The Genetic Architecture of Hybrid Male Sterility in the *Drosophila pseudoobscura* Species Group

by

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Dissertation submitted in partial fulfillment of
the requirements for the degree of Doctor
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ABSTRACT

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Abstract

Biodiversity is generated by the process of speciation. Because biological species are defined as populations that are unable to exchange genes with one another, the study of the evolution of reproductive isolation occupies the center of speciation research. A key to deciphering how reproductive isolation evolves is to understand the genetic changes that underlie these barriers to gene flow. Intrinsic postzygotic barriers, such as hybrid sterility or inviability, are known to impede gene flow and especially lend themselves to genetic analysis because of their ease of study in a laboratory setting. Because hybrid sterility likely evolves before hybrid inviability, it potentially plays an important role in the cessation of gene flow. Yet, while their X-linked counterparts have been precisely localized, we remain ignorant of the numbers of and interactions among dominant autosomal loci that are predicted to contribute to F_1 hybrid male sterility.

To address this conceptual void, I examine the genetic architecture of hybrid male sterility between the allopatric sister species *Drosophila persimilis* and *D. pseudoobscura bogotana*. First, using a large-scale backcross analysis, I fine-map autosomal QTL from *D. persimilis* that confer sterility in male hybrids. This fine-mapping shows that loci contributing to hybrid male sterility reside outside chromosomal rearrangements (i.e., regions of reduced recombination) in this allopatric species pairs. In contrast, these QTL do not contribute to hybrid male sterility in the comparable sympatric hybridizing species *D. persimilis* and *D. pseudoobscura*, as predicted by models that suggest that hybridizing species persist because of broad regions of reduced recombination. Next, I use a serial backcross design to introgress these sterility-conferring QTL from *D. persimilis* into a *D.*

p. bogatana genetic background devoid of other alleles from *D. persimilis*. This introgression study tested a prediction of the dominance theory proposed to explain Haldane's rule: dominant-acting autosomal loci should interact with recessive-acting X-linked loci to produce sterile hybrid males. Surprisingly, the results demonstrated that the "composite" dominance of the autosomal QTL is more important than the dominance of individual QTL for producing Haldane's rule: epistasis among loci elevated their dominant effects on sterility such that individually-recessive-acting autosomal QTL can contribute to F_1 male infertility. Finally, using recombination to generate independent lines bearing only small segments of the identified QTL regions, I examine whether single or multiple loci within these regions contribute to the overall effect of hybrid sterility. While the effect of one QTL depends on epistasis between several loci within that small region, the effect of the other QTL appears to derive from a single genetic factor. These results suggest that estimates of the number of genes that contribute to reproductive isolation are at best, likely too low and, at worst, unattainable with the mapping resolution attainable by standard backcross and introgression approaches.

This dissertation addresses both evolutionary and genetic hypotheses of intrinsic postzygotic isolation. Hybrid male sterility between *D. persimilis* and *D. p. bogotana* clearly involves highly specific and complex interactions between homospecific loci. The mapping results presented here also lay the foundation for the identification and cloning of multiple autosomal sterility-conferring "speciation genes."

Dedication

In memory of Professor Ching-huei Chang.

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1. The Genetics of Hybrid Male Sterility Between the Allopatric Species Pair *Drosophila persimilis* and *D. pseudoobscura bogotana*: Dominant Sterility Alleles in Collinear Autosomal Regions

1.1 Introduction

HALDANE's (1922) rule observes that, in general, when one sex of hybrids between species is sterile or inviable, it is more frequently the heterogametic sex. The causes of this rule have been studied extensively (see reviews by WU *et al.* 1996; LAURIE 1997; ORR 1997), and much of the pattern seems to be explained by the "dominance theory" (MULLER 1942; ORR 1993; TURELLI and ORR 1995), which posits that sterility or inviability in the heterogametic sex often results from deleterious interactions between loci on a hemizygous X chromosome and dominant-acting loci on the autosomes. While several X-linked loci contributing to hybrid male sterility have been precisely localized (e.g., GUENET *et al.* 1990; OKA *et al.* 2004; STORCHOVA *et al.* 2004), especially within Drosophila (e.g., CABOT *et al.* 1994; PEREZ and WU 1995; MacDONALD and GOLDSTEIN 1999; ORR and IRVING 2001), dominant autosomal effects have typically been crudely localized or of comparatively small effect (e.g., MOEHRING *et al*. 2006; but see SLOTMAN *et al*. 2004).

The genetics of hybrid male sterility has been studied in the *Drosophila pseudoobscura-D. persimilis* species pair for over 70 years. The USA subspecies of *D. pseudoobscura* (*D. p. pseudoobscura*) co-occurs with *D. persimilis* on the west coast of North America. The two species have been shown to hybridize in natural populations at very low levels (DOBZHANSKY 1973; POWELL 1983), and variable amounts of

introgression have been detected across regions of their genomes (MACHADO *et al.* 2002; MACHADO and HEY 2003; HEY and NIELSEN 2004). The Bogota subspecies of *D. pseudoobscura*, *D. p. bogotana*, occurs allopatrically in South America. *D. pseudoobscura* and *D. persimilis* diverged between 0.5 and 1.0 million years ago (AQUADRO *et al*. 1991; WANG *et al.* 1997; LEMAN *et al*. 2005) while *D. pseudoobscura* and *D. p. bogotana* diverged between 150,000 and 200,000 years ago (SCHAEFFER and MILLER 1991; WANG *et al*. 1997). Hybrid males between *D. persimilis* and either *D. pseudoobscura* subspecies are viable but sterile, while hybrid females are fertile, consistent with HALDANE's (1922) rule. Previous studies have mapped the underlying genetic factors that contribute to hybrid sterility between the sympatric species pair (DOBZHANSKY 1936; ORR 1987, 1989; NOOR *et al.* 2001). These factors are strongly associated with three inversions (two on the X and one on the 2nd chromosome) that are fixed or nearly fixed, differentiating *D. pseudoobscura* and *D. persimilis*: Because these sterility-conferring loci are associated with inversions, they have not been precisely localized.

Additionally, because *D. pseudoobscura* and *D. persimilis* differ by these inversions and show multiple forms of pre- and postzygotic isolation, they have been a suitable system in which to study the effect of recombination on the evolution and maintenance of reproductive isolation. Recombination between genomes can potentially prevent the evolution or persistence of co-adapted gene complexes that confer species-specific adaptations and/or reproductive isolation between species. Thus, suppressing such recombination can allow the persistence of species despite occasional gene flow. One

means for suppressing recombination and for facilitating species persistence is through chromosomal rearrangements: crossover products are not recovered from heterozygotes (hybrids) for such rearrangements.

Several empirical studies (e.g., RIESEBERG *et al.* 1999; FEDER *et al*. 2003, PANITHANARAK *et al*. 2004, STUMP *et al*. 2005), including studies in the *D. pseudoobscura* group (NOOR *et al.* 2001), support the role for chromosomal rearrangements and other regions of suppressed recombination (e.g., centromeric regions; STUMP *et al.* 2005) in hybrids in preserving gene complexes that confer reproductive isolation (see reviews in ORTIZ-BARRIENTOS *et al.* 2002; BUTLIN 2005). Furthermore, theoretical models show that chromosomal rearrangements can facilitate the accumulation of hybrid incompatibilities between parapatric populations (e.g., NAVARRO and BARTON 2003; KIRKPATRICK and BARTON 2006). In the *D. pseudoobscura* group, BROWN *et al*. (2004) show that genetic factors contributing to pre- and postzygotic isolation are associated with inverted regions of the genome in the sympatric species *D. pseudoobscura* and *D. persimilis* but are associated with both inverted and probably uninverted (i.e., collinear) regions in the allopatric species *D. p. bogotana* and *D. persimilis*. The reduced recombination model of speciation directly predicts this association. However, these putative collinear-region effects were not mapped and may have been complicated by an additional fixed inversion difference between *D. p. bogotana* and *D. persimilis* on the third chromosome.

Here, we build on the results of previous studies in two significant ways. First, we use 26 microsatellite markers to demonstrate that hybrid male sterility between the

allopatric species *D. p. bogotana* and *D. persimilis* maps to dominant-acting autosomal regions of the genome outside of the inversions that distinguish these species. We show that at least some of these regions do not confer hybrid sterility between the sympatric species *D. pseudoobscura* and *D. persimilis*, as predicted by the reduced recombination models of speciation. Second, we localize these dominant autosomal genetic factors to regions of the 2nd, 3rd, and 4th chromosomes using a large-scale backcross analysis. These factors are among the first dominant autosomal factors contributing to hybrid male sterility to be precisely mapped, and this study thus provides the basis for introgression mapping and ultimately, molecular cloning, of interacting genes that contribute to F_1 hybrid sterility.

1.2 Materials and Methods

1.2.1 Fly Stocks and Crosses

Drosophila pseudoobscura bogotana females carrying a *white* eye mutation (hereafter bogw) were collected as virgins and maintained for seven days. On day eight, bogw were crossed to *D. persimilis* MSH 1993 (hereafter per) males. F_1 females were backcrossed to bogw males to generate backcross males (hereafter BCbogw males) for fertility assays. Only male progeny bearing the white mutation were scored. The bogw strain is a subculture of *the D. p. bogotana* El Recreo line collected in 1978 (provided by H. A. ORR). The per line was derived from females collected at Mt. St. Helena, California in 1993 (NOOR 1995). All crosses were performed on standard sugar/yeast/agar medium at 20 ± 1° C and 85% relative humidity.

1.2.2 Fertility Assays of BCbogw Males

BCbogw males were collected as virgins and maintained for seven days in vials containing 10 to 20 males. On day eight, the fertility of each backcross male was assessed by dissection of the testes in Ringer's solution following the method of COYNE (1984). A male was scored "fertile" if at least one motile sperm was observed and "sterile" if no motile sperm were observed. Treating fertility as a binary trait has been shown to be conservative (CAMPBELL and NOOR 2001), though other methods of scoring fertility exist (e.g., WHITE-COOPER 2004). All dissected BCbogw males were labeled and stored at –20^{o} C.

1.2.3 Microsatellite Genotyping of BCbogw Males

DNA was extracted from all dissected BCbogw males following the protocol of GLOOR and ENGELS (1992). Microsatellite genotyping was performed in two steps. First, all 4853 BCbogw males were genotyped for markers associated with each inversion that distinguishes *D. pseudoobscura* (and *D. p. bogotana*) and *D. persimilis*. The markers used for this initial screen were: DPSX002 (chromosome arm XL); DPSX030 (XR); and *bicoid* (bcd; 2) (ORTIZ-BARRIENTOS *et al.* 2006). These markers identified the inversion arrangement on these chromosome arms. Second, because we were interested in localizing sterility-conferring alleles that map outside the inverted regions between these species, only those 1102 BCbogw males that were hemi- or homozygous for the *D. p. bogotana* allele at the three inversion markers (hereafter BCbogwLim males) were further genotyped. This procedure thus would identify dominant *D. persimilis* alleles that interact with a predominantly *D. p. bogotana* genetic background. Surveys of other

markers along the X-chromosome showed that this procedure also essentially selected for an almost complete *D. p. bogotana* X-chromosome as well as much of the second chromosome.

BCbogwLim males were genotyped for 23 microsatellite markers distributed evenly on the 2nd, 3rd, and 4th chromosomes. Primer sequences for all markers used in this study are available at http://www.genetics.org/cgi/content/full/genetics.106.067314/DC1. PCR amplification followed a touchdown protocol: 95° for 1 min; 3 cycles of 94° for 30 sec, 56° for 30s, 72° for 30 sec; 3 cycles of 94° for 30 sec, 56° for 30 sec, 72° for 30 sec; and 30 cycles of 94° for 30 sec, 50° for 30 sec, 72° for 30 sec. PCRs were visualized on acrylamide gels on LiCor 4200 DNA sequencer/analyzers.

1.2.4 Mapping Hybrid Male Sterility

QTL mapping was first performed with composite interval mapping (CIM) (ZENG 1994) using Windows QTL Cartographer V. 2.5 (WANG *et al*. 2006). We focus on our CIM results rather than our other analysis (see below) because of the longer history of confirmation of effects initially mapped using CIM. Fertility was treated as a binary trait (the presence or absence of sperm, see above). Though this violates the assumption of normality in CIM, a previous study (MOEHRING *et al*. 2004) has shown that this treatment gives essentially the same result as when a trait is continuous, if CIM is based on logistic regression (e.g., XU and ATCHLEY 1996). Thresholds for significance were set by permutations (experiment-wise $P = 0.05$ and N = 1000).

Nonetheless, because our dataset does violate an assumption of the CIM procedure, the QTLs detected using CIM were further confirmed using the new binary multiple

interval mapping (bMIM) (LI *et al.* 2006) procedure in Windows QTL Cartographer V.2.5 (WANG *et al.* 2006). Results from both forward and backward regression methods on markers are reported.

F_1 hybrid male sterility is thought to result from epistatic interactions between recessive X-linked and dominant autosomal loci (MULLER 1942; ORR 1993; TURELLI and ORR 1995). We do not explicitly test for epistasis in this study because we have limited the dataset to just those males bearing an X-chromosome from *D. p. bogotana*, hence identifying dominant autosomal loci contributing to sterility derived from *D. persimilis*. While there may be epistasis among autosomal loci, bMIM currently does not include a test for epistasis (S. WANG, personal communication), and such a test is beyond the scope of the hypotheses we examine in this study.

We evaluated whether the same QTLs are associated with hybrid male sterility in backcross hybrids between per and *D. pseudoobscura* (hereafter, ps) vs. between per and *D. p. bogotana* (hereafter, bog) using a three-way contingency test based on a log-linear model (SOKAL and ROHLF 1995). For the per-ps hybridization, we used the raw data from our previous mapping study (NOOR *et al.* 2001), limited the dataset to those backcross hybrid males bearing the three inversion-associated markers from *D. pseudoobscura*, and examined the effects of markers closest to the ones surveyed in our per-bog backcross. Markers DPS2003 and DPS3001 were surveyed in both crosses and their associations with hybrid male sterility were compared in this manner. On the 4th chromosome, we did not have data for markers immediately adjacent to the per-bog sterility QTL in the per-ps backcross. Thus, for the 4th chromosome QTL, we performed

a more conservative three-way contingency test using a marker even further from the sterility QTL in per-bog (DPS4G1e) than the nearest marker surveyed in per-ps (*Adh*).

1.3 Results

Controlling for the effects of 3 microsatellite markers (DPSX002, DPSX030, and *bcd*; see MATERIALS AND METHODS) associated with the inversion differences between *D. persimilis* and *D. p. bogotana* allowed us to detect QTLs that confer hybrid male sterility occurring outside the chromosomal rearrangements that distinguish these species. Using 26 microsatellite markers, we mapped using CIM at least four autosomal dominant QTLs with large effects on hybrid male sterility that interact with a predominantly *D. p. bogotana* genetic background. Furthermore, we were able to localize two of these four QTLs to relatively small regions: On the 2nd chromosome, a QTL was localized to an interval of about 840 Kb between markers DPS2-390p and DPS2-534j (Fig. 1A). The annotated part of this region contained 104 genes, based on sequence homology to *D. melanogaster* (GILBERT 2005; see Supplementary Table 1 at http://www.genetics.org/cgi/content/full/genetics.106.067314/DC1). The second QTL on this chromosome is associated with marker DPS2-1206e; we could not localize the size of this genomic region due to a lack of markers beyond DPS2-1206e, which lies about 17 Kb from the centromeric end of the 2nd chromosome sequence assembly. On the 4th chromosome, a QTL was localized to an interval of about 1.2 Mb between markers DPS4G1h and DPS4033b (Fig. 1B) and is closely associated with marker DPS4G1a. The annotated part of this region contained 136 genes (GILBERT 2005; see Supplementary

Table 1 at http://www.genetics.org/cgi/content/full/genetics.106.067314/DC1). We were unable to refine the location of the 3rd chromosome QTL further because of an inversion difference between *D. persimilis* and *D. p. bogotana*. The biological and molecular functions of the 239 genes contained in the two smaller QTL intervals were evaluated using PANTHER (MI et al. 2005) software and are included in Supplementary Table 2 at http://www.genetics.org/cgi/content/full/genetics.106.067314/DC1. Figure 2 shows the relative effects of these QTLs individually and in combination on hybrid male sterility.

We further confirmed the presence and location of these QTLs using bMIM. Table 1 shows the additive effects associated with the three major QTLs, as well as the microsatellite markers flanking each QTL, for both regression forward and backward selection on markers. The results from bMIM (Table 1) and those from CIM (Figure 1) were highly comparable, aside from minor movements of the exact peak location. Using a backward regression method in bMIM also detected the fourth QTL near the centromeric end of chromosome 2 (data not shown), as did CIM.

Previously, BROWN *et al.* (2004) showed that genes likely associated with collinear regions significantly decreased fertility in hybrids between *D. persimilis* and *D. p. bogotana* but do not have detectable effects on fertility in hybrids between *D. persimilis* and *D. pseudoobscura*. This analysis was based on a comparison between the proportion of sterile males from a *D. p. bogotana* backcross and the proportion of sterile males from a *D. pseudoobscura* backcross. Here, we directly evaluate whether the same collinear-region QTLs are associated with hybrid male sterility in hybrids between the two hybridizations by using a three-way contingency test based on a log-linear model

(SOKAL and ROHLF 1995). In backcross hybrids, the major QTLs on the 2nd, 3rd, and 4th chromosomes all contribute significantly to hybrid male sterility in hybrids between *D. persimilis* and *D. p. bogotana* but do not contribute significantly to sterility between *D. persimilis* and *D. pseudoobscura,* and the differences in effect are all statistically significant ($G^2 = 94.05$, $P < 0.001$; $G^2 = 159.97$, $P < 0.001$; and $G^2 = 61.72$, $P < 0.001$, respectively; Supplementary Table 1 at http://www.genetics.org/cgi/content/full/genetics.106.067314/DC1). Thus, these genomic regions contribute significantly more to hybrid male sterility in hybridizations of the allopatric species than in hybridizations of the sympatric species, as predicted by the reduced recombination model of speciation.

1.4 Discussion

In this study, we fine-map at least three *Drosophila persimilis* autosomal dominant QTLs that confer hybrid male sterility in a *D. p. bogotana* genetic background. These QTLs are located outside the chromosomal inversions that differentiate the two arms of the X-chromosome and on the 2nd chromosome in *D. persimilis* and *D. p. bogotana*. Furthermore, we demonstrate that the effects of these QTLs on hybrid male sterility are greater in crosses between *D. persimilis* and *D. p. bogotana* than between *D. persimilis* and *D. pseudoobscura*.

These results confirm a prediction of the reduced recombination model of speciation: in allopatric species where the potential for gene flow does not exist, genetic factors associated with reproductive isolation should reside both within and outside genomic regions experiencing reduced recombination (in this case, within and outside

chromosomal inversions); in sympatric species, factors associated with reproductive isolation should reside preferentially within genomic regions experiencing reduced recombination (within inversions). Thus, in the *D. pseudoobscura* species group, chromosomal rearrangements appear to contribute to the maintenance of species persistence by restricting recombination between genomic regions that contain genetic factors underlying reproductive isolation, while gene flow has homogenized collinear regions.

Chromosomal rearrangements have also been proposed to contribute directly to reproductive isolation via strong underdominance (WHITE 1969) resulting from meiotic difficulties. This type of chromosomal speciation model is distinct from the recombination-reduction models in that hybrid sterility results from the chromosomal rearrangements directly, not from effects of loci captured within the rearrangement. Although bearing some recent support (DELNERI *et al.* 2003), this model remains controversial (COYNE and ORR 2004). In contrast, our results directly support a prediction of the reduced recombination chromosomal speciation models.

A previous study by BROWN *et al.* (2004) showed that almost all hybrid males between *D. persimilis* and *D. p. pseudoobscura* homozygous or hemizygous for the three inverted regions were fertile. In contrast, only about one-third of the male hybrids between *D. persimilis* and *D. p. bogotana* homozygous or hemizygous for the three inverted regions were sterile, suggesting that other factors conferring sterility likely occurs in collinear regions. By selecting only those *D. p. bogotana* backcross hybrid males that were homozygous or heterozygous for the three inversions, we built on this

work by directly localizing these additional hybrid male sterility loci to small regions on the 2nd and 4th chromosomes and a region of the 3rd chromosome. Our findings are also consistent with DNA sequence surveys of these species, which indicate extensive introgression in collinear regions between *D. pseudoobscura* and *D. persimilis* but probably not between *D. persimilis* and *D. p. bogotana* (MACHADO and HEY 2003).

The sterility effects of these three autosomal regions are dominant, as hybrid males heterozygous (i.e., carrying both the *D. persimilis* and the *D. p. bogotana* allele) for these loci are more likely to be sterile. The dominance theory proposed to explain Haldane's rule suggests that F_1 hybrid male sterility often results from a deleterious interaction between a hemizygous (recessive) sex-chromosome effect and dominant autosomal effects. While many studies have precisely localized the recessive X-chromosomal effects (GUENET *et al.* 1990; CABOT *et al.* 1994; PEREZ and WU 1995; MacDONALD and GOLDSTEIN 1999; ORR and IRVING 2001; OKA et al. 2004; STORCHOVA *et al.* 2004; MOEHRING *et al*. 2006), most have failed to pinpoint the locations of individually significant dominant autosomal effects with high confidence (but see SLOTMAN *et al.* 2004).

Although we identify at least three such dominant autosomal regions, the effect of each individual allele appears to be weak as well (Figure 2): no single factor caused complete or nearly complete sterility. Figure 2 shows that the effect on the sterility phenotype increases with the addition of each QTL, though they must interact with *D. p. bogotana* factors that were not identified in this study (such as the X-chromosome). As

expected, the highest proportion of hybrid male sterility (83%) occurred in those hybrid males that were heterozygous for all four of the QTLs.

To attempt to examine whether genetic introgression has occurred between *D. persimilis* and *D. p. bogotana* in the major QTL intervals on the 2nd and 4th chromosomes, two 900-bp regions (one within each interval) were randomly selected for amplification and sequencing. The region on the 2nd chromosome contains coding DNA while the region on the 4th chromosome contains only non-coding DNA. These sequences were obtained for two *D. persimilis* strains, two *D. p. bogotana* strains, two *D. pseudoobscura* strains, and one *D. miranda* (outgroup) strain. Sequences obtained are available in GenBank (accession numbers EF392818 to EF392831). No fixed differences were detected between *D. persimilis* and *D. p. bogotana* or between *D. persimilis* and *D. pseudoobscura* in the ~1800-bp amplified so it appears that the differentiation associated with sterility between these species may be rather localized. We refrained for further analysis of our sequence data because of the presently still coarse scale of our QTL mapping.

Because the QTLs identified in this study were mapped only in a single line of *D. persimilis* (MSH 1993) and in a single line of *D. p. bogotana* (El Recreo), it is also possible that the alleles detected in these hybrids could reflect intraspecific polymorphism for alleles associated with sterility. However, BROWN *et al.* (2004) did not observe dramatic differences in hybrid male sterility among three backcross lineages of *D. pseudoobscura* or among three backcross lineages of *D. p. bogotana*, suggesting that our mapping results may be representative of much of these species. Further,

because precision-mapping of any phenotypic trait typically involves scoring hundreds, if not thousands, of individuals for that trait, this caveat applies to almost all QTL mapping studies published to date. Because this study localizes the QTLs to relatively small autosomal regions, it does provide a basis for future introgression studies of these factors that cause hybrid male sterility between *D. persimilis* and *D. p. bogotana* and for the eventual cloning of "sterility genes."

Table 1. Location and Additive Effect of the Three Major QTL Detected Using Binary Multiple-Interval Mapping (bMIM)

Forward regression

QTL	Chromosome	Additivity	Flanking Markers
1	2	2.634	DPS2-390p ; DPS2-534j
2	3	2.049	DPS3001 ; DPS3026
3	4	1.784	DPS4G1a ; DPS4033b

Backward regression

QTL	Chromosome	Additivity	Flanking Markers
1	2	2.047	DPS2-390p ; DPS2-534j
2	3	2.008	DPS3001 ; DPS3026
3	4	1.756	DPS4G1h ; DPS4G1a

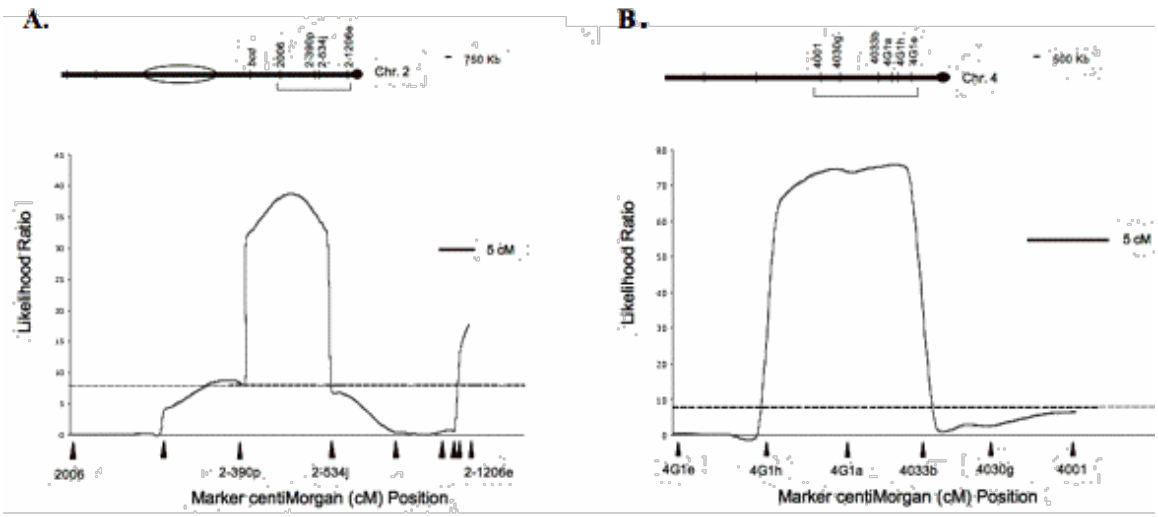

Figure 1: QTLs Associated with Hybrid Male Sterility on the (A) 2nd and (B) 4th Chromosomes.

Plots are the likelihood-ratio (LR) test statistic as determined by composite interval mapping on the y-axis. The significance thresholds were determined by permutation testing to be approximately LR = 7.8 and are indicated by horizontal dashed lines. Marker locations are represented by black triangles on the x-axis of the QTL plot. The markers flanking the significant QTLs are indicated by name. Above the QTL plots are diagrams of the chromosomes with the physical location of relevant markers indicated by hatch marks. An inversion is represented by the presence of an open oval. A bracket indicates the area magnified in the QTL plot below. Scale bars (representing either recombinational or physical distance) are given.

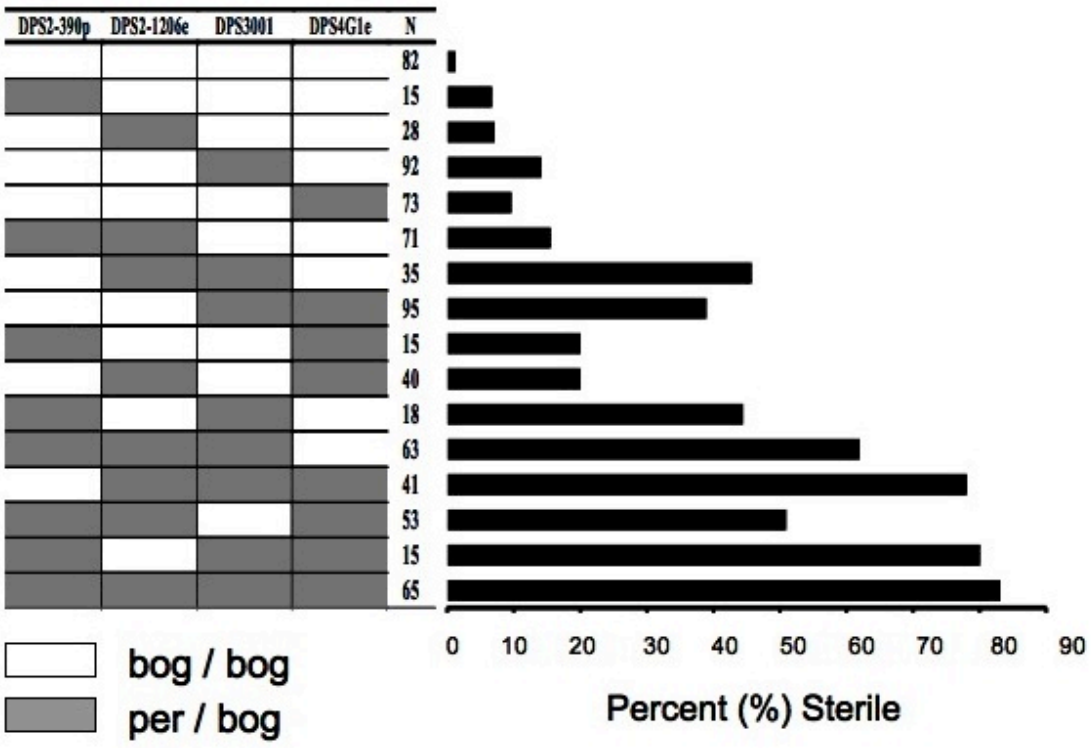

Figure 2: Relative Effects of Each QTL on Hybrid Male Sterility.

Hybrid male genotypes are given on the y-axis and sterility (as determined by sperm motility) is given on the x-axis. Heterozygous QTLs (hybrid males carry both the *D. persimilis* and *D. p. bogotana* alleles) are indicated by grey rectangles and homozygous QTLs (hybrid males carry only the *D. p. bogotana* allele) are indicated by white rectangles. A marker associated with each QTL is used to determine the average sterility of males hetero- or homozygous for that QTL. N is the number of males of a given genotype.

2. Epistasis Modifies the Dominance of Loci Causing Hybrid Male Sterility in the *Drosophila pseudoobscura* Species Group

2.1 Introduction

The genetic changes underlying the evolution of reproductive isolation has long been an important research topic in the study of speciation. Gene flow between populations can be restricted either via prezygotic barriers that act before the fusion of the egg and the sperm or postzygotic barriers that act after gamete fusion. Postzygotic barriers, such as hybrid sterility or inviability, are thought to result from negative epistatic interactions between the genomes of two diverging populations (DOBZHANSKY 1936; MULLER 1942). To date, many studies on postzygotic reproductive isolation have been devoted specifically to elucidating the causes of Haldane's (1922) rule and more generally to the causes of F_1 problems in hybrid males (COYNE and ORR 2004).

Postzygotic isolating barriers between disparate taxa largely conform to Haldane's (1922) rule, which observes that when hybrids of one sex are sterile or inviabile, it is more often the heterogametic (XY or ZW) sex. While several theories have been proposed to explain this asymmetry (CHARLESWORTH *et al.* 1987; WU and DAVIS 1993; WU *et al.* 1996), the dominance theory (MULLER 1940; MULLER 1942; TURELLI and ORR 1995; TURELLI and ORR 2000) appears to explain the majority of the observed cases, as it applies whether males or females are the heterogametic sex. The dominance theory simply posits that alleles underlying hybrid incompatibilities are, on average, partially recessive. Under this condition of partial recessivity, the expression of

X-linked incompatible recessive alleles in the heterogametic sex (e.g., XY) outweighs the potentially greater number of incompatibilities suffered by the homogametic sex (e.g., XX, which could carry twice as many incompatibility alleles as the XY sex), resulting in Haldane's rule.

The dominance theory predicts that recessive X-linked loci from one species interact with dominant autosomal loci from another species to produce incompatibilities in heterogametic-sex hybrids. This simple prediction suggests two important corollaries. First, the evolution of hybrid incompatibilities necessarily involves epistasis between loci from different species (figure 3a) and could also involve epistasis between multiple loci from one or both species (figures 3b and 3c). This corollary is well-supported by empirical evidence (see references below). Second, genetic analyses of hybrid incompatibilities should be able to localize both those recessive X-linked loci and the dominant-acting autosomal loci. However, while the former have been relatively accessible (CABOT *et al.* 1994; GUENET *et al.* 1990; MACDONALD and GOLDSTEIN 1999; MASLY and PRESGRAVES 2007; OKA *et al.* 2004; ORR and IRVING 2001; PEREZ and WU 1995; STORCHOVA *et al.* 2004; TRUE *et al.* 1996), their dominant autosomal counterparts have remained far more elusive (but see SLOTMAN *et al.* 2004).

Several studies of Drosophila species pairs have highlighted the importance of epistatic interactions when considering the causes of hybrid male sterility. Hybrid males between *D. simulans* and its sister species *D. mauritiana* are rendered completely sterile only if the *D. mauritiana* allele of the X-linked gene *Odysseus* and an adjacent X-

chromosome region interact with one or more *D. simulans* autosomal regions (DAVIS and WU 1996; PALOPOLI and WU 1994; PEREZ and WU 1995). Similarly, sterility of hybrid males between the Bogota and USA subspecies of *D. pseudoobscura* is conditional upon interactions between Bogota alleles at two or more X-chromosome loci and USA alleles at two or more autosomal loci (ORR and IRVING 2001). Finally, Naveira and Fontdevila (1986; 1991) noted that hybrid male sterility between the species of the *D. buzzatii* species group results from epistasis between promiscuous (i.e., interchangeable) segments of the autosomes. Yet, though these studies have acknowledged the significant contribution of epistasis to hybrid male sterility, none have explicitly examined the effects of epistasis among putatively dominant autosomal sterility-conferring loci that should contribute to Haldane's rule.

While epistasis must contribute to Haldane's rule, the pattern of interactions causing sterility in hybrids is less clear. Recent studies (reviewed in COYNE and ORR 2004) suggest that F_1 hybrid male sterility likely results not from "major genes" of large effect but instead from multiple factors that individually have small effects and interact epistatically to cause sterility. Under the "additive threshold" model (NAVEIRA and MASIDE 1998), sterility arises from epistasis between promiscuous genetic factors that together exceed a threshold level of foreign introgression. In contrast, under the "weak allele-strong interaction" model (PALOPOLI and WU 1994; PEREZ and WU 1995; WU and HOLLOCHER 1998), sterility results from epistasis between non-interchangeable conspecific alleles. It is important to note that both polygenic models predict that

introgression of individual factors have little or no effect on hybrid male sterility. Instead, sterility results only from epistasis between multiple co-introgressed factors.

Here, we examine the pattern of epistasis and its effect on the dominance of three autosomal QTL underlying hybrid male sterility between *Drosophila persimilis* and *D. pseudoobscura bogotana*. We also determine which of the two polygenic hypotheses better explain hybrid male sterility between *D. persimilis* and *D. p. bogotana*. Results from our previous study (CHANG and NOOR 2007) suggest that three autosomal QTL (one on each major autosome) from *D. persimilis* interact with a *D. p. bogotana* background to produce sterile hybrid males. Specific epistatic interactions between these regions (i.e., between the three QTL) were not carefully examined in our previous study because of the variable genetic background of our backcross population.

The genetics underlying hybrid male sterility between *D. persimilis* and *D. p. bogotana* thus lends itself to addressing the two aforementioned corollaries of the dominance theory. In this study, we first introgressed QTL from *D. persimilis* into an otherwise completely *D. p. bogotana* genetic background to assess the dominance of each QTL individually. Second, we introgressed heterozygous and homozygous combinations of QTL to evaluate the effect of epistasis on the dominance of the sterility-conferring QTL. Results from this study show strong epistasis between these autosomal factors, and, unexpectedly, that epistasis modifies the apparent dominance of loci underlying hybrid male sterility. These results suggest that assessments of the dominance of individually examined QTL are not necessarily applicable when considering the causes of

Haldane's rule. In addition, we show that hybrid male sterility between *D. persimilis* and *D. p. bogotana* results at least in part from epistasis between non-interchangeable *D. persimilis* regions of the genome against a *D. p. bogotana* genetic background.

2.2 Materials and Methods

F_1 hybrid females between *D. persimilis* and *D. p. bogotana* were backcrossed to *D. p. bogotana* males. The females were then genotyped at three microsatellite markers linked to the three chromosomal inversions (two on the X-chromosome and one on the 2^{nd}-chromosome) distinguishing these species. Those females hemizygous or homozygous for the *D. p. bogotana* arrangement were subsequently genotyped for microsatellite markers flanking each of 3 QTL, one on each autosome, conferring hybrid male sterility. Selecting for the *D. p. bogotana* arrangement ensured that the X-chromosome in the subsequent backcross hybrid males derived only from *D. p. bogotana*, as the two inversions and their recombination-suppression effects on the X-chromosome span the majority of the chromosome. Furthermore, this process also guaranteed that the sterility-conferring loci mapped in this study reside *outside* the inversions and are comparable to those fine-mapped in Chang and Noor (2007).

Females heterozygous for the sterility loci markers were selected and used for successive backcrosses to *D. p. bogotana* males: three individual "single introgression" lines (Q2, Q3, and Q4, with the numbers designating the autosome on which the QTL resides) were created such that each introgression line contained a QTL from *D. persimilis* that conferred hybrid male sterility against a *D. p. bogotana* genetic

background. Ten generations of backcross were performed to ensure that the genetic background was purged of *D. persimilis* alleles other than those at the three sterility-conferring QTL (see figure 4a). Males from the 11th generation of backcross were assayed for fertility following the methods of Coyne (1984): testes were dissected in Ringer's solution and sperm motility was determined. The males were then genotyped at the relevant microsatellite markers (see DNA preparation and genotyping methods in CHANG and NOOR 2007). Primer sequences for all markers used in this study are available in Table S1.

To create homozygous introgression lines for each of the three autosomal QTL, males and females from within each single introgression line were self-crossed. This was possible because none of the introgression lines bearing one copy of the *D. persimilis* allele produced sterile hybrid males (see Results). Male offspring from these crosses were then assayed for fertility and genotyped at the relevant markers.

To examine the effect of epistasis on the dominance of sterility-conferring loci, males from one introgression line were crossed to females of another introgression line to produce "combination introgression" lines. For example, to assay the heterozygous effect (if any) of both the 2nd- and 3rd-chromosome QTL (i.e., Q2 + Q3) on hybrid male fertility, heterozygous Q3 males were crossed to heterozygous Q2 females and vice versa (figure 4b). These Q2 + Q3 males were assayed for fertility and genotyped at the relevant markers. Though 13 total heterozygous/homozygous combinations of QTL were

possible, not all were produced and assayed, as males homozygous for Q2 and homozygous for Q3 were nearly completely sterile (see Results).

2.3 Results

2.3.1 Dominance of Individual QTL Introgressions

To assess the dominance of individual autosomal QTL, each sterility-conferring QTL from *D. persimilis* was introgressed via 11 generations of backcross into an otherwise *D. p. bogotana* genetic background. In contrast to the results from the original backcross analysis (CHANG and NOOR 2007), our data here suggest that two of the three QTL (Q2 and Q3, the 2nd- and 3rd-chromosome QTL, respectively) behaved recessively when examined alone: Q2 and Q3 produced 3.8% and 0% sterility, respectively, when heterozygous (figure 5, genotypes 2 and 4) but 90.9% and 100% sterility when homozygous (figure 5, genotypes 3 and 5). In dramatic contrast, Q4, the 4th-chromosome QTL, showed no effect on hybrid male sterility (figure 5, genotypes 6 and 7). Indeed, a vigorous self-perpetuating strain was constructed that is homozygous for the Q4 introgression.

2.3.2 Epistasis and Dominance of Multiple Inter-Chromosomal QTL Introgressions

To detect epistatic effects on dominance of hybrid male sterility QTL, co-introgressions of either one (heterozygous) or two (homozygous) copies of the *D. persimilis* allele for multiple QTL were performed. While 13 combinations of heterozygous and/or homozygous introgressions (in addition to what is equivalent to the

wild-type genotype) are possible, not all genotypes were assayed for fertility (see above).

Figure 4 shows the results of co-introgressing heterozygous and homozygous combinations of *D. persimilis* QTL on hybrid male sterility. In contrast to an individual heterozygous introgression of Q2, which yielded only 3.8% males sterile, co-introgression of heterozygous Q2 with heterozygous Q3 (which alone caused 0% of the males to be sterile) rendered 43.0% of the males sterile, suggesting strong epistasis between the *D. persimilis* alleles at these two QTL. However, heterozygous Q4, which had no effect when introgressed alone, failed to significantly increase male sterility when co-introgressed with heterozygous Q2. Similarly, no effect on sterility was apparent in co-introgressions of heterozygous Q4 with heterozygous Q3. Thus, heterozygous introgressions of two of the three QTL suggest that epistasis between Q2 and Q3 produced an elevated "composite dominance" of these two QTL while two-locus epistasis (i.e., Q2 + Q4 and Q3 + Q4) appeared to have no effect on Q4.

To further investigate the role of Q4 in producing hybrid male sterility, we introgressed two copies of the *D. persimilis* allele into a *D. p. bogotana* background in combination with heterozygous introgressions of Q2 and Q3. 15% of males with heterozygous Q2 and homozygous Q4 introgressions were sterile, in comparison to 4% of sterile males with heterozygous Q2 and Q4 introgressions; this difference is not statistically significant (figure 6, genotype 5 vs. genotype 3; $p = 0.16$, Fisher's Exact test). In addition, 0% of males with heterozygous Q3 and homozygous Q4 introgressions were sterile, which is not significantly different from the 5% of sterile males with

heterozygous Q3 and Q4 introgressions previously observed (figure 6, genotype 6 vs. genotype 4; p = 0.25, Fisher's Exact test). These data suggest that Q4 had no effect on hybrid male sterility between *D. persimilis* and *D. p. bogotana* when in combination with only one of the two other QTL conferring hybrid male sterility between *D. persimilis* and *D. p. bogotana*.

In contrast to those male flies carrying only two heterozygous introgressions of QTL from *D. persimilis*, 94% of males bearing heterozygous introgressions of Q2, Q3, and Q4 (figure 6, genotype 7) were sterile. This strongly suggests that Q4 interacted epistatically with Q2 and Q3 to produce nearly complete sterility and confirms the results from our original backcross analysis (Chang and Noor 2007) in which Q4 contributed significantly to hybrid male sterility. Very interestingly, this dominant effect of Q4 was manifest only in combination with a single copy of the *D. persimilis* allele of *both* Q2 and Q3.

2.3.3 Effect of Introgression Size on Hybrid Male Sterility

To test whether the sterility we observed in hybrid males between *D. persimilis* and *D. p. bogotana* was caused by the size of the introgression itself (the polygenic "additive threshold" model; (NAVEIRA and MASIDE 1998)) or by interactions of specific loci (the "weak allele-strong interaction" model; (WU and HOLLOCHER 1998)), we introgressed a 3.23 Mb region from the *D. persimilis* 2nd-chromosome into an otherwise *D. p. bogotana* background. This introgressed region is greater in size than the

introgression Q2 by 1.47 Mb. Introgression of this segment of chromosome 2 in combination Q3 and Q4 failed to produce any sterile hybrid males, showing that the hybrid male sterility we observe in this study was not a direct result of the sizes of the individual introgressed regions.

2.4 Discussion

While epistasis is widely considered to play a role in hybrid incompatibilities, few studies have formally identified specific epistatic interactions between autosomal loci that contribute to hybrid unfitness. Here, using an introgression approach, we explicitly test the nature of epistasis between the three autosomal QTL (one on each major autosome) from *D. persimilis* that confer hybrid male sterility in an otherwise completely *D. p. bogotana* genetic background. We show that none of the three QTL is capable of producing hybrid sterility when a single (i.e., heterozygous) *D. persimilis* allele is introgressed. However, almost 50% of males carrying both the 2^{nd}- and 3^{rd}-chromosome QTL introgressions are sterile. Introgressions of the 2^{nd}- and 4^{th}-chromsome QTL or the 3^{rd}- and 4^{th}-chromosome QTL have almost no effect on male sterility, but almost 100% of males carrying one copy of all three QTL are sterile.

These results demonstrate that manifestation of hybrid male sterility between *D. persimilis* and *D. p. bogotana* depends critically on epistasis that is highly specific in nature. That we failed to observe sterility when a large region not associated with a QTL was introgressed along with two of our focal QTL further underscores the importance of QTL identity. Our results thus do not support the "additive threshold" model, as the

sterility-conferring genetic factors do not appear to be interchangeable. Instead, hybrid male sterility between these species appears to conform more closely to the "weak allele-strong interaction" hypothesis: the individually weak QTL have a formidable effect on male fertility when all three are co-introgressed.

Our results further suggest that epistasis between the hybrid sterility QTL modifies the apparent dominance of these QTL. Both the chromosome-2 and chromosome-3 QTL from *D. persimilis* behave completely recessively when introgressed individually into a *D. p. bogotana* background. Epistasis appears to elevate the "composite dominance" of these two QTL, as nearly half the males carrying co-introgressions of these QTL are sterile. The addition of one copy of the 4th-chromsome QTL, which alone had no effect on hybrid male sterility even when homozygous, to the other two QTL produced nearly complete male sterility. Thus, the 4th-chromosome QTL, via epistasis, plays an indispensible role in the establishment of a postzygotic barrier to gene flow.

Together, these results demonstrate that the dominance of a single locus can be misleading when considering how hybrid incompatibilities can arise between species (see also (FITZPATRICK 2008)). The dominance theory explaining Haldane's rule (MULLER 1940; MULLER 1942; TURELLI and ORR 1995; TURELLI and ORR 2000) predicts that F_1 genetic incompatibilities, such as hybrid male sterility, are often caused by interactions between recessive X-linked loci from one species and dominant autosomal loci from another. Based on this theory, genetic studies should be able to localize dominant

autosomal loci precisely, as well as to map their recessive X-linked counterparts. Contrary to these expectations, however, the former have been comparatively difficult to detect, though several recessive autosomal loci (MASLY and PRESGRAVES 2007; TAO *et al.* 2003a; TAO and HARTL 2003; TAO *et al.* 2003b; TRUE *et al.* 1996) underlying hybrid incompatibilities have been isolated from exhaustive introgression studies.

We offer a possible explanation for this discrepancy: strong epistasis among foreign autosomal loci may moderate their detectable effects in backcross or introgression analyses. As we observe in this study, individual QTL may behave recessively in isolation but dominantly in combination. Only by taking into account epistatic interactions between the QTL were we able to detect "dominant" effects of these loci that would operate in an F_1 hybrid. Introgression studies in particular may often entirely miss QTL underlying hybrid incompatibilities. Loci that behave similarly to the chromosome-4 QTL in this study may appear to have no effect though, through epistasis, they may dramatically contribute to hybrid unfitness in an F_1 hybrid.

Furthermore, autosomal recessive "speciation genes" that have previously been isolated (e.g., *Nup96* (PRESGRAVES *et al.* 2003); *Tmy* (TAO *et al.* 2001; TAO *et al.* 2003b)) may also contribute directly to F_1 male unfitness. Our data suggest that, in the case of speciation genes such as *Nup96* and *Tmy*, should their autosomal interactors be cloned, the composite effect of these genes and their interactors could potentially cause lethality or sterility in F_1 hybrids. Introgression studies by Tao et al. (TAO *et al.* 2003a; TAO and HARTL 2003; TAO *et al.* 2003b) offer another intriguing opportunity to assess the

applicability of these results: Tao et al. (TAO *et al.* 2003a; TAO and HARTL 2003; TAO *et al.* 2003b) mapped many autosomal loci causing hybrid male sterility between *D. simulans* and *D. mauritiana*. While all these loci behaved recessively, the F_1 males between these species were completely sterile. Consistent with our hypothesis, "trans-heterozygotes" of some pairs of alleles they studied showed some reduction in fertility (TAO and HARTL 2003). It would be enlightening to examine whether epistasis between more of their sterility QTL can elevate their composite dominance to complete.

In this study, we only address epistasis between alleles from *D. persimilis* in a *D. p. bogotana* genetic background. Certainly, these *D. persimilis* alleles must interact with one or more *D. p. bogotana* alleles to produce sterile hybrid males between these two species. Furthermore, *D. p. bogotana* alleles could interact with each other as well. However, neither of these latter two classes of interactions is within the scope of this study. As a result, we can only say that interactions between a minimum of 5 genes are required to cause hybrid male sterility in this species pair, but likely many more loci are involved.

We also note that our current study examines the effect of hybrid male sterility QTL on a group and not on individuals. In other words, our results rely on observations of the number of males that are sterile *vs.* the number of males that are fertile rather than number of nonfunctional vs. functional sperm within an individual. This latter approach was impractical for our studies: hybrid male sterility between *D. persimilis* and *D. p. bogotana* could originally only be detected in our introgressions when large sample sizes

were utilized, thus rendering sperm counts of individual males unfeasible. Yet, the effects of the QTL and of epistasis between them were present when using the group approach, though we acknowledge that we may have missed other contributing loci.

In summary, epistasis between hybrid male sterility QTL appears indispensable for the manifestation of this incompatibility between *D. persimilis* and *D. p. bogotana.* In the absence of epistatic interactions, individually recessive-acting loci cannot contribute to F_1 hybrid sterility. Whether this observation applies more broadly to hybrid incompatibilities between other species pairs can only be resolved by additional future studies. The paucity of dominant-acting "speciation loci" to date suggests that perhaps their necessary interactors have not yet been detected. The elucidation of the genetic and cellular mechanisms causing hybrid incompatibilities will depend on identification of all members of the interacting genetic complex.

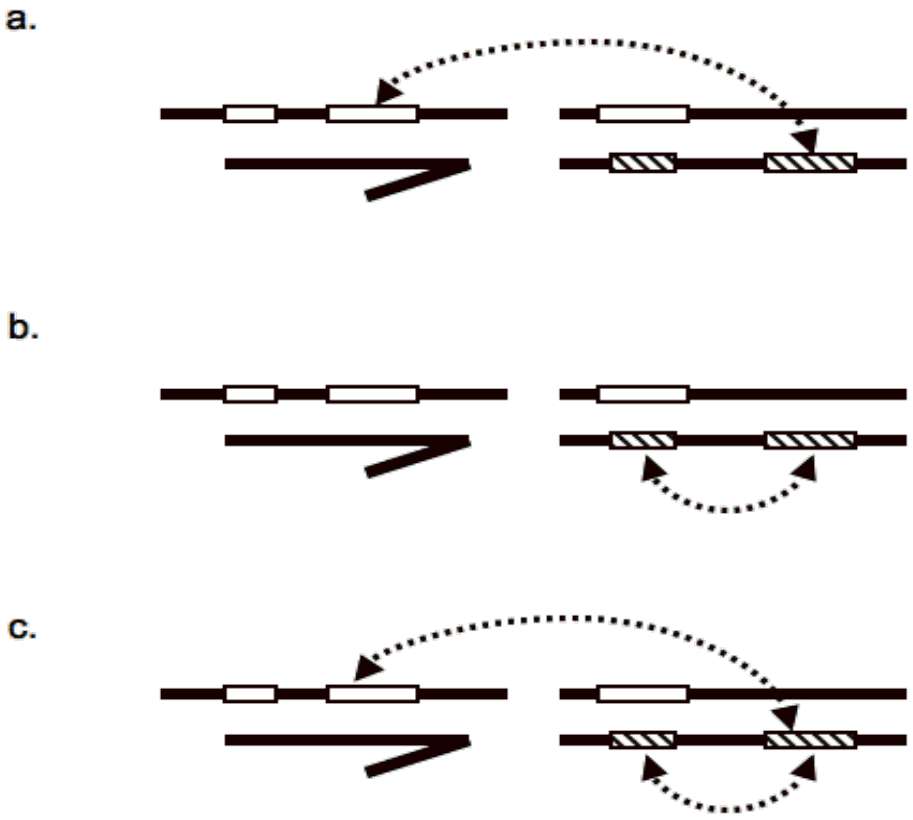

Figure 3. Epistasis Between Hybrid Incompatibility QTL.

Epistasis necessarily occurs between incompatibility-conferring loci along chromosomes from different species (a). However, epistasis may also occur between multiple loci within a single species (b) and/or between multiple loci from both species (c). Open rectangles indicate incompatibility loci from Species 1; filled rectangles indicate incompatibility loci from Species 2. Dashed arrows represent epistatic interactions between loci. Note that not all possible interactions are depicted.

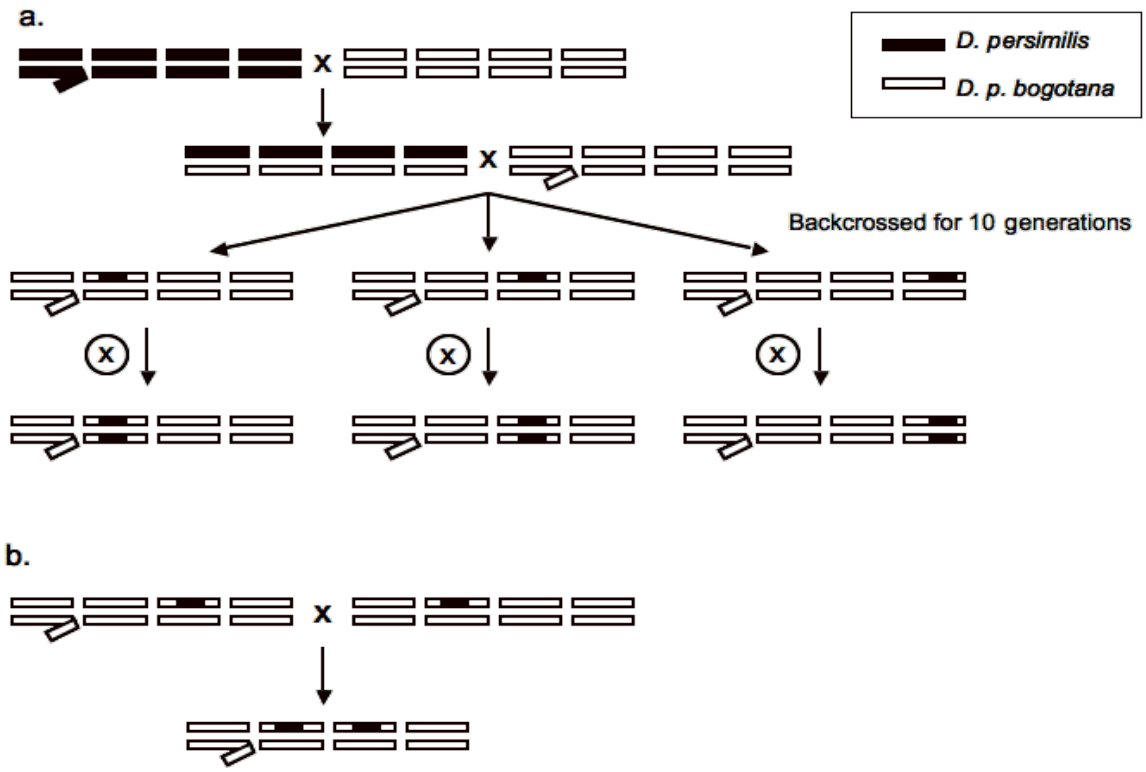

Figure 4. Crossing Scheme for Assessing Dominance

For single introgressions (a), *D. persimilis* males were crossed to *D. p. bogotana* females. F_1 females were backcrossed to *D. p. bogotana* males for 10 generations until the genetic background was devoid of *D. persimilis* alleles except for the QTL of interest. Three introgression lines were created for each autosomal QTL (Q2, Q3, and Q4). Males homozygous for each QTL introgression were generated by self-crossing within each line. Figure 2(b) shows an example of a cross to generate male flies heterozygous for both the 2nd - and the 3rd-chromosome QTLs.

Genotype	Q2	Q3	Q4	Fertile	Sterile	% Sterile
1				30	0	0.0
2				100	4	3.8
3				2	20	90.9
4				50	0	0.0
5				0	44	100.0
6				93	0	0.0
7				51	0	0.0

Figure 5. Effect on Male Sterility of Single QTL Introgressions.

Percent of males sterile for each genotype at the three QTLs is given. White rectangles represent homozygous *D. p. bogotana* genotype. Grey rectangles represent heterozygous *D. persimilis / D. p. bogotana* genotype. Black rectangles represent homozygous *D. persimilis* genotype.

Genotype	Q2	Q3	Q4	Fertile	Sterile	% Sterile
1				30	0	0.0
2				65	49	43.0
3				43	2	4.4
4				37	2	5.1
5				17	3	15.0
6				39	0	0.0
7				2	33	94.3

Figure 6. Effect on Male Sterility of Multiple QTL Introgressions.

Percent of males sterile for each genotype at the three QTLs is given. White rectangles represent homozygous *D. p. bogotana* genotype. Grey rectangles represent heterozygous *D. persimilis / D. p. bogotana* genotype. Black rectangles represent homozygous *D. persimilis* genotype.

3. The Nature of Homospecific Epistasis Among Factors Conferring Hybrid Male Sterility Between *Drosophila persimilis* and *D. pseudoobscura bogotana*

3.1 Introduction

The evolution of hybrid sterility in animals is generally thought to occur as a pleiotropic by-product of genic divergence between populations. Populations that have become separated accumulate changes in their nucleotide sequences, either as a result of selection (e.g., in response to new environments) or as a result of genetic drift. The Bateson-Dobzhansky-Muller model (BATESON 1909; DOBZHANSKY 1936; MULLER 1942) describes how, when these largely differentiated populations are brought back together, genes that have evolved in one genomic context possibly fail or work less efficiently in the context of the other genome, thus leading to sterility of the hybrid offspring.

While further efforts in identifying such individual "speciation genes" may eventually reveal the types of genes that underlie reproductive isolation between species, they do not answer some long-standing and fundamental questions: How many loci are necessary to produce hybrid male sterility? What role, if any, does epistasis between these loci play in causing hybrid dysfunction? What is the linkage relationship between loci conferring hybrid sterility? For each of these questions, we have some preliminary answers. For example, studies have shown that hybrid male sterility sometimes results from complex epistasis between multiple loci and not from single factors of large effect

(CABOT *et al.* 1994; DAVIS and WU 1996; GADAU *et al.* 1999; ORR and IRVING 2001; PALOPOLI and WU 1994; SAWAMURA *et al.* 2004; TAO *et al.* 2003a; TAO *et al.* 2001). In some instances, tightly linked homospecific alleles appear crucial for the production of infertile hybrid males (DAVIS and WU 1996; PEREZ and WU 1995). Yet, we are far from being able to generalize from these single or few examples the nature of interaction between hybrid sterility loci.

In this study, we explore possible reasons why these fundamental questions have persisted despite extensive mapping efforts and technological innovations. Mapping factors that cause hybrid sterility often employs methodology similar to those for mapping quantitative traits, but one distinction is important: unlike quantitative traits, once an individual is sterile, further "sterility" effects cannot be observed. Specific alleles at several loci may need to interact together to cause sterility. However, multiple such interactions may also exist: alleles at loci A and B can interact to cause sterility, and a separate interaction between loci C and D may also be sufficient. Standard backcross mapping studies often do not distinguish between these scenarios.

To overcome the intrinsic limitations of backcross analyses, much recent progress in identifying and counting hybrid sterility loci has thus come from introgression analyses (PALOPOLI and WU 1994; SAWAMURA *et al.* 2000; TAO *et al.* 2003a; TAO and HARTL 2003; TAO *et al.* 2003b; TRUE *et al.* 1996). Such analyses allow the evaluation of the contributions of individual or groups of genetic factors, as well as the interactions between them. Alleles from one species that are introgressed into another species

background necessarily interact with their native counterparts. However, the foreign (i.e., introgressed) factors may also interact with each other. This "homospecific epistasis" is often implicitly assumed to be absent in introgression analyses. Yet, the results from chapter 2 show that, at the between-chromosome level, additional sterility effects are detected with the introgression of multiple foreign segments, suggesting that homospecific epistasis is important.

This study builds on the results from previous chapters and further dissects two of the three individual QTL that confer hybrid male sterility when introgressed from *D. persimilis* into a *D. pseudoobscura bogotana* genetic background. These two species diverged approximately 0.5 to 1 million years ago (LEMAN *et al.* 2005; WANG *et al.* 1997) and are distinguished by four chromosomal inversions: two on the X chromsome, one on the 2nd-chromosome, and one on the 3rd-chromosome. (The previously mapped QTL that is not discussed here is linked to the chromosome-3 inversion and thus cannot be further broken apart by recombination.) One of the two QTL addressed in this study resides outside the inverted region on chromosome-2 while the other resides on chromosome-4.

Here, I show that, between *D. persimilis* and *D. p. bogotana*, a larger number of loci than could be estimated from a standard QTL or introgression mapping design confer infertility of backcross hybrid males. Furthermore, tightly linked homospecific loci sometimes, but not always, contribute to hybrid malfunction.

3.2 Materials and Methods

3.2.1 Fly Stocks and Culturing Conditions

Drosophila pseudoobscura bogotana carrying a *white* eye mutation were used in the crosses described below, as the *white* locus is linked to an inversion that distinguishes between *D. p. bogotana* and *D. persimilis*. The *D. p. bogotana white* strain is a subculture of the *D. p. bogotana* El Recreo line collected in 1978 (provided by H. A. ORR). *The D. persimilis* MSH1993 line was derived from females collected at Mt. St. Helena, California in 1993 (NOOR 1995). All crosses were performed on standard sugar/yeast/agar medium at 20 ± 1^{o} C and 85% relative humidity.

3.2.2 Fine-Mapping of Sterility Factors Within the Chromosome-2 and Chromosome-4 QTL

Following the methods described in chapter 2, two independent introgression lines were created, one for the chromosome-2 QTL (hereafter Q2) and the other for the chromosome-4 QTL (hereafter Q4). Each line contained a single copy of the *D. persimilis* allele and a single copy of the *D. p. bogotana* allele between positions 26,679,247 and 28,477,289 on chromosome 2 and positions 3,490,400 and 4,721,639 on group 1 of chromosome 4, respectively. Briefly, F_1 females were backcrossed to *D. p. bogotana* males for ten generations to clear the background of *D. persimilis* alleles at regions other than those coinciding with the two QTL. During this process, the *D. p. bogotana* chromosomal arrangements were selected for the two inversions on the X-chromosome and the one inversion on the 2nd-chromosome that differentiate the two species. This ensures that the mapping results obtained here most recapture effects detected in the original QTL-mapping study (see Chapter 1 and CHANG and NOOR 2007).

Selection for the introgressions and for the rearrangements was completed by microsatellite genotyping of markers delineating the QTL and the rearrangements.

For each introgressed *D. persimilis* QTL region, we sought to generate recombinant segments and assay their fertility alone and in combination with the other QTL. Additional mapping lines were created by repeatedly crossing females of the introgression line (i.e., Q2 or Q4) with *D. p. bogotana* males. Recombination between the *D. persimilis* and *D. p. bogotana* genomes within each QTL generated multiple independent lines; additional microsatellite markers were designed to differentiate between these lines. Because we previously identified an interaction between the QTL on each of the three major autosomes as necessary for complete sterility, females from mapping lines were then crossed to males heterozygous for the chromosome-3 and either the chromosome-4 or the chromosome-2 QTL. Recombinant male flies heterozygous for one portion of the QTL of interest (e.g., Q2) and heterozygous for the other two QTL (e.g., Q3 and Q4) were assayed for fertility following the methods of COYNE (1984). Lines resulting in sterile males were maintained in heterozygous state while lines resulting in fertile males were made homozygous for the *D. persimilis* allele in the absence of the other two QTL.

3.3 Results

3.3.1 Fine-Mapping Dominant Chromosome-2 Factors Underlying Hybrid Male Sterility

Using independent introgressions generated by recombination between the *D. persimilis* and *D. p. bogotana* genomes (see Materials and Methods), we assessed the effect on hybrid male sterility of different regions within the chromosome-2 QTL, which spans a region of almost 2 Mb near the centromere. All introgressions described below involved a single (i.e., heterozygous) copy of the *D. persimilis* allele. Introgression of a *D. persimilis* segment of approximately 1.5 Mb (line 1 in figure 7) resulted in nearly complete hybrid male sterility when hybrid males were heterozygous for this and Q3 and Q4 (see chapter 2). However, introgression of an overlapping segment between 0.5 Mb and 0.8 Mb in size (line 3 in figure 7) dramatically decreased the proportion of sterile males to approximately 33%, suggesting that at least one sterility factor resides between positions 26,680,000 and 27,790,000 of chromosome 2. This factor alone is necessary for causing near-complete sterility, as its absence had a significant effect on the proportion of sterile males. Furthermore, splitting this second introgression (line 3) into two smaller but overlapping introgressions (lines 6 and 8 in figure 7) resulted in 13% male sterility in line 6 but full fertility of all males in line 8. The difference in fertility between line 3 and line 6 is statistically significant ($G^2 = 26.3$, $p < 0.0001$, log-linear three-way contingency test (SOKAL and ROHLF 1995), suggesting the presence of at least two additional sterility-conferring loci within this region. At this smaller genomic scale (within a single autosomal QTL), epistasis between loci is critical to the extent of resultant hybrid male sterility. However, the highly specific nature of epistasis prevents

the identification of dominant-acting genetic factors on chromosome-2 that underlie hybrid male sterility.

3.3.2 The Chromosome-2 QTL Contains at Least One Recessive Genetic Factor that Can Confer Hybrid Male Sterility in the Absence of Homospecific Epistasis

In addition to the dominant-acting sterility factors within the chromosome-2 QTL, at least one recessive-acting sterility factor also resides in this 2-Mb region. Line 7 in figure 7 shows the likely location of this factor. All males heterozygous for only this introgression were fertile, as were all males heterozygous for this and for the Q3 and Q4 introgressions. However, all males homozygous for only this introgression (i.e., do not also carry the Q3 and Q4 introgressions) were sterile; in contrast, only 2 of 20 males heterozygous for only this introgression were sterile. This difference is significant ($p = 0.00565$, Fisher's Exact test). Furthermore, repeated attempts to create a stable homozygous line of this introgression failed, likely resulting from the sterility of males homozygous for the *D. persimilis* allele at this locus.

3.3.3 A Single Factor Within the Chromosome-4 QTL Causes Hybrid Male Sterility

In contrast to the chromosome-2 QTL, which appears to harbor at least three factors that interact with each other, the chromosome-4 QTL appears to contain a single locus contributing to hybrid male sterility. This locus resides between positions 4,500,000 and 4,800,000 on group 1 of chromosome 4. It is important to note that hybrid

male sterility is manifest only when this chromosome-4 factor interacts with the chromosome-2 QTL and with the chromosome-3 QTL (see chapter 2). When a single copy of this factor is co-introgressed with single copies of the 2nd- and 3rd-chromsome QTL, 60% of the resulting male flies are sterile. Co-introgressions of other regions within the chromosome-4 QTL fail to produce sterile males (see figure 8).

3.4 Discussion

In the previous chapter, we show that hybrid male sterility between *D. persimilis* and *D. pseudoobscura bogotana* results from highly specific epistasis between three autosomal QTL. Those results suggest that, at least between these species, hybrid male sterility does not require many interchangeable and interacting loci of small effect that together exceed some threshold. However, because few studies have actually dissected individual QTL contributing to hybrid sterility, the number of genetic factors that underlie this form of intrinsic postzygotic isolation remains unclear. By fine-mapping the sterility loci residing within two of the three QTL, we show that the sterility phenotype results from interactions between more homospecific genetic factors than previously inferred. Furthermore, these results suggest some, but not all, sterility factors are closely linked to other factors modifying their effects.

3.4.1 How Many Homospecific Loci Are Necessary to Cause Hybrid Male Sterility?

To date, only a small number of studies have precisely localized genes that underlie hybrid male sterility between closely related species. Most of the examples that

exist are restricted to the genus Drosophila (MASLY *et al.* 2006; PHADNIS and ORR 2009; TAO *et al.* 2003b; TING *et al.* 1998) except for one in the house mouse (MIHOLA *et al.* 2009). The first gene identified, *OdsH*, causes a 50% reduction in fertility in hybrid males when the *D. mauritiana* allele is introgressed into a *D. simulans* genetic background (PEREZ and WU 1995). For complete sterility, *OdsH* must be co-introgressed with other closely linked factors; these factors remain unidentified.

Using the same species, Tao et al.'s (2003b) study examining hybrid male sterility uncovered at least 19 factors on chromosome 3 that, when introgressed from *D. mauritiana* into a *D. simulans* background, resulted in sterile males. Interestingly, none of these 19 factors alone could cause infertility in hybrid males. Instead, at least two or three factors must be co-introgressed for appreciable sterility. Only one region on the 3rd chromosome (*tmy*) was capable of causing significant sterility on its own. Furthermore, none of the regions identified conferred sterility when heterozygous, which would be necessary for producing the F_1 hybrid male sterility observed.

Most recently, Phadnis and Orr (2009) identified *Overdrive*, an X-linked gene that causes both hybrid male sterility and meiotic drive between the two subspecies (USA and Bogota) of *D. pseudoobscura*. However, this gene must interact with at least two other unidentified X-linked loci from the Bogota subspecies for significant sterility/drive to occur. Thus, between these two young subspecies, a few homospecific loci appear necessary for hybrid male sterility. In contrast, between subspecies of house mouse, a

single autosomal gene, *Prdm9*, is sufficient in disrupting spermatogenesis (MIHOLA *et al.* 2009).

Together, these studies reveal a fundamental gap in our knowledge of the genetics underlying postzygotic barriers to gene flow such as hybrid male sterility. While the identities and thus the function of these "speciation genes" are becoming available, the number of interactions among genes that confer these hybrid dysfunctions remains unknown. Addressing this deficiency is far from trivial, as it requires simultaneous introgression of, and generating recombinants of, multiple sterility-conferring QTL regions.

In this study, dissection of the chromosome-2 and chromosome-4 QTL suggests that the number of factors residing within QTL conferring sterility can vary. In the case of the former QTL, three, or perhaps more, sterility factors must interact in a highly specific nature for the entire chromosome-2 QTL to contribute significantly to male infertility. The extent of epistasis here precludes localization of single genes (or other genetic factors such as *cis*-regulatory binding domains) that can be considered "sterility genes." The probability of capturing the exact combination of loci that together produce the effect of the 2nd-chromosome QTL is small when the fine-mapping method relies on recombination within this QTL to generate introgressions of different sizes, many of which are overlapping. While the chromosome-2 QTL appears to harbor several genetic factors that together produce the sterility effect shown by this QTL, the chromosome-4

QTL seems to only contain a single locus underlying this QTL's contribution, given its localization to under 0.3 Mb.

The results of fine-mapping presented here imply that we have largely underestimated the number of loci that contribute to hybrid dysfunction. As the case of the chromosome-2 QTL region demonstrates, a single QTL can harbor more than one locus underlying hybrid sterility. Furthermore, it is important to point out that, between *D. persimilis* and *D. p. bogotana*, an accurate estimate of sterility loci is also confounded by the presence of four chromosomal inversion differences between these species. One of the sterility-conferring QTL that we originally mapped is linked to the 3rd-chromosome inversion, thus precluding fine-mapping as no recombination in this region can occur. Additionally, the two inversions on the X chromosome and the one inversion on the 2nd-chromosome may also contain multiple loci contributing to hybrid sterility.

3.4.2 Role of Linkage Between Homospecific Genetic Factors in Hybrid Male Sterility

In the *D. pseudoobscura* species group, whether linkage between homospecific loci is important appears to depend on the difference in divergence times between the species pairs. In the more recent divergence between the two subspecies of *D. pseudoobscura*, close linkage seems to be unnecessary: *Overdrive*, which is located on the right arm of the X chromosome, must interact with other loci from *D. p. bogotana* but those loci reside on the left arm of the X chromosome (ORR and IRVING 2001; PHADNIS and ORR 2009). However, between the more distantly related species *D. persimilis* and

D. p. bogotana, closely linked factors (i.e., within the same 2nd-chromosome QTL region) are essential at least some of the time. (Note that these two QTL must interact also with each other and with the 3rd-chromosome QTL for hybrid male sterility to occur.)

Early studies of *OdsH* in the *D. mauritiana-D. simulans* species pair seemed to suggest that linkage between homospecific loci played an essential role in hybrid male sterility. *OdsH* itself had minimal effect on fertility unless a proximal region on the X-chromosome of *D. mauritiana* was co-introgressed with *OdsH* into an otherwise *D. simulans* genetic background (PEREZ and WU 1995). Since then, however, the role of linkage on hybrid dysfunction has become more controversial. TAO *et al.*'s (2003b) study did not explicitly examine the linkage relationship between the 19 putative loci that contribute to hybrid male sterility in these species. However, given all 19 of those loci reside on the 3rd-chromosome and that sterility appears to result from complex patterns of epistasis between some of those loci, it is likely that linkage may exist amongst some, conferring sterility together.

3.4.3 Identification of a Hybrid Sterility Gene

This study utilized an introgression approach to identify a single genetic factor on chromosome 4 that, when the *D. persimilis* allele is placed into an otherwise *D. p. bogotana* background, interacts with the chromosome-2 and chromosome-3 sterility-conferring QTL to produce infertile hybrid males. The data thus far suggest that such a factor resides in a 0.3 Mb region on group 1 of chromosome 4. Three predicted genes (GA19742, GA19717, and GA16363) are contained within this region and are therefore

good candidates for another hybrid sterility gene. Unfortunately, we currently have no information on the function of these three candidates.

As with *tmy* (TAO *et al.* 2003b), this factor appears to produce a sterility effect without homospecific epistasis. The extreme epistasis described in this study may be more important for the effects of heterozygous sterility alleles than for homozygous alleles. This may be a consequence of the predominantly recessive nature of factors contributing to hybrid dysfunctions and further emphasizes why later generation hybrid breakdown (which can involve homozygous homospecific factors) may evolve earlier in divergence than F_1 hybrid problems (which requires accumulated dominant effects of multiple homospecific factors).

Identification of an additional "speciation gene" will, in the long term, shed light on the types of genes that are important in population divergence. The small handful of genes that contribute to hybrid sterility and inviability currently available yields only premature conclusions regarding gene classes causal to speciation and tentative conclusions regarding the evolutionary forces that act on these genes. As gene ontologies become increasingly available and molecular functions are assigned to more individual genes, studies of the patterns of speciation will finally be able to interface with studies of molecular genetics.

Though we are currently beginning to uncover the identity of genes that are involved in hybrid incompatibilities, we have yet to answer a much more fundamental

question: How many genetic changes are necessary for reproductive isolation and thus for species formation? The results presented here suggest that we are only beginning to understand the intricacies of interactions between genetic factors causing hybrid dysfunction. The resolution limitations imposed by available mapping techniques places us just at the tip of the iceberg.

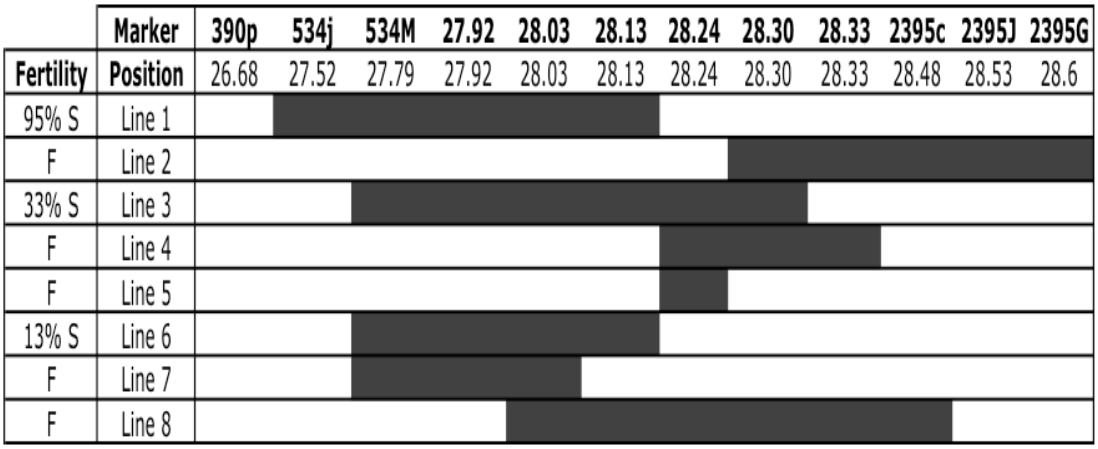

Figure 7. Effect on Male Sterility of Introgressions Within the Chromosome-2 QTL Region.

Individual *D. persimilis* introgressions are represented by grey bars. Microsatellite markers and marker positions delineating each introgression are given.

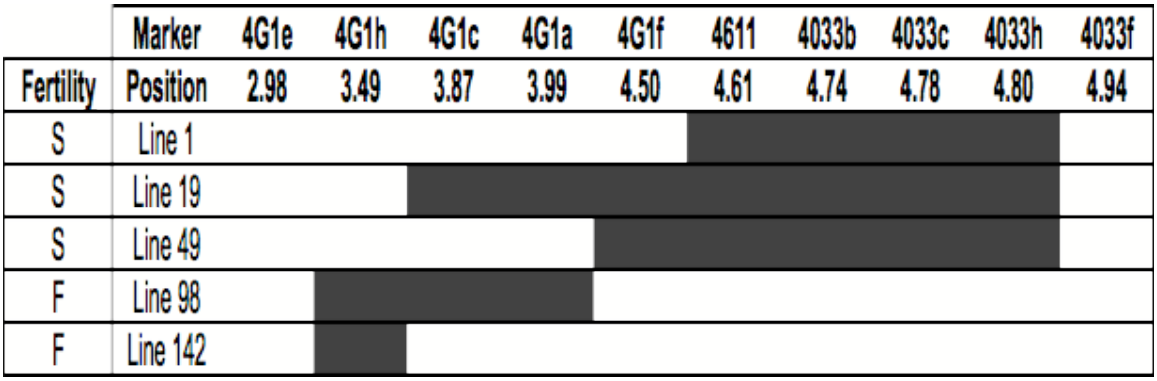

Figure 8. Effect on Male Sterility of Introgressions Within the Chromosome-4 QTL Region.

Individual *D. persimilis* introgressions are represented by grey bars. Microsatellite markers and marker positions delineating each introgression are given.

4. Conclusions

Because the process of speciation generates biodiversity, its study is among the most active and enduring in evolutionary biology. Early research efforts sought to elucidate the reproductive barriers that arise between diverging populations, as the evolution of such barriers is largely equivalent to the processing of establishing "good" species. Studies on broad patterns of speciation soon followed: we now know that population divergence more often occurs when populations are geographically separated from one another, for example, though divergence in sympatry may also play a role in speciation in some instances. Various prezygotic and postzygotic barriers to gene flow have been characterized in multiple taxa and we often understand their contributions to reproductive isolation. Finally, we are beginning to identify the genes that underlie in species hybrids, thus providing data to address models and hypotheses first proposed by Dobzhansky, Muller, and Haldane.

Using the *Drosophila pseudoobscura* species group as a study system, this dissertation addresses three fundamental questions on the genetic architecture of hybrid male sterility:

1. How does reproductive isolation and hence population divergence occur in the face of gene flow?
2. How does epistasis affect the dominance of autosomal alleles contributing to hybrid male sterility?

3. What is the nature of epistasis and linkage between homospecific alleles that underlie hybrid male sterility?

In chapter 1, I test an evolutionary model proposed to explain how populations can remain distinct despite the exchange of genetic material between them. In the absence of strong selection, gene flow (i.e., recombination) between populations should "swamp out" differences that accumulate (e.g., from natural selection or drift) in each lineage. Selection would eliminate alleles conferring hybrid dysfunction and thus would homogenize the genomes. As such, the genomes would be fully compatible and preclude the maintenance of reproductive isolation. Yet, many examples of species that remain distinct despite gene flow exist. Independently, NOOR *et al.* (2001) and RIESEBERG (2001) proposed an elegant solution to this conundrum: by preventing recombination over long stretches of the genome, chromosomal inversions allow the persistence of alleles conferring incomptability of divergence adaptations.

By mapping hybrid male sterility loci between *D. persimilis* and its sister species *D. p. bogotana*, I confirm one of the key predictions of the "reduced recombination" model: between these allopatric species, such loci map to regions of the genome that do not contain inversions. Between these species, three major QTL, one on each autosome, confer hybrid male sterility when the *D. persimilis* alleles interact with a *D. p. bogotana* background. Furthermore, these QTL do not confer sterility in the sympatric hybridization between *D. persimilis* and *D. pseudoobscura*. This study follows up on an initial study (BROWN *et al.* 2004) in this species group that showed that QTL conferring

reproductive isolation were located both within and outside inversions in the allopatric species pair but were located only within inversions in the sympatric species pair (i.e., *D. persimilis* and *D. pseudoobscura*). Together, these results delegate a novel role for chromosomal rearrangments in the process of speciation.

Using the results obtained from the initial mapping study (Chapter 1), I introgressed the *D. persimilis* alleles of three sterility-conferring QTL into an otherwise *D. p. bogotana* background to test a genetic hypothesis. The dominance theory (MULLER 1940; MULLER 1942; TURELLI and ORR 1995; TURELLI and ORR 2000) proposed to explain Haldane's (1922) rule posits that sex-linked alleles contributing to incompatibilities in hybrids are, on average, partially recessive. This property of incompatibility alleles is what causes the asymmetry in F_1 hybrid male and female fitness; as Haldane observed, when one hybrid sex is sterile or inviable, it is more often the heterogametic sex. These recessive X-linked alleles interact with dominant autosomal alleles from the other species to produce F_1 hybrid unfitness in the heterogametic. The apparently dominant nature of the *D. persimilis* sterility QTL strongly suggested that such autosomal loci could be characterized.

Surprisingly, introgression analysis of the dominance of those QTL produced significantly different results than that from backcross analysis: When singly introgressed, the *D. persimilis* alleles had virtually no effect on hybrid male sterility. In contrast, when those alleles were introduced by a single generation of backcrossing, each QTL had a detectable effect on sterility. The simple explanation for this discrepancy is

that many epistatic interactors with those QTL that were present in the backcross population were lost when the QTL were inserted into a *D. p. bogotana* genetic background void of all other *D. persimilis* alleles (i.e., including those interactors). However, further characterization of those *D. persimilis* QTL revealed that 1) epistasis between those alleles are highly specific and 2) epistasis plays a critical role in modulating the dominance of the sterility alleles. These unexpected results thus imply that recessive-acting "speciation genes" thought *not* to participate in F_1 hybrid problems may warrant further examination with respect to epistasis.

Finally, this dissertation focuses on individual QTL regions to examine the extent and nature of epistasis within sterility-conferring loci from one species in the other species' genetic background. Dissection of two of the three QTL from *D. persimilis* produce contrasting results: one of the QTL appears to harbor several genetic factors that interact with each other to produce the overall effect of the introgressed QTL that was observed in Chapter 2. The effect of the other QTL appears attributable to a single factor (or at least a very small region) residing within that QTL. These results suggest that, quite likely, no discernible "rule" applies to QTL/alleles contributing to hybrid problems. Furthermore, currently available mapping resolution may compromise our ability to accurately estimate the number of genetic changes required to induce reproductive isolation.

The study of speciation has made impressive progress since the advent of modern genetics. A century and a half after the publication of *On the Origin of Species* (DARWIN

1859), we are finally beginning to know how species can form after populations are separated genetically from one another and are reproductively isolated. A mere three-quarters of a century after Dobzhansky's (1937) seminal *Genetics and the Origin of Species*, genes actually producing hybrid incompatibilities have been cloned and characterized. Yet, while many of the "big questions" have been answered, more details remain to be elucidated. Here, using the *D. pseudoobscura* species group (a favorite of Dobzhansky's), I examine some of the fundamental aspects of the genetic architecture of hybrid male sterility. I test an evolutionary model that allows species persistence in the face of gene flow, a scenario intermediate to the traditional, "extreme" models of geographic allopatry *vs*. sympatry. In addition to localizing individual genes that cause this form of hybrid dysfunction, I address the broader genetic interactions between loci that underlie hybrid male sterility.

References

AQUADRO, C. F., A. L. WEAVER, S. W. SCHAEFFER and W. W. ANDERSON, 1991 Molecular evolution of inversions in *Drosophila pseudoobscura*: The amylase gene region. Proc. Natl. Acad. Sci. USA **88:** 305-309.

BASTEN, C. J., B. S. WEIR and Z.-B. ZENG, 1999 *QTL Cartographer: A Reference Manual and Tutorial for QTL Mapping*. North Carolina State University, Raleigh, North Carolina (http://statgen.ncsu.edu).

BATESON, W., 1909 Heredity and variation in modern lights in *Darwin and Modern Science*, edited by A. C. SEWARD. Cambridge University Press, Cambridge.

BROWN, K. M., L. M. BURK, L. M. HENAGAN and M. A. F. NOOR, 2004 A test of the chromosomal rearrangement model of speciation in *Drosophila pseudoobscura*. Evolution **58:** 1856-1860.

BUTLIN, R. K., 2005 Recombination and speciation. Mol. Ecol. **14:** 2621-2635.

CABOT, E. L., A. W. DAVIS, N. A. JOHNSON and C.-I. WU, 1994 Genetics of reproductive isolation in the *Drosophila simulans* clade: complex epistasis underlying hybrid male sterility. Genetics **137:** 175-189.

CAMPBELL, R. V., and M. A. F. NOOR, 2001 Assessing hybrid male fertility in Drosophila species: Correlation between sperm motility and production of offspring. Drosoph. Inf. Serv. **84:** 6-9.

CHANG, A. S., and M. A. NOOR, 2007 The genetics of hybrid male sterility between the allopatric species pair *Drosophila persimilis* and *D. pseudoobscura bogotana*: dominant sterility alleles in collinear autosomal regions. Genetics **176:** 343-349.

CHARLESWORTH, B., J. A. COYNE and N. H. BARTON, 1987 The relative rates of evolution of sex chromosomes and autosomes. Am. Nat. **130:** 113-146.

COYNE, J. A., 1984 Genetic basis of male sterility in hybrids between two closely related species of *Drosophila*. Proc. Natl. Acad. Sci., USA **81:** 4444-4447.

COYNE, J. A., and H. A. ORR, 2004 *Speciation*. Sinauer Associates, Inc., Sunderland, MA.

DARWIN, C. R., 1859 *On the origin of species by means of natural selection, or the preservation of favoured races in the struggle for life*. John Murray, London.

DAVIS, A. W., and C.-I. WU, 1996 The broom of the sorcerer's apprentice: the fine structure of a chromosomal region causing reproductive isolation between two sibling species of Drosophila. Genetics **143:** 1287-1298.

DOBZHANSKY, T., 1936 Studies of hybrid sterility. II. Localization of sterility factors in *Drosophila pseudoobscura* hybrids. Genetics **21:** 113-135.

DOBZHANSKY, T., 1937 *Genetics and the Origin of Species*. Columbia University Press, New York.

DOBZHANSKY, T., 1973 Is there gene exchange between *Drosophila pseudoobscura* and *Drosophila persimilis* in their natural habitats? Am. Nat. **107:** 312-314.

FEDER, J. L., J. B. ROETHELE, K. FILCHAK, J. NIEDBALSKI and J. ROMERO-SEVERSON, 2003 Evidence of inversion polymorphism related to sympatric host race formation in the apple maggot fly, *Rhagoletis pomonella*. Genetics **163:** 939-953.

FITZPATRICK, B. M., 2008 Hybrid dysfunction: Population genetic and quantitative genetic perspectives. American Naturalist **171:** 491-498.

GADAU, J., J. R.E. PAGE and J. H. WERREN, 1999 Mapping of hybrid incompatibility loci in *Nasonia*. Genetics **153:** 1731-1741.

GILBERT, D. G., 2005 DroSpeGe, a public database of Drosophila species genomes

GLOOR, G. B., and W. R. ENGELS, 1992 Single-fly DNA preps for PCR. Drosoph. Inf. Serv. **71:** 148-149.

GUENET, J., C. NAGAMINE, D. SIMON-CHAZOTTES, X. MONTAGUTELLI and F. BONHOMME, 1990 *Hst-3*: an X-linked hybrid sterility gene. Genetical Research **53:** 163-165.

HALDANE, J. B. S., 1922 Sex ratio and unisexual sterility in hybrid animals. J. Genet. **12:** 101-109.

HEY, J., and R. NIELSEN, 2004 Multilocus methods for estimating population sizes, migration rates and divergence time, with applications to the divergence of *Drosophila pseudoobscura* and *D. persimilis*. Genetics **167:** 747-760.

KIRKPATRICK, M., and N. BARTON, 2006 Chromosome inversions, local adaptation and speciation. Genetics **173:** 419-434.

LAURIE, C. C., 1997 The weaker sex is heterogametic: 75 years of Haldane's rule. Genetics **147:** 937-951.

LEMAN, S. C., Y. CHEN, J. E. STAJICH, M. A. F. NOOR and M. K. UYENOYAMA, 2005 Likelihoods from summary statistics: Recent divergence between species. Genetics **171:** 1419-1436.

LI, J., S. WANG and Z. B. ZENG, 2006 Multiple-interval mapping for ordinal traits. Genetics **173:** 1649-1663.

MACDONALD, S. J., and D. B. GOLDSTEIN, 1999 A quantitative genetic analysis of male sexual traits distinguishing the sibling species *Drosophila simulans* and *D. sechellia*. Genetics **153:** 1683-1699.

MACHADO, C. A., and J. HEY, 2003 The causes of phylogenetic conflict in a classic Drosophila species group. Proc. R. Soc. Lond. B **270:** 1193-1202.

MACHADO, C. A., R. M. KLIMAN, J. A. MARKERT and J. HEY, 2002 Inferring the history of speciation from multilocus sequence data: the case of *Drosophila pseudoobscura* and its close relatives. Mol. Biol. Evol. **19:** 472-488.

MASLY, J. P., C. D. JONES, M. A. F. NOOR, J. LOCKE and H. A. ORR, 2006 Gene transposition as a cause of hybrid sterility in Drosophila. Science **313:** 1448-1450.

MASLY, J. P., and D. C. PRESGRAVES, 2007 High-resolution genome-wide dissection of the two rules of speciation in Drosophila. PLoS Biology **5:** e243.

MI, H., B. LAZAREVA-ULITSKY, R. LOO, A. KEJARIWAL, J. VANDERGRIFF *et al.*, 2005 The PANTHER database of protein families, subfamilies, functions and pathways. Nucleic Acids Res **33:** D284-D288.

MIHOLA, O., Z. TRACHTULEC, C. VLCEK, J. C. SCHIMENTI and J. FOREJT, 2009 A mouse speciation gene encodes a meiotic histone H3 methyltransferase. Science **323:** 373-375.

MOEHRING, A. J., J. LI, M. D. SCHUG, S. G. SMITH, M. DEANGELIS *et al.*, 2004 Quantitative trait loci for sexual isolation between *Drosophila simulans* and *D. mauritiana*. Genetics **167:** 1265-1274.

MOEHRING, A. J., A. LLOPART, S. ELWYN, J. A. COYNE and T. F. MACKAY, 2006 The genetic basis of postzygotic reproductive isolation between *Drosophila santomea* and *D. yakuba* due to hybrid male sterility. Genetics **173:** 225-233.

MULLER, H. J., 1940 Bearings of the Drosophila work on systematics, pp. 185-268 in *New Systematics*, edited by J. HUXLEY. Clarendon Press, Oxford.

MULLER, H. J., 1942 Isolating mechanisms, evolution and temperature. Biol. Symp. **6:** 71-125.

NAVARRO, A., and N. H. BARTON, 2003 Accumulating postzygotic isolation in parapatry: A new twist on chromosomal speciation. Evolution **57:** 447-459.

NAVEIRA, H., and A. FONTDEVILA, 1986 The evolutionary history of *Drosophila buzzatii*. XII. The genetic basis of sterility in hybrids between *D. buzzatii* and its sibling *D. serido* from Argentina. Genetics **114:** 841-857.

NAVEIRA, H., and A. FONTDEVILA, 1991 The evolutionary history of *D. buzzatii*. XXI. Cumulative action of multiple sterility factors on spermatogenesis in hybrids of *D. buzzatii* and *D. koepferae*. Heredity **67:** 57-72.

NAVEIRA, H. F., and X. R. MASIDE, 1998 *The genetics of hybrid male sterility in Drosophila*. Oxford University Press, Oxford.

NOOR, M. A., 1995 Speciation driven by natural selection in *Drosophila*. Nature **375:** 674-675.

NOOR, M. A. F., K. L. GRAMS, L. A. BERTUCCI, Y. ALMENDAREZ, J. REILAND *et al.*, 2001 The genetics of reproductive isolation and the potential for gene exchange between *Drosophila pseudoobscura* and *D. persimilis* via backcross hybrid males. Evolution **55:** 512-521.

OKA, A., A. MITA, N. SAKURAI-YAMATANI, H. YAMAMOTO, N. TAKAGI *et al.*, 2004 Hybrid breakdown caused by substitution of the X chromosome between two mouse subspecies. Genetics **166:** 913-924.

ORR, H. A., 1987 Genetics of male and female sterility in hybrids of *Drosophila pseudoobscura* and *D. persimilis*. Genetics **116:** 555-563.

ORR, H. A., 1989 Localization of genes causing postzygotic isolation in two hybridizations involving *Drosophila pseudoobscura*. Heredity **63:** 231-237.

ORR, H. A., 1993 A mathematical model of Haldane's Rule. Evolution **47:** 1606-1611.

ORR, H. A., 1997 Haldane's Rule. Annu. Rev. Ecol. Syst. **28:** 195-218.

ORR, H. A., and S. IRVING, 2001 Complex epistasis and the genetic basis of hybrid sterility in the *Drosophila pseudoobscura* Bogota-USA hybridization. Genetics **158:** 1089-1100.

ORTIZ-BARRIENTOS, D., A. S. CHANG and M. A. F. NOOR, 2006 A recombinational portrait of the *Drosophila pseudoobscura* genome. Genet. Res., Camb. **87:** 23-31.

ORTIZ-BARRIENTOS, D., J. REILAND, J. HEY and M. A. F. NOOR, 2002 Recombination and the divergence of hybridizing species. Genetica **116:** 167-178.

PALOPOLI, M. F., and C.-I. WU, 1994 Genetics of hybrid male sterility between Drosophila sibling species: a complex web of epistasis is revealed in interspecific studies. Genetics **138:** 329-341.

PANITHANARAK, T., H. C. HAUFFE, J. F. DALLAS, A. GLOVER, R. G. WARD *et al.*, 2004 Linkage-dependent gene flow in a house mouse chromosomal hybrid zone. Evolution **58:** 184-192.

PEREZ, D. E., and C.-I. WU, 1995 Further characterization of the *Odysseus* locus of hybrid sterility in Drosophila: One gene is not enough. Genetics **140:** 201-206.

PHADNIS, N., and H. A. ORR, 2009 A single gene causes both male sterility and segregation distortion in Drosophila hybrids. Science **323:** 376-379.

POWELL, J. R., 1983 Interspecific cytoplasmic gene flow in the absence of nuclear gene flow: Evidence from *Drosophila*. Proc. Natl. Acad. Sci. USA **80:** 492-495.

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.

RIESEBERG, L. H., 2001 Chromosomal rearrangements and speciation. Trends Ecol. Evol. **16:** 351-358.

RIESEBERG, L. H., J. WHITTON and K. GARDNER, 1999 Hybrid zones and the genetic architecture of a barrier to gene flow between two sunflower species. Genetics **152:** 713-727.

SAWAMURA, K., A. W. DAVIS and C.-I. WU, 2000 Genetic analysis of speciation by means of introgression into *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA **97:** 2652-2655.

SAWAMURA, K., J. ROOTE, C. I. WU and M. T. YAMAMOTO, 2004 Genetic complexity underlying hybrid male sterility in Drosophila. Genetics **166:** 789-796.

SCHAEFFER, S. W., and E. L. MILLER, 1991 Nucleotide sequence analysis of *Adh* genes estimates the time of geographic isolation of the Bogota population of *Drosophila pseudoobscura*. Proc. Natl. Acad. Sci. USA **88:** 6097-6101.

SLOTMAN, M., A. DELLATORRE and J. R. POWELL, 2004 The genetics of inviability and male sterility in hybrids between *Anopheles gambiae* and *A. arabiensis*. Genetics **167:** 275-287.

SOKAL, R. R., and F. J. ROHLF, 1995 *Biometry*. W.H. Freeman and Company, New York.

STORCHOVA, R., S. GREGOROVA, D. BUCKIOVA, V. KYSELOVA, P. DIVINA *et al.*, 2004 Genetic analysis of X-linked hybrid sterility in the house mouse. Mamm. Genome **15:** 515-524.

STUMP, A. D., M. C. FITZPATRICK, N. F. LOBO, S. TRAORE, N. SAGNON *et al.*, 2005 Centromere-proximal differentiation and speciation in *Anopheles gambiae*. Proc Natl Acad Sci U S A **102:** 15930-15935.

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.

TAO, Y., S. CHEN, D. L. HARTL and C. C. LAURIE, 2003a Genetic dissection of hybrid incompatibilities between *Drosophila simulans* and *D. mauritiana*. I. Differential accumulation of hybrid male sterility effects on the X and autosomes. Genetics **164:** 1383-1398.

TAO, Y., and D. L. HARTL, 2003 Genetic dissection of hybrid incompatibilities between *Drosophila simulans* and *D. mauritiana*. III. Heterogeneous accumulation of hybrid incompatibilities, degree of dominance, and implications for Haldane's Rule. Evolution **57:** 2580-2598.

TAO, Y., D. L. HARTL and C. C. LAURIE, 2001 Sex-ratio segregation distortion associated with reproductive isolation in Drosophila. Proc. Natl. Acad. Sci. USA **98:** 13183-13188.

TAO, Y., Z.-B. ZENG, J. LI, D. L. HARTL and C. C. LAURIE, 2003b Genetic dissection of hybrid incompatibilities between *Drosophila simulans* and *D. mauritiana*. II. Mapping hybrid male sterility loci on the third chromosome. Genetics **164:** 1399-1418.

TING, C.-T., S.-C. TSAUR, M.-L. WU and C.-I. WU, 1998 A rapidly evolving homeobox at the site of a hybrid sterility gene. Science **282:** 1501-1504.

TRUE, J. R., B. S. WEIR and C. C. LAURIE, 1996 A genome-wide survey of hybrid incompatibility factors by the introgression of marked segments of *Drosophila mauritiana* chromosomes into *Drosophila simulans*. Genetics **142:** 819-837.

TURELLI, M., and H. A. ORR, 1995 The dominance theory of Haldane's Rule. Genetics **140:** 389-402.

TURELLI, M., and H. A. ORR, 2000 Dominance, epistasis and the genetics of postzygotic isolation. Genetics **154:** 1663-1679.

WANG, R. L., J. WAKELEY and J. HEY, 1997 Gene flow and natural selection in the origin of *Drosophila pseudoobscura* and close relatives. Genetics **147:** 1091-1106.

WHITE, M. J. D., 1969 Chromosomal rearrangements and speciation in animals. Annu. Rev. Genet. **3:** 75-98.

WHITE-COOPER, H., 2004 Spermatogenesis: Analysis of meiosis and morphogenesis, pp. 45-75 in *Drosophila Cytogenetics Protocols*, edited by D. S. HENDERSON. Humana Press, Totowa, New Jersey.

WU, C.-I., and A. W. DAVIS, 1993 Evolution of postmating reproductive isolation: The composite nature of Haldane's Rule and its genetic bases. Am. Nat. **142:** 187-212.

WU, C.-I., and H. HOLLOCHER, 1998 Subtle is nature: The genetics of species differentiation and speciation, pp. 339-351 in *Endless Forms: Species and Speciation*, edited by D. J. HOWARD and S. H. BERLOCHER. Oxford University Press, New York.

WU, C.-I., N. A. JOHNSON and M. F. PALOPOLI, 1996 Haldane's rule and its legacy: why are there so many sterile males? Trends Ecol. Evol. **11:** 281-284.

XU, S., and W. R. ATCHLEY, 1996 Mapping quantitative trait loci for complex binary diseases using line crosses. Genetics **143:** 1417-1424.

ZENG, Z. B., 1994 Precision mapping of quantitative trait loci. Genetics **136:** 1457-1468.

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Noor, M. A. F., and **A. S. Chang**. 2006. Evolutionary genetics: Jumping into a new species. Current Biology, 16: R890-R892.

Ortiz-Barrientos, D., **A. S. Chang**, and M. A. F. Noor. 2006. A recombinational portrait of the *Drosophila pseudoobscura* genome. Genetical Research, 87: 23-31

Chang, A. S. 2004. Conspecific sperm precedence in sister species of *Drosophila* with overlapping ranges. Evolution 58: 781-789

Coyne, J. A., S. Y. Kim, **A. S. Chang**, D. Lachaise, and S. Elwyn. 2002. Sexual isolation between two sibling species with overlapping ranges: *Drosophila santomea* and *Drosophila yakuba*. Evolution 56: 2424-2434

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