. Steward, ed. Darwin and modern science. Cambridge Univ. Press, Cambridge, U.K.

Budar, F., P. Touzet, and R. De Paepe. 2003. The nucleo-mitochondrial conflict in cytoplasmic male sterilities revisited. Genetica 117:3–16.

Charlesworth, D. 1981. A further study of the problem of the maintenance of females in gynodioecious species. Heredity 46:27–39.

———. 2002. What maintains male sterility factors in plant populations? Heredity 89:408–409.

Charlesworth, D., and B. Charlesworth. 1978. A model for the evolution of dioecy and gynodioecy. Am. Nat. 112:975–997.

Charlesworth, D., and V. Laporte. 1998. The male sterility polymorphism of *Silene vulgaris*: analysis of genetic data from two populations and comparison with *Thymus vulgaris*. Genetics 150: 1267–1282.

Darwin, C. R. 1877. The different forms of flowers on plants of the same species. Murray, London.

Diaz, A., and M. R. Macnair. 1999. Pollen tube competition as a mechanism of prezygotic reproductive isolation between *Mimulus nasutus* and its presumed progenitor *M. guttatus*. New Phytol. 144:471–478.

Dobzhansky, T. 1937. Genetics and the origin of species. Columbia Univ. Press, New York.

Dudle, D. A., P. Mutikainen, and L. F. Delph. 2001. Genetics of sex determination in the gynodioecious species *Lobelia siphilitica*: evidence from two populations. Heredity 86:265–276.

Edmands, S., and R. S. Burton. 1999. Cytochrome C oxidase activity in interpopulation hybrids of a marine copepod: A test for nuclear-nuclear or nuclear-cytoplasmic coadaptation. Evolution 53:1972–1978.

Fenster, C. B., and D. E. Carr. 1997. Genetics of sex allocation in *Mimulus* (Scrophulariaceae). J. Evol. Biol. 10:641–661.

Fishman, L., and J. H. Willis. 2001. Evidence for Dobzhansky-Muller incompatibilities contributing to the sterility of hybrids between *Mimulus guttatus* and *M. nasutus*. Evolution 55: 1932–1942.

Fishman, L., A. Kelly, E. Morgan, and J. H. Willis. 2001. A genetic map in the *Mimulus guttatus* species complex reveals transmission ratio distortion due to heterospecific interactions. Genetics 159:1701–1716.

Fishman, L., A. J. Kelly, and J. H. Willis. 2002. Minor quantitative trait loci underlie floral traits associated with mating system divergence in *Mimulus*. Evolution 56:2138–2155.

Frank, S. A. 1989. The evolutionary dynamics of cytoplasmic male sterility. Am. Nat. 133:345–376.

———. 1991. Divergence of meiotic drive-suppression systems as an explanation for sex biased hybrid sterility and inviability. Evolution 45:262–267.

Gavrilets, S. 2003. Models of speciation: what have we learned in 40 years? Evolution 57:2197–2215.

Hanson, M. R., and S. Bentolila. 2004. Interactions of mitochondrial and nuclear genes that affect male gametophyte development. Plant Cell 16:s154–s169.

Henikoff, S., K. Ahmad, and H. S. Malik. 2001. The centromere paradox: stable inheritance with rapidly evolving DNA. Science 293:1098–1102.

Hurst, G. D. D., and M. Schilthuizen. 1997. Selfish genetic elements and speciation. Heredity 80:2–8.

Hurst, L. D., and A. Pomiankowski. 1991. Causes of sex ratio bias may account for unisexual sterility in hybrids: a new explanation for Haldane's rule and related phenomena. Genetics 128: 841–858.

Kaul, M. L. H. 1988. Male sterility in higher plants. Springer, Berlin.

Koelewijn, H. P., and J. M. M. Van Damme. 1995. Genetics of male sterility in gynodioecious *Plantago coronopus*. II. Nuclear genetic variation. Genetics 139:1759–1775.

Lander, E. S., P. Green, J. Abrahamson, A. Barlow, M. Daly, S. Lincoln, and L. Newburg. 1987. MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181.

Laser, K. D., and N. R. Lersten. 1972. Anatomy and cytology of microsporogenesis in cytoplasmic male sterile angiosperms. Bot. Rev. 38:425–454.

Levin, D. A. 2003. The cytoplasmic factor in plant speciation. Syst. Bot. 28:5–11.

Lewis, D. 1941. Male sterility in natural populations of hermaphrodite plants. New. Phytol. 40:56–63.

Lloyd, D. G. 1974. Theoretical sex ratios in dioecious and gynodioecious angiosperms. Heredity 32:11–31.

Martin, N. H. 2003. Flower size preferences of honeybee (*Apis mellifera*) foraging on *Mimulus guttatus*. Evol. Ecol. Res. 6: 777–782.

Martin, N. H. 2004. Evolution, genetics, and maintenance of reproductive isolation in *Mimulus guttatus* and *M. nasutus*. Ph.D. diss. Duke University, Durham, NC.

McCauley, D. E. 1997. The relative contributions of seed and pollen movement to the local genetic structure of *Silene alba*. J. Hered. 88:257–263.

McCauley, D. E., and D. R. Taylor. 1997. Local population structure and sex ratio: evolution in gynodioecious plants. Am. Nat. 150: 406–419.

Muller, H. J. 1942. Isolating mechanisms, evolution and temperature. Biol. Symp. 6:71–125.

Orr, H. A. 1995. The population genetics of speciation: the evolution of hybrid incompatibilities. Genetics 139:1805–1813.

———. 2005. The genetic basis of reproductive isolation: insights from *Drosophila*. Proc. Natl. Acad. Sci. USA 102:6522–6526.

Orr, H. A., and S. Irving. 2005. Segregation distortion in hybrids between Bogota and USA subspecies of *Drosophila pseudoobscura*. Genetics 169:671–682.

Orr, H. A., and D. C. Presgraves. 2000. Speciation by postzygotic isolation: forces, genes, and molecules. Bioessays 22: 1085–1094.

Presgraves, D. C., L. Balagopalan, S. M. Abmayr, and H. A. Orr. 2003. Adaptive evolution drives divergence of a hybrid inviability gene between two species of *Drosophila*. Nature 423: 715–719.

Rand, D. M., R. A. Haney, and A. D. Fry. 2004. Cytonuclear coevolution: the genomics of cooperation. Trends Ecol. Evol. 19: 645–653.

Ritland, K. 1991. A genetic approach to measuring pollen discounting in natural plant populations. Am. Nat. 138:1049–1057.

Ritland, C., and K. Ritland. 1989. Variation of sex allocation among eight taxa of the *Mimulus guttatus* complex (Scrophulariaceae). Am. J. Bot. 76:1731–1739.

Sackton, T. B., R. A. Haney, and D. M. Rand. 2003. Cytonuclear coadaptation in *Drosophila*: disruption of cytochrome c oxidase activity in backcross genotypes. Evolution 57:2315–2325.

Saur, N., M. S. Jacobs, and M. J. Wade. 2003. A synthetic review of the theory of gynodioecy. Am. Nat. 161:837–851.

Schnable, P. S., and R. P. Wise. 1998. The molecular basis of cytoplasmic male sterility and fertility restoration. Trends Plant Sci. 3:176–180.

Sun, S., C. T. Ting, and C. I. Wu. 2004. The normal function of a speciation gene, Odysseus, and its hybrid sterility effect. Science 305:81–83.

Sweigart, A. L., and J. H. Willis. 2003. Patterns of nucleotide diversity are affected by mating system and asymmetric introgression in two species of *Mimulus*. Evolution 57:2490–2506.

Sweigart, A. L., L. Fishman, and J. H. Willis. 2006. A simple genetic incompatibility causes hybrid male sterility in *Mimulus*. Genetics 172:2465–2479.

Tao, Y., D. L. Hartl, and C. C. Laurie. 2001. Sex-ratio segregation distortion associated with reproductive isolation in *Drosophila*. Proc. Nat. Acad. Sci. USA 98:13183–13188.

Taylor, D. R., M. S. Olson, and D. E. McCauley. 2001. A quantitative genetic analysis of nuclear-cytoplasmic male sterility in structured populations of *Silene vulgaris*. Genetics 158:833–841.

Tiffin, P., M. S. Olson, and L. C. Moyle. 2001. Asymmetrical crossing barriers in angiosperms. Proc. Roy. Soc. Lond. B 26: 861–867.

Turelli, M., and H. A. Orr. 2000. Dominance, epistasis and the genetics of postzygotic isolation. Genetics 154:1663–1679.

Vickery, R. K., Jr. 1978. Case studies in the evolution of species complexes in *Mimulus*. Evol. Biol. 11:405–507.

Wang, S., C. J. Basten, and Z. -B. Zeng. 2005. Windows QTL cartographer 2.5. Deptartment of Statistics, North Carolina State University, Raleigh, NC.

Willett, C. S., and R. S. Burton. 2001. Viability of Cytochrome c genotypes depends on cytoplasmic backgrounds in *Tigriopus californicus*. Evolution 55:1592–1599.

Willis, J. H. 1993. Partial self-fertilization and inbreeding depression in two populations of *Mimulus guttatus*. Heredity 71: 145–154.

———. 1999. The role of genes of large effect in inbreeding depression in *Mimulus guttatus*. Evolution 53:1678–1691.

Wittbrodt, J., D. Adam, B. Malitschek, W. Maueler, F. Raulf, A. Telling, S. M. Robertson, and M. Schartl. 1989. Novel putative receptor tyrosine kinase encoded by the melanoma-inducing *Tu* locus in *Xiphophorus*. Nature 341:415–421.

Zeh, J. A., and D. W. Zeh. 2005. Maternal inheritance, sexual conflict, and the maladapted male. Trends Genet. 21:281–286.

Corresponding Editor: J. Kohn