Genetic Architecture of Male Sterility and Segregation Distortion in *Drosophila pseudoobscura* Bogota–USA Hybrids

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ABSTRACT Understanding the genetic basis of reproductive isolation between recently diverged species is a central problem in evolutionary genetics. Here, I present analyses of the genetic architecture underlying hybrid male sterility and segregation distortion between the Bogota and USA subspecies of *Drosophila pseudoobscura*. Previously, a single gene, *Overdrive* (*Ovd*), was shown to be necessary but not sufficient for both male sterility and segregation distortion in F_1 hybrids between these subspecies, requiring several interacting partner loci for full manifestation of hybrid phenomena. I map these partner loci separately on the Bogota *X* chromosome and USA autosomes using a combination of different mapping strategies. I find that hybrid sterility involves a single hybrid incompatibility of at least seven interacting partner genes that includes three large-effect loci. Segregation distortion involves three loci on the Bogota *X* chromosome and one locus on the autosomes. The genetic bases of hybrid sterility and segregation distortion are at least partially—but not completely—overlapping. My results lay the foundation for fine-mapping experiments to identify the complete set of genes that interact with *Overdrive*. While individual genes that cause hybrid sterility or inviability have been identified in a few cases, my analysis provides a comprehensive look at the genetic architecture of all components of a hybrid incompatibility underlying F_1 hybrid sterility. Such an analysis would likely be unfeasible for most species pairs due to their divergence time and emphasizes the importance of young species pairs such as the *D. pseudoobscura* subspecies studied here.

STUDYING the genetics and evolution of reproductive isolating barriers between populations is key to understanding speciation. Over the past two decades, we have gained a good understanding of various aspects of the evolution of reproductive isolation and made particularly rapid progress in our understanding of the genetics of intrinsic postzygotic isolation, *i.e.*, hybrid sterility and inviability (Coyne and Orr 2004). We have advanced from understanding several key comparative patterns characterizing intrinsic isolation—*e.g.*, Haldane's rule and the preferential sterility and inviability of hybrids of the heterogametic sex (Haldane 1922)—to identifying and characterizing the genes that cause hybrid sterility and hybrid inviability.

Bateson, Dobzhansky, and Muller independently described a model for the evolution of genetic hybrid incompatibilities that underlie intrinsic postzygotic isolation (Bateson 1909; Dobzhansky 1937; Muller 1942; Orr 1996). A hybrid incompatibility involves a negative epistatic interaction among genes from two species that causes hybrid sterility or hybrid inviability. Under the simplest version of their model, functional divergence at only two loci is sufficient to cause a hybrid incompatibility. Theory and empirical data suggest, however, that hybrid incompatibilities—particularly those involved in hybrid male sterility—may often be complex; *i.e.*, hybrids may have to carry the "correct" alleles at three or more loci for sterility or inviability to arise (Orr 1995; Davis and Wu 1996). This pattern is known as complex epistasis, as several alleles must come from the appropriate species for the manifestation of full hybrid sterility.

Despite the identification of several genes that cause hybrid sterility or inviability (Presgraves 2010), our understanding of the complex genetic architecture of hybrid incompatibilities is still lagging. Although a single incompatibility consisting of two genes can, in theory, cause postzygotic isolation, we have little understanding of how many incompatibilities typically separate species. Furthermore, we do not know how many partner genes typically interact in

single incompatibility. While mapping and identifying individual genes that cause hybrid sterility or inviability in species hybrids is extremely informative for many important questions in speciation, it provides few clues about the number and effect sizes of genes that take part in incompatibilities and the number of distinct incompatibilities that cause postzygotic reproductive isolation. Indeed, of all the hybrid incompatibility genes that have been identified so far, we do not have a single case where we know how many partner loci these genes interact with to complete the hybrid incompatibility.

According to theory and recent genetic evidence, hybrid incompatibilities accumulate between taxa faster than linearly with divergence, a pattern known as the "snowball effect" (Orr 1995; Matute *et al.* 2010; Moyle and Nakazato 2010). This rapid accumulation of genic incompatibilities continues even after reproductive isolation is complete. This process can, obviously, confound the interpretation of the number of genes that cause intrinsic reproductive isolation. In particular, it is difficult to say which incompatibilities were important during speciation *vs.* which accumulated after the completion of reproductive isolation. It is clear that the genetic dissection of interacting partner genes that form Dobzhansky–Muller incompatibilities in young species is crucial to gaining an understanding of the genetic architecture and evolution of hybrid sterility.

Drosophila pseudoobscura pseudoobscura (hereafter USA) and *Drosophila pseudoobscura bogotana* (hereafter Bogota) provide a powerful system for addressing these questions. USA and Bogota are young subspecies estimated to have diverged between 150,000 and 230,000 years (Schaeffer and Miller 1991; Wang *et al.* 1997). The USA and Bogota subspecies are geographically separated by >2000 km: the USA subspecies is distributed across western North America, whereas the Bogota subspecies is found in regions of high elevation near Bogota, Colombia (Dobzhansky *et al.* 1963). These taxa are incompletely reproductively isolated: hybrid F_1 males from Bogota mothers are sterile, whereas reciprocal hybrid males and hybrid females from both directions of the cross are fertile (Prakash 1972). There is no detectable prezygotic isolation between these subspecies (Noor 1995).

The genetic basis of hybrid male sterility between these subspecies involves a small number of chromosomal regions that interact in a complex pattern (Orr and Irving 2001). Regions on the *XL* and *XR* from Bogota (Muller elements A and D, respectively) were found to interact with dominant factors on the second and third autosomes from USA (Muller elements E and C, respectively) to cause hybrid sterility (Orr and Irving 2001). The fourth autosome and fifth dot chromosome (Muller elements B and F, respectively) have no detectable effect on postzygotic isolation. None of the important regions shows much effect singly on hybrid sterility: all interacting partners must be simultaneously present for the full expression of hybrid sterility.

More recently, Bogota–USA hybrid F_1 males were discovered to be slightly fertile (Orr and Irving 2005). Orr and Irving (2005) found that "sterile" F_1 hybrid males become very weakly fertile when aged. Surprisingly, these F_1 hybrid males produce almost all daughters. Several lines of evidence show that this sex-ratio distortion is caused not by hybrid male inviability, but by an overrepresentation of *X*-bearing sperm among the functional gametes of hybrid males (Orr and Irving 2005). The precise stage at which this segregation distortion arises is unclear. The genetic basis of segregation distortion appears similar to that of hybrid male sterility (Orr and Irving 2001, 2005; Phadnis and Orr 2009). Hybrid segregation distortion involves the same approximate regions on the Bogota *XL* and *XR*, but the effects of particular autosomes have not been characterized (Orr and Irving 2005). Pure Bogota individuals show normal segregation because they are homozygous for recessive autosomal suppressors.

Phadnis and Orr (2009) performed fine-scale mapping of the *sepia* (*se*) region on Bogota *XR*—a region that has a large effect on both male sterility and segregation distortion in the hybrid F_1 males. Through a series of recombination mapping and transgenic experiments in *Drosophila pseudoobscura*, they showed that a single gene, *Overdrive* (*Ovd*), plays a causal role in both hybrid sterility and hybrid segregation distortion (Phadnis and Orr 2009). These results indicate that hybrid male sterility and segregation distortion in Bogota–USA hybrids share a common genetic basis, raising the possibility that genetic conflict involving segregation distorters and their suppressors may drive the evolution of hybrid sterility.

Ovd is necessary but not sufficient to cause hybrid male sterility or segregation distortion; it requires the "correct" alleles at several interacting loci to yield a strong hybrid incompatibility. The number and locations of these interacting partners of *Ovd* that are essential for both hybrid phenotypes remain unknown. Indeed, we cannot even be sure about the number of relevant loci on the Bogota *X*—the best-studied chromosome in this hybridization—that are involved in hybrid sterility. Even less is known about the genetic basis of segregation distortion in hybrids and its suppression in pure Bogota individuals.

Here, I perform mapping of hybrid sterility and hybrid segregation distortion genes on the *X*, second, and third chromosomes, chromosomes previously shown to be important for hybrid sterility and segregation distortion (Orr and Irving 2001, 2005). I perform two independent crosses and analyses for the *X* and the autosomes, using different mapping strategies that are optimal for each chromosome.

My study departs from and builds on previous analyses in several ways. First, whereas previous studies were limited by the small number of visible markers available in *D. pseudoobscura*, I use molecular markers to increase marker density. Second, I employ distinct crossing designs that capitalize on information about gene locations from previous studies, allowing me to focus on mapping genes that cause F_1 hybrid phenomena and to avoid the confounding effects of any recessively acting genes that appear in F_2 or backcross-like genotypes. Complex epistatic interactions,
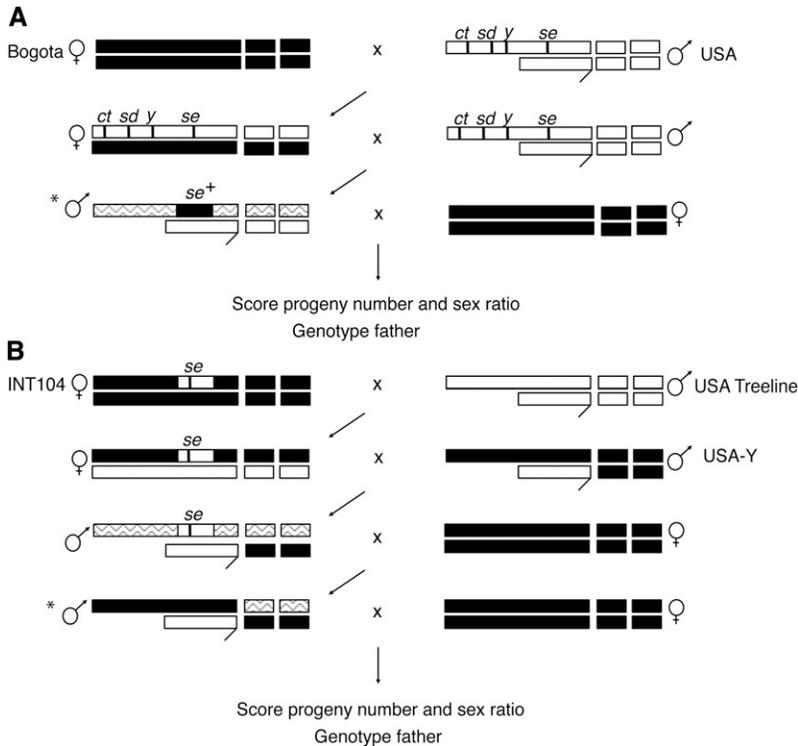
Figure 1 Crossing designs for mapping loci that cause male sterility and segregation distortion in F_1 hybrids between *D. pseudoobscura* Bogota and USA. Bogota material is solid, USA material is open, and recombinant chromosomal regions are shaded. The large metacentric *X* chromosome of *D. pseudoobscura* is denoted as a long bar, the *Y* chromosome as a hooked bar, and the autosomes as small bars. Only the second and third autosomes are shown for simplicity; the fourth autosome and the fifth dot chromosome play no role in postzygotic isolation between these subspecies (Orr and Irving 2001). (A) Crosses for mapping loci on the Bogota *X* chromosome. The focal males (indicated by *) bear one set of unrecombined USA autosomes that carry dominant sterility factors, Bogota material at *se*, and a recombinant *X* chromosome. (B) Crosses for mapping loci on the autosomes. The focal males (indicated by *) bear an unrecombined Bogota *X* chromosome, a USA *Y* chromosome, an entire set of unrecombined Bogota autosomes, and a set of recombined autosomes. INT104 is an introgression line that carries USA material near *se* in an otherwise Bogota genetic background. This *se* introgression, which includes *Ovd*, is used to make sure that males in the intermediate crossing generations are fertile. The Y^{USA} stock has a USA *Y* chromosome in an otherwise Bogota genetic background.

such as those that may underlie hybrid sterility, can dramatically reduce mapping power. I therefore use two separate crossing designs to exert greater control over the mapping population and thus provide an increase in mapping power—despite the lack of good balancer chromosomes in *D. pseudoobscura*. Finally, while previous studies used sperm motility to measure hybrid fertility, I count progeny. This allows for the simultaneous mapping of hybrid sterility and hybrid segregation distortions in single crosses and allows comparison with earlier findings. It also allows me, for the first time, to address the relationship between hybrid sterility and hybrid segregation distortion on the autosomes. From these studies, a coherent picture emerges that implicates three major-effect loci (and four minor-effect loci) on Bogota *X* chromosome and USA autosomes as the genetic basis of hybrid sterility. I also find a strong, but not perfect, overlap in regions implicated in segregation distortion, confirming previous findings for a shared genetic basis of both hybrid phenotypes.

Materials and Methods

Fly stocks

All flies were maintained and crosses performed at 22° using standard cornmeal–sugar–yeast–agar food. Most strains used are as in Orr and Irving (2001, 2005). Mapping of *X*-linked factors was performed between the Bogota-ER strain and a USA strain with a multiply marked *X* chromosome *cut* (*ct*; 1–22.5), *scalloped* (*sd*; 1–43.0), *yellow* (*y*; 1–74.5), and *se* (1–156.5). Mapping of autosomal genes was performed between Bogota-ER and Treeline *Lobe* (*L*; 3–13.3) strain

from USA. These strains were chosen as they carry collinear third chromosomes (*Treeline* arrangement), allowing free recombination along the entire third chromosome between Bogota and USA.

Autosomal mapping also involved a *se* introgression line, INT104, produced in previous work (Phadnis and Orr 2009), and a newly constructed Y^{USA} stock. Briefly, INT104 is a *se* introgression line that derives its genetic material almost entirely from Bogota except for a small region near *se*, which is derived from USA (Phadnis and Orr 2009). Hybrid males produced by crossing INT104 females to USA males are completely fertile and show normal segregation as they carry the USA allele at *Ovd*, near *se*. The Y^{USA} stock was constructed by first crossing males from a USA strain with individually marked chromosomes *y* (1–74.5), *glass* (*gl*; 2–83.3), *orange* (*or*; 3–0.0), and *incomplete* (*inc*; 4–0.0) males to INT104 females. Multiple lines were established by repeatedly backcrossing the resulting males (carrying unrecombined autosomes) to Bogota females for 10 generations. The Y^{USA} stock has a genome entirely derived from Bogota, except the *Y* chromosome, which derives from USA. This was confirmed in two ways. First, I crossed virgin females from the Y^{USA} strain to *y*, *gl*, *or*, and *inc* males and confirmed that all major chromosomes were homozygous for Bogota (the fifth dot chromosome was unmarked). Second, I sequenced a *Y*-linked region and confirmed that it was derived from USA. In particular, I scored five diagnostic SNPs in the *Y*-linked gene *CG12218Y* using the forward primer 5′-GCAGTCGAAC CAGTGCAAT-3′ and the reverse primer 5′-GTGCGGGCAATG GATAAT-3′ (Carvalho and Clark 2005). Y^{USA} males are fertile and show normal sex-chromosome segregation.

Crosses

Male sterility in Bogota–USA hybrids was previously shown to involve complex epistasis between 5 and 15 loci (Orr and Irving 2001). Because highly epistatic interactions can dramatically reduce mapping power, separate crosses that yielded mapping populations enriched for individuals of appropriate genotypes were used to map the *X*-linked and autosomal loci. The mapping crosses were designed to take advantage of information about the genetics of hybrid sterility and hybrid segregation distortion from previous studies (Orr and Irving 2001, 2005; Phadnis and Orr 2009).

For *X*-linked mapping, Bogota-ER females were crossed to *ct sd y se* males (Figure 1A). The resulting F_1 hybrid females were backcrossed to *ct sd y se* males. Because *se* hybrid males carry USA material at *Ovd*, they are almost always fertile and show normal segregation regardless of genotype at other hybrid sterility loci (Phadnis and Orr 2009). Such males are therefore uninformative for mapping partner hybrid sterility and segregation distortion genes. Because *se* hybrid males are uninformative and constitute 50% of a backcross population, inclusion of these males in the mapping population can severely reduce mapping power. Therefore, only se^+ backcross hybrid males, which carry the Bogota material at *Ovd*, were scored for progeny number and sex ratio.

Mapping of dominant autosomal genes for hybrid sterility and segregation distortion requires a mapping population that has one set of recombinant autosomes along with a nonrecombinant Bogota *X* chromosome, a USA *Y* chromosome, and at least one copy of nonrecombinant Bogota autosomes. In most hybridizations, generating individuals of such a genotype is unfeasible because of the sterility of individuals in the intermediate generations of crosses. I circumvent this problem in producing the autosomal mapping population by utilizing the introgression line INT104 analogous to a hybrid fertility rescue mutation. More precisely, introgression line INT104, which carries a USA allele of *Ovd* in an otherwise Bogota genetic background, ensures that recombinant hybrid males in the penultimate generation are fertile (Figure 1B). The crossing scheme used here also produces males that are homozygous for the nonrecombinant Bogota autosomes, similar to pure Bogota individuals. Because these males are uninformative for mapping, they were excluded from the mapping population.

Fertility and segregation distortion assay

Five-day-old focal virgin hybrid males were mated singly to two three-day-old Bogota-ER virgin females. Males were frozen at −80° and females were discarded after 7 days. Progeny number and sex ratio were scored 21 days after the parents were removed.

Markers and genotyping

DNA extraction was performed using the protocol described by Gloor *et al.* (1993). Primers for amplification of micro-satellite loci were based on previously published marker locations (Ortiz-Barrientos *et al.* 2006; Chang and Noor 2007) or designed from the *D. pseudoobscura* whole-genome sequence (Richards *et al.* 2005) using the web-based program Tandem Repeats Finder (Benson 1999) and Primer 3 (Rozen and Skaletsky 2000) (Table S1). An attempt was made to distribute the markers evenly across linkage groups using previously published local recombination rate data (Ortiz-Barrientos *et al.* 2006). Fluorescently labeled primers were used to amplify microsatellite loci from the extracted DNA following a touchdown PCR protocol: 95° for 2 min; 5 cycles of 95° for 30 sec, 55° for 30 sec, 72° for 30 sec; 10 cycles of 95° for 30 sec, 52° for 30 sec, 72° for 30 sec; and 15 cycles of 95° for 30 sec, 49° for 30 sec, 72° for 30 sec. Up to five markers were multiplexed in single PCR reactions when possible. The PCR products were analyzed for fragment size using the Applied Biosystems 3730 Genetic Analyzer.

Recombination mapping

Genotype data were extracted using GeneMapper (Applied Biosystems) and confirmed by visual inspection. Linkage maps for the markers were constructed using JoinMap (Kyazma). Composite interval mapping (CIM) (Zeng 1994) was performed using Windows QTL Cartographer V 2.5 (Wang *et al.* 2011). Fertility was analyzed in two ways: as a continuous trait and as a binary trait (males producing no offspring *vs.* some offspring). The latter method produced data similar to sperm motility assays that are typically used in such experiments. Sex-ratio distortion is measured as the ratio of the number of females to total progeny number, and mapping was performed using data from males that produced five or more progeny. Because distortion is measured as a ratio of the number of females to the total number of progeny, data from crosses that produced very few progeny are obviously meaningless. Significance thresholds were calculated using permutations of data in QTL Cartographer ($P = 0.05$ and $n = 500$). Because only individuals that produced more than five progeny were used in the analysis for segregation distortion, the sample sizes for these analyses are smaller than those used for analysis of hybrid sterility. One consequence is reduced mapping power for segregation distortion relative to that for hybrid sterility. A total of 576 males were used for mapping the *X* chromosome, and 480 males were used for the autosomal mapping.

Results

Mapping X-linked Bogota factors

The *D. pseudoobscura X* is a large metacentric chromosome that constitutes nearly 40% of the entire genome. I mapped loci affecting hybrid sterility and segregation distortion on the Bogota *X* chromosome using 14 markers, 4 of which were visible, including *se*. The first striking observation of the mapping population prior to genotyping was that hybrid sterility is a nearly Mendelian trait. Nearly half of the
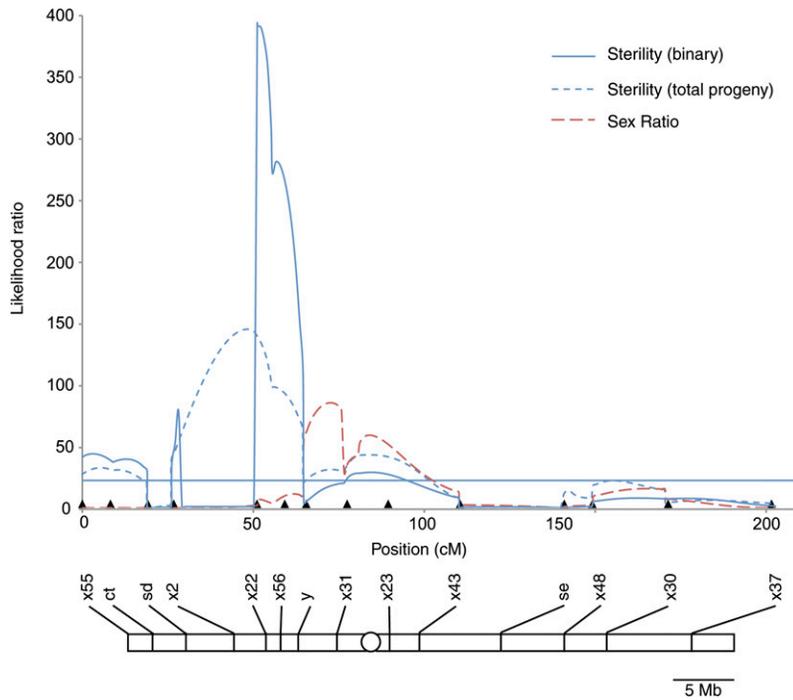
Figure 2 Mapping of loci on the Bogota *X* chromosome that affect fertility and segregation distortion in hybrids. The *X* chromosome of *D. pseudoobscura* is a large metacentric chromosome that represents 40% of the entire genome. Likelihood ratio (LR) test statistic profiles from CIM of male fertility and segregation distortion in the *X*-chrosomosome mapping population. All genotyped males are Bogota at the *sepia* locus to control for the effects of *Overdrive* (*Ovd*), and therefore this prominent locus does not appear in this analysis. Solid blue line represents mapping of hybrid sterility as a binary trait, dashed blue line represents mapping of hybrid sterility as a continuous trait based on total progeny numbers, and red dashed line represents mapping of segregation distortion loci. LR significance thresholds from permutation tests for all phenotypes were close to 25.00 and are shown as a single horizontal line for clarity. Marker locations are shown as solid triangles. Markers names are presented below their respective solid triangles, and their positions on the physical map are shown to scale. The centromere is represented as an open circle.

mapping population (which is controlled for the large effect of *Ovd* on *XR*) is completely sterile, indicating the possibility of a single large-effect locus affecting hybrid sterility. This large effect manifests in the CIM as a large peak tightly linked to the marker *X22* on *XL* (Figure 2). Indeed, when Bogota *Ovd* is present, *X22* is a strong predictor of whether a recombinant male is fertile or sterile; nearly all males that carry the Bogota allele at *X22* are sterile and vice versa. Previous studies showed a small, but nonsignificant, effect of the *ct* region (Orr and Irving 2001). Consistent with this previous report, I find this small but now statistically significant effect of the *ct* region in my analysis. I also detect two other small-effect loci near *X2* and *X23*, respectively. These results are consistent with previous studies (Orr and Irving 2001, 2005), where single-marker analysis revealed a large effect of *XL* on hybrid sterility. To simplify analysis and to render my results comparable with those from previous studies, I rely on CIM of *X*-linked loci that treat hybrid sterility as a binary trait. I conclude from these mapping studies that, in addition to *Ovd* on *XR*, there is one major-effect locus contributing to hybrid sterility on Bogota *XL* at *X22* and three loci of modest effect at *ct*, *X2*, and *X23* (Figure 2).

Analysis of segregation distortion reveals peaks near *X31* and *X23*, a region that flanks the centromere of the *X* chromosome and that also affects hybrid sterility. No peak for segregation distortion is detected near *X22*, the region that has the largest effect on hybrid sterility. This suggests that the genetic bases of hybrid sterility and segregation distortion may be separable at *X22*. However, it is important to note that mapping power to detect the segregation distortion effect of a locus that has a large effect on hybrid sterility is—essentially by definition—reduced in this experimental design. Put differently, if *X22* has a large effect on both

hybrid sterility and segregation distortion, then the progeny count data necessary to detect the effect of this locus on segregation distortion will be reduced as nearly all males carrying the Bogota allele at *X22* are sterile. My mapping results indicate that the genetic bases of hybrid sterility and segregation distortion are at least partially overlapping, but the extent of this overlap cannot be addressed further with this experimental design.

Mapping dominant USA autosomal factors

The second chromosome is the largest autosome in *D. pseudoobscura*. I mapped loci for hybrid sterility and segregation distortion on the second chromosome using seven markers. CIM reveals a single large-effect QTL for both hybrid sterility and segregation distortion at marker *2_390* (Figure 3A).

I also mapped loci for hybrid sterility and segregation on the third chromosome using 10 markers to genotype the autosomal mapping population. I detected one locus with a small effect on hybrid sterility between the markers *3001* and *3002* (Figure 3B). This region lies in the collinear part of the third chromosome that is not tied up in a chromosomal inversion difference. CIM does not detect regions with an effect on segregation distortion on chromosome 3.

I tested the patterns of epistasis between the loci on the second and third chromosomes (Figure 4). In presence of a Bogota *X* chromosome, the *2_390* region on the second chromosome alone has a large effect on hybrid sterility and segregation distortion. The *3001* region on the third chromosome has a small effect of hybrid sterility. Full expression of hybrid sterility and segregation distortion is seen when both second and third chromosome loci carry material from USA, highlighting the epistatic interactions between these loci.
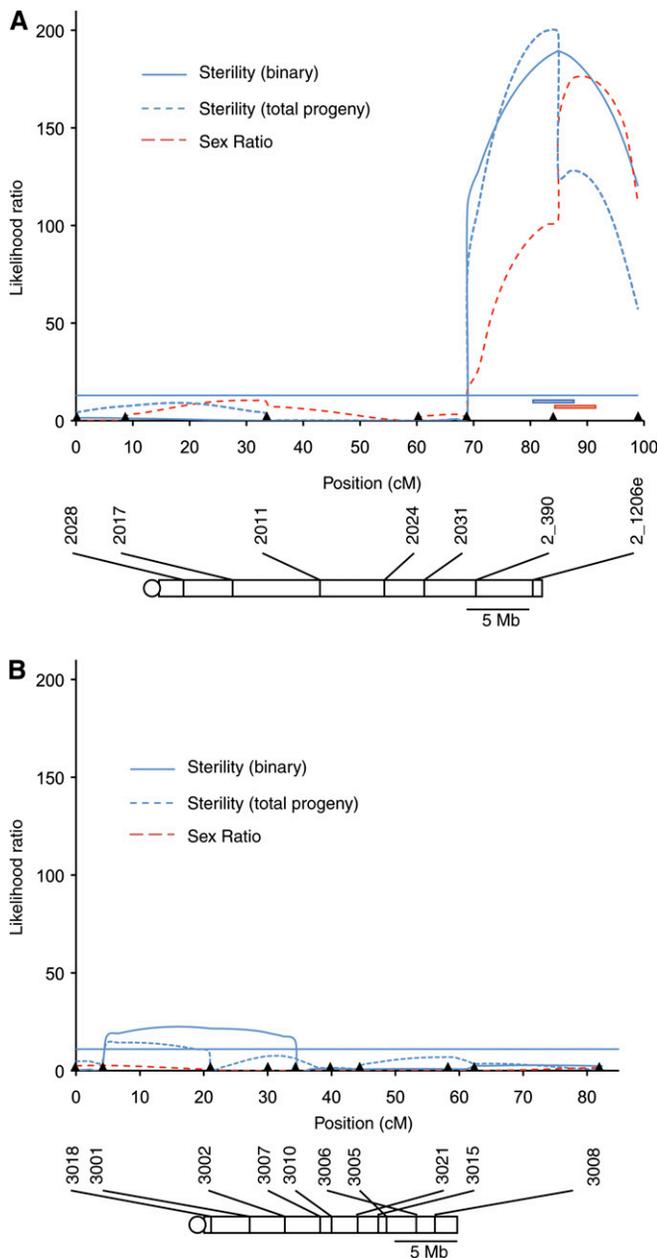
Figure 3 Mapping of dominantly acting USA autosomal loci. Likelihood ratio (LR) test statistic profiles from CIM of male fertility and segregation distortion in the autosomal mapping population. Solid blue line represents mapping of hybrid sterility as a binary trait, dashed blue line represents mapping of hybrid sterility as a continuous trait based on total progeny numbers, and red dashed line represents mapping of segregation distortion loci. LR significance thresholds from permutation tests for all phenotypes were around 10.00 and are shown as a single horizontal line for clarity. Conventions for marker and centromere locations are as in Figure 2. (A) Mapping of factors on the second chromosome. The blue and red bars show one standard deviation around the QTL for hybrid sterility and segregation distortion, respectively. (B) Mapping of factors on the third chromosome. A scrambling of marker order between *3006* and *3021* is observed because the linkage map is based on the *Treeline* arrangement in the mapping population and the physical map is based on the sequenced strain of *D. pseudoobscura*, which harbors the *Arrowhead* arrangement. *Treeline* and *Arrowhead* differ by multiple overlapping inversions on the distal part of the third chromosome.

Discussion

The Dobzhansky–Muller model elegantly describes the evolution of intrinsic reproductive barriers between species. Much effort has been devoted to identifying single genes involved in such incompatibilities, and several are now known. However, several outstanding questions remain about the structure of Dobzhansky–Muller interactions that separate species. In particular, it is difficult to say how many genetically independent incompatibilities typically cause postzygotic isolation between taxa and how many loci typically interact in a single incompatibility. Taken together with past studies (Orr and Irving 2001, 2005; Phadnis and Orr 2009), my results provide a more complete picture of the complex genetic architecture that underlies hybrid F_1 male sterility between the Bogota and USA subspecies of *D. pseudoobscura*. Bogota and USA are young taxa in the earliest stages of speciation, and the genes that cause sterility of hybrid males are essential for reproductive isolation between these taxa. We can map this set of genes that are essential for reproductive isolation and can say with some certainty how many incompatibilities underlie hybrid sterility, how many individual loci are involved in Dobzhansky–Muller interactions, and the extent to which hybrid sterility may share a genetic basis with segregation distortion in these young species (Figure 5).

A single Dobzhansky–Muller incompatibility causes hybrid F_1 male sterility between Bogota and USA: replacing an incompatible allele with a compatible allele at any single hybrid incompatibility locus results in restored fertility. For example, replacing the incompatible Bogota allele at *X22* in an F_1 hybrid male with a compatible USA allele results in nearly completely restored fertility. Similarly, replacing the incompatible USA allele at *2_390* in an F_1 hybrid male with a compatible Bogota allele results in nearly completely restored fertility. This single incompatibility involves a relatively modest number of loci that interact in a complex pattern. A total of seven loci—two large-effect loci (*X22* and *Ovd*) and three small-effect loci (*ct*, *X2*, and *X23*) on the Bogota X chromosome along with two dominantly acting loci on the USA autosomes (*2_390* and *3001*)—interact to cause hybrid F_1 male sterility (Figure 5). Interactions between these Bogota X chromosomal alleles and USA autosomal alleles form the basis of this hybrid incompatibility. The Bogota–USA hybridization is one of a few cases in which the Y plays little or no role in hybrid male sterility.

Two different explanations have been offered to explain the pattern of complex epistasis that often characterizes hybrid sterility. According to the first explanation, sterility is caused by the cumulative effects of a large number of factors, each of small effect (Naveira and Fontdevila 1986, 1991 Davis and Wu 1996). An extreme version of this hypothesis maintains that even the identities of the genes may not be important: sterility is caused when the amount of foreign genetic material crosses a threshold (Naveira and Fontdevila 1986, 1991). According to the second explanation, hybrid
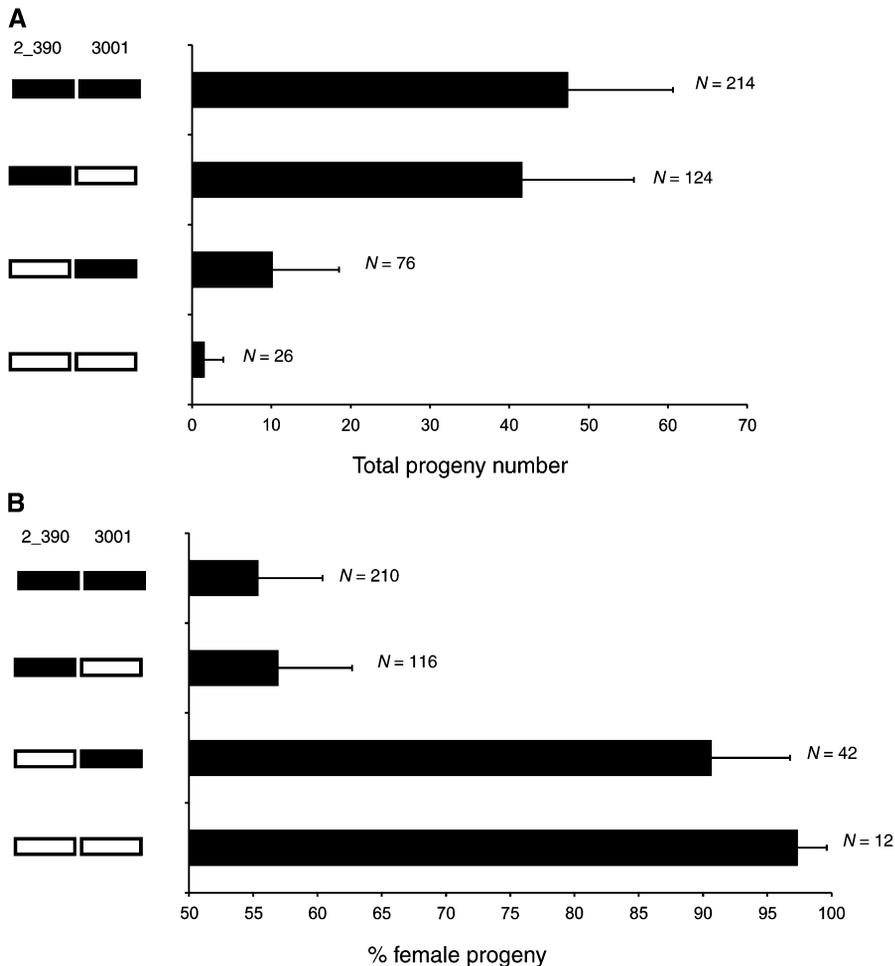
A

B

Figure 4 Pattern of conspecific epistasis between the autosomes. Bogota material is solid and USA material is open. *2_390* is a QTL on the second chromosome and *3001* is a QTL on the third chromosome. While the *2_390* region on the second has a large effect and the *3001* region on the third chromosome has a small effect on hybrid sterility and segregation distortion, the full expression of both phenotypes requires both regions from USA to be present simultaneously. (A) Pattern of epistasis for hybrid sterility. Fertility for each genotype is presented as the mean and standard deviation of the total progeny count. (B) Pattern of epistasis for hybrid segregation distortion. Segregation distortion for each genotype is presented as the mean and standard deviation of the percentage of female progeny.

sterility may be caused by the interactions of a few genes of large effect (Orr 1995). Here, I found a modest number of loci involved in hybrid sterility, with only three large-effect loci accounting for most of the effect (*X22*, *Ovd*, and *2_390*). These results support the idea that few genes of large effect may underlie hybrid male sterility. The number of incompatibilities is predicted to increase faster than linearly with time [the "snowball effect" (Orr 1995; Matute *et al.* 2010; Moyle and Nakazato 2010)]. It may, therefore, be difficult to tease apart the effects of individual hybrid sterility loci in older taxa, where many distinct incompatibilities may underlie

postzygotic isolation. This further highlights the importance of genetic analysis of postzygotic isolation in very young taxa that are in the early stages of speciation.

In addition, my analyses find that the genetic basis of hybrid segregation distortion and its suppression also appears to be fairly simple. Sex-chromosome segregation distortion requires three loci on the *X* chromosome: loci near *X31* on *XL* and *X23* and *Ovd* on *XR* cause sex chromosome distortion. This distortion is almost completely suppressed by a recessive suppressor(s) near *2_390* on the Bogota second chromosome.
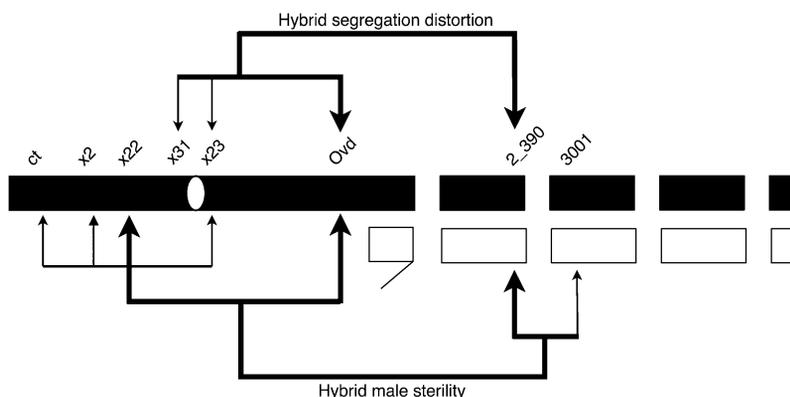

Figure 5 The genetic architecture of male sterility and segregation distortion in hybrid F$_1$ males between Bogota and USA. Hybrid male sterility is caused by loci on the Bogota *X* chromosome that interact with dominantly acting loci on the USA autosomes. Segregation distortion is caused by loci on the Bogota *X* chromosome; Bogota material at *2_390* acts as a recessive suppressor of this segregation distortion. Hybrid male sterility and segregation distortion show a partial overlap in their genetic bases. Bogota material is solid, and USA material is open. The large metacentric *X* chromosome of *D. pseudoobscura* is denoted as a long bar and the centromere as an open oval, the *Y* chromosome as a hooked bar, and the autosomes as small bars. Thick lines denote large-effect loci, and thin lines denote small-effect loci.

A comparison of QTL locations for hybrid sterility and segregation distortion suggests that the two phenotypes at least partially share their genetic bases. Although some loci, such as *ct* and *X22* on *XL*, appear exclusive to hybrid sterility, other loci such as *X23* on *XR* and *2_390* have an effect on both phenotypes. As noted earlier, however, mapping power for genes causing segregation distortion decreases in regions that have a strong effect on hybrid sterility in this experimental design. These results are consistent with the previously observed correlation between hybrid sterility and segregation distortion (Orr and Irving 2005; Phadnis and Orr 2009). It is important to note that, although loci that cause hybrid sterility and segregation distortion reside near each other, they need not always share exactly the same genetic basis. Instead, the genetic bases of the two hybrid phenomena may only partially overlap. Determining whether the same genes cause both hybrid sterility and segregation distortion will require fine mapping of individual loci and characterization of the effect of the genes on both phenotypes (*e.g.*, Phadnis and Orr 2009).

It is important to emphasize that the hybrid incompatibility loci studied here play an essential role in the sterility of hybrid F_1 males. Studies of genes that cause hybrid F_1 sterility or inviability—as opposed to those of recessive loci that cause problems in specific F_2-like genotypes—are rare (Barbash *et al.* 2003; Slotman *et al.* 2004; Brideau *et al.* 2006; Chang and Noor 2007; Phadnis and Orr 2009). Genome-wide introgression studies that rely on introducing single chromosomal regions from one species into another detect abundant recessive hybrid sterility loci, but miss dominant loci that underlie hybrid F_1 sterility (True *et al.* 1996; Tao *et al.* 2003; Masly and Presgraves 2007). A likely reason for this discrepancy is that complex conspecific epistasis between several genes may underlie F_1 hybrid sterility. More precisely, because multiple loci from the same species may be required together to complete a hybrid incompatibility, moving single chromosomal regions alone from one species into another may cause no F_1 hybrid sterility.

This problem can be seen more clearly in the light of the *D. pseudoobscura* Bogota–USA hybridization. For example, the Bogota alleles at *X22* and *Ovd* together have large effects on hybrid sterility, but have little or no effect when considered singly. Introgressing either region singly from Bogota into USA is not expected to show any hybrid phenotype. On the other hand, when only a single incompatibility is involved in postzygotic isolation, removing any single essential partner breaks the incompatibility and results in fully restored fertility or viability, *e.g.*, *Hmr* (Barbash *et al.* 2003) and *Lhr* (Brideau *et al.* 2006) in *D. melanogaster–D. simulans* hybrids and *Ovd* in Bogota–USA hybrids (Phadnis and Orr 2009). Also, Chang and Noor (2010) suggest that the dominance of loci that cause F_1 hybrid sterility may be modified by epistatic interaction with other Dobzhansky–Muller partners. If so, this phenomenon would also complicate the mapping of dominant partners involved in postzygotic isolation. This hypothesis would predict, for example, that homozygous USA material

at *3001* in an otherwise Bogota background would be sufficient to cause hybrid male sterility even in the absence of the USA material at *2_390*, and vice versa. Constructing introgression lines that move the autosomal regions at *2_390* and *3001* from USA into Bogota will allow a test of the hypothesis in this hybridization.

The locus near *2_390* on the second USA chromosome that acts dominantly to cause hybrid sterility in Bogota–USA hybrids is in the same region as a locus that causes hybrid sterility between *Drosophila persimilis* and *D. pseudoobscura* Bogota (Chang and Noor 2007). *D. persimilis* and *D. pseudoobscura* USA are sympatric species with incomplete reproductive isolation and are known to exchange genes, albeit rarely, in the collinear regions of the genomes (Wang *et al.* 1997; Noor *et al.* 2001). Because this locus near marker *2_390* has little or no effect on the sterility of hybrids between *D. persimilis* and *D. pseudoobscura* USA, genes in this region may be exchanged between these species with little opposition from natural selection (Noor *et al.* 2001). These observations raise the possibility that gene flow between *D. persimilis* and *D. pseudoobscura* USA may have led them to share alleles at the same gene(s) at *2_390* that cause hybrid sterility when crossed to Bogota (McDermott and Noor 2011). Higher-resolution mapping of this large-effect locus may determine whether the same gene(s) cause hybrid sterility in *D. pseudoobscura* Bogota–USA hybrids and in *D. persimilis–D. pseudoobscura* Bogota hybrids.

The study of individual components of hybrid incompatibilities underlying hybrid sterility and inviability has led to significant insights into the likely biological underpinnings of hybrid defects. Yet, studies of individual genes in hybrid incompatibilities have left unanswered important questions about the nature of the Dobzhansky–Muller hybrid incompatibility itself: How many genes are involved and what are their effect sizes? Indeed, given that hybrid incompatibilities accumulate with a snowball effect (Orr 1995; Matute *et al.* 2010; Moyle and Nakazato 2010), such a comprehensive view may be out of reach for all but the youngest diverged species pairs. My results lay the foundation for fine-mapping experiments to identify *all* the genes that interact with *Ovd* to cause male sterility and segregation distortion in hybrid F_1 males between the Bogota and USA subspecies of *D. pseudoobscura*. Since postzygotic isolation between Bogota and USA involves a single incompatibility with a modest number of interacting loci, identification and characterization of these genes will provide important insight into the molecular nature of postzygotic isolation between species.

Acknowledgments

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GENETICS

Genetic Architecture of Male Sterility and Segregation Distortion in *Drosophila pseudoobscura* Bogota–USA Hybrids

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Table S1 Primer sequences for markers used in this study.

Forward primer	Primer sequence	Reverse primer	Primer sequence	Location
NDPSX55F	GGTGATCCACCAAGCAACC	NDPSX55R	TCCAACGAGAGATAAAAAGAGG	XL_group1a: 6768563
NDPSX2F	AGTACGGTCCACCCCTTACC	NDPSX2R	GGAAATGGAAATGGAGAGCA	XL_group1e: 10966474
NDPSX22F	GTGCTGCACTCCGTTCGATA	NDPSX22R	CCAAAGTTGAAGGGTCCAGA	XL_group1e: 6553449
NDPSX56F	TCTCTCTCTGCATGCTCGAA	NDPSX56R	GATGGTTAAATTTAGGCGATGA	XL_group1e: 5892976
NDPSX31F	TGAGGCTCGCGAACTAAAAT	NDPSX31R	GGACATTCTGCCATGTCCTT	XL_group1e: 1271074
NDPSX23F	GTTGCTGTCGCCAGAGTACA	NDPSX23R	CATACTCCACCTCCTGCACA	XL_group3a: 2511422
NDPSX43F	CCCAAGGAAGGCAAGAAGTC	NDPSX43R	AGAATACCCCCAACGATTCC	XR_group6: 5826743
NDPSX48F	AAATTGCCCACGGAAAAAGT	NDPSX48R	ACCTGCGACCACAATTAAGC	XR_group6: 11342391
NDPSX30F	TATGCAGCCCACAAAGGATT	NDPSX30R	TTAGAGCGTTCCTTGCTGGT	XR_group8: 2789114
NDPSX037NF	CGAGAATGGTAGAAGGAGGAA	NDPSX037NR	GACTCGATGAAAGGCAAAGG	XR_group8: 5051028
DPS2028F	TCAGCCTCCGCTTCGATTG	DPS2028R	CGCCTACCTCGTACCTATACAGCAT	2:1518641
DPS2017F	ACCTCGCTTACCATTTTCCTCCA	DPS2017R	GGGAAATTTGTGCAGCTTGTGA	2:4790187
DPS2011F	ACTTGTCTGCAGCTGTCAGACAGA	DPS2011R	AATTGCACTTTGCGCTGATG	2:12296317
DPS2024F	TGTCCAAATCCCACGCAGAT	DPS2024R	ATGCTCCAAATGGCCGATG	2:18462645
DPS2031F	TGTTGACAATTTGGCGATACCC	DPS2031R	GCTGCCTCATTTGCATTGGTT	2:21656284
DPS2_390pF	AAACAAGGCAGAATCCCACACAAAC	DPS2_390pR	TTTTGTTTGCCAAATATGCTCGGCG	2:26679247
DPS2_1206eF	TTTTGCCATTTTATTGAAGGCTGCATCTG	DPS2_1206eR	ACGAAAGACCGTAAAGGAAAGGCAC	2:30776914
NDPS3018F	TCAGCCCACTCTGGAACTCT	NDPS3018R	CAGCAACAACTTGCAGCATT	3:321319
NDPS3001F	TAGCTTGCAGAGCGAGTGG	NDPS3001R	TCCAACAAGCACCGTAATTTT	3:2761278
NDPS3002F	ATGGCAACAATTTCCAAACC	NDPS3002R	TTGTGACCCGTTGACTACCA	3:5540542
			TTTGCAAGGGCACTAAAAGC	
DPS3007F	TTAAGCAGATGGGGGATGA	DPS3007R		3:9187186
NDPS3010F	GCTGACGGTACGATGGATTT	NDPS3010R	CGGTCAAAGAGGAAATGGAA	3:9917710
NDPS3006F	CAGAACAGGTTATCAAGTTGTCG	NDPS3006R	TGAGGCTGGGCCTTATTTAG	3:17425745
NDPS3005F	ATCAGCAGCATCATCAGCAA	NDPS3005R	GCAGAGGATGGGTATGGATG	3:14604447
NDPS3021F	AAGTAGCAGGCTGTGGGTGT	NDPS3021R	TGGAATGCGAGAAGGAAAAC	3:11734192
NDPS3015F	AGTGGCTGCCCTCCATTATT	NDPS3015R	TGTAAGGCGAGCAATGAGTG	3:13340670
NDPS3008F	ACGTCCGTAGGCAACAAAAA	NDPS3008R	AGAGGCAACGACGAGTGAGT	3:18701139