Segregation distortion in hybrids between the Bogota and USA subspecies of *Drosophila pseudoobscura*

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Running head: Segregation distortion in hybrids

Keywords: hybrid sterility, postzygotic isolation, meiotic drive, segregation distortion, speciation

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ABSTRACT

We show that, contrary to claims in the literature, “sterile” males resulting from cross of the Bogota and USA subspecies of *Drosophila pseudoobscura* are weakly fertile. Surprisingly, these hybrid males produce almost all daughters when crossed to females of any genotype (pure Bogota, pure USA, hybrid F₁). Several lines of evidence suggest that this sex ratio distortion is caused by sex chromosome segregation distortion in hybrid males. We genetically analyze this normally-cryptic segregation distortion and show that it involves several regions of the Bogota X chromosome that show strong epistatic interactions with each other. We further show that segregation distortion is normally masked within the Bogota subspecies by autosomal suppressors. Our analysis shows that the genetic basis of hybrid segregation distortion is similar to that of hybrid male sterility between the same subspecies. Indeed the severity of segregation distortion is correlated with the severity of sterility among hybrids. We discuss the possibility that hybrid sterility in this paradigmatic case of incipient speciation is caused by segregation distortion.
INTRODUCTION

The notion that selfish genetic elements may play a role in the origin of species has proved both popular and controversial (Grun 1976, pp. 352-254; Hurst and Pomiankowski 1991; Cosmides and Tooby 1981; Hurst and Werren 2001; Coyne and Orr 2004). While the idea has appeared in many guises, involving transposable elements, infectious endosymbionts like Wolbachia, and mitochondrial mutations that cause cytoplasmic male sterility, one version has proved particularly attractive. According to this idea, alleles that cause meiotic drive might systematically give rise to postzygotic isolation generally and to hybrid sterility specifically (Frank 1991; Hurst and Pomiankowski 1991; Tao et al. 2001; Tao and Hartl 2003; Henikoff et al. 2001; Henikoff and Malik 2002).

Mutations that cause meiotic drive distort Mendelian ratios to their own advantage, usually by inactivating sperm that carry a homologous chromosome. X chromosome-bearing sperm might, for instance, inactivate Y chromosome-bearing sperm. While obviously advantageous for the driving mutation, meiotic drive imposes a fertility cost on its bearers (since many gametes are rendered nonfunctional) as well as a fitness cost on most other genes in the genome (Lyttle 1991). When residing on the sex chromosomes, mutations causing meiotic drive also bias sex ratios away from the 50:50 favored by Fisherian selection. For all of these reasons, there will usually be strong selection to suppress meiotic drive (Sandler and Novitski 1957; Lyttle 1991; Jaenike 2001).
It is easy to imagine how mutations causing meiotic drive could ultimately give rise to intrinsic postzygotic isolation between taxa: two allopatric populations might each be invaded by different meiotic drive mutations; if each mutation later becomes suppressed, both populations will return to normal segregation ratios; if, however, these populations later come into secondary geographic contact and hybridize, this normally-cryptic meiotic drive could become re-expressed (assuming that the suppressors of meiotic drive are less than fully dominant). In the simplest (though not only) scenario, X-linked drive alleles might inactivate Y-bearing sperm in hybrids, while Y-linked alleles would inactivate X-bearing sperm, rendering XY hybrids sterile. Such a scenario might even help to explain “Haldane’s rule,” the preferential sterility or inviability of hybrids of the heterogametic (XY) sex, an idea that was proposed independently by FRANK (1991) and HURST and POMIANKOWSKI (1991).

TAO and HARTL (2003) and HENIKOFF et al. (2001) and HENIKOFF and MALIK (2002) have recently suggested variations on the meiotic drive theory of postzygotic isolation. In TAO and HARTL’s scenario, struggles over sex ratio are especially acute in the heterogametic sex as different portions of the genome in heterogametic individuals “prefer” different sex ratios (the Y chromosome in Drosophila, for instance, prefers more sons). In HENIKOFF and colleagues’ scenario, struggles over which chromosome segregates into an egg (and not into a polar body) in female meiosis give rise to bouts of meiotic drive followed by suppression of drive; these bouts, they suggest, involve evolution at centromeric sequences and at the special histones that bind these sequences. Despite their differences, these models all share a central theme: segregation distorters appear and are then suppressed within species, only to be re-expressed in species hybrids.
Although the meiotic drive theory of postzygotic isolation is attractive—especially as segregation distortion occurs in a wide variety of organisms, including insects, mammals, plants, and fungi (LYTTEL 1991)—it fell out of favor in the early 1990’s. The main reason was that experiments by COYNE (1986), JOHNSON and WU (1992), COYNE and ORR (1993) found no segregation distortion in hybrids between several evolutionarily independently pairs of Drosophila species. (These hybridizations produce partially fertile F1 hybrids, allowing tests of segregation distortion in hybrid gametogenesis.) These findings were widely viewed as fatal to the meiotic drive theory of speciation (a view once shared by the senior author).

More recent studies, however, suggest that COYNE, ORR, JOHNSON, and Wu may have been unlucky in their choice of species pairs or of hybrid genotypes. Several cases of normally-cryptic segregation distortion have now been described. All occur in Drosophila, presumably reflecting the intense genetic scrutiny of this genus. By far the best studied of these cases involves the species pair *D. simulans* and *D. mauritiana*. TAO et al. (2001) showed that otherwise *D. simulans* males that are homozygous for a small region of the *D. mauritiana* third chromosome suffer sex-ratio segregation distortion, producing approximately 80% daughters. TAO et al. suggest that the *D. simulans* genome carries X-linked meiotic drive factor(s) that are normally suppressed within species by a dominant autosomal suppressor on the *D. simulans* third chromosome. When this dominant suppressor is replaced by recessive autosomal material from *D. mauritiana*, sex chromosome meiotic drive results. TAO et al. (2001) map this autosomal suppressor to less than 80kb of DNA; they call the putative suppressor gene residing in this region *too much yin* (*tmy*). Similarly, DERMITZAKIS et al. (2000) showed that certain hybrid
introgression lines between *D. simulans* and *D. sechellia* suffer male meiotic drive, which causes sex ratio distortion among their progeny. Although several different introgression lines show such distortion, complementation tests suggested that the same autosomal region is involved in all lines (DERMITZAKIS *et al.* 2000). While the above cases involve hybrids between named “good” species, other cases involve hybrids between populations or strains within species. In one, *D. simulans* flies produced by crossing individuals from Tunisia with individuals from Seychelles or New Caledonia suffer meiotic drive, while pure-population individuals do not (MERCOT *et al.* 1995; CAZEMAJOR *et al.* 1997; MONTCHAMP-MOREAU and JOLY 1997). In another, hybrids between certain stocks of *D. subobscura* suffer meiotic drive, while pure-stock individuals do not (HAUSCHTECK-JUNGEN 1990).

Here we report the discovery of segregation distortion in hybrids between the Bogota and USA subspecies of *D. pseudoobscura*, taxa that have often been viewed as paradigmatic of the earliest stages of speciation, *e.g.*, LEWONTIN (1974). The Bogota subspecies is restricted to high elevations near Bogota, Colombia and is geographically isolated from the USA subspecies of North and Central America by more than 2000 km (PRAKASH 1972). The Bogota-USA system represents an especially young hybridization: DNA sequence analysis shows that the Bogota and USA subspecies may have separated as recently as 155,000 to 230,000 years ago (SCHAEFFER and MILLER 1991; WANG *et al.* 1997; MACHADO *et al.* 2002; MACHADO and HEY 2003). Not suprisingly, the Bogota and USA subspecies are incompletely reproductively isolated: they show little or no prezygotic isolation (PRAKASH 1972) or conspecific sperm precedence (DIXON *et al.* 2003) and produce completely fertile female hybrids. Male hybrids are also fertile in
one direction of the hybridization (USA mothers), while male hybrids from the reciprocal
direction of the hybridization (Bogota mothers) have traditionally been described as
completely sterile. This hybrid male sterility has been the subject of several genetic
studies (PRAKASH 1972; DOBZHANSKY 1974; ORR 1989ab; ORR and IRVING 2001).

Here we show that hybrid males having Bogota mothers are not, in fact,
completely sterile; instead these hybrids become weakly fertile when aged. Surprisingly,
these F\textsubscript{1} hybrid males produce almost all daughters. The results presented below suggest
that this sex ratio distortion reflects normally-cryptic segregation distortion in hybrid
males. We also present the results of a preliminary genetic analysis of this distortion.

**MATERIALS AND METHODS**

**Stocks and crosses:** Our methods generally follow those of ORR (1989ab) and
ORR and IRVING (2001). These papers also describe many of the stocks used. The *Sex
Ratio (SR)* and *Standard (ST)* arrangement stocks were kindly provided by DR. JOHN
JAENIKE, and were collected in Tucson, AZ. The wildtype isofemale lines of *D.
pseudoobscura* USA were kindly provided by DR. MOHAMED NOOR. All crosses were
performed at room temperature unless otherwise indicated. All map positions are from
ANDERSON and NORMAN (1977), except for the X chromosome, which are from ORR

**Male fertility:** Male fertility was measured by assessing sperm motility, which is
standard in studies of hybrid male sterility in Drosophila, *e.g.*, COYNE (1985),
Testes were dissected from four day old virgin males and examined under a compound microscope with dark field optics. Males were classified into three sperm motility classes: Many, wherein a male had a large number of motile sperm that filled the field of vision; Few, wherein small pockets of motile sperm were seen; and None, wherein no motile sperm were seen. While sperm motility is not equivalent to fertility, the two are strongly correlated (Orr 1987). In one large cross described below, male fertility was measured by counting number of offspring produced, as these offspring were produced for other reasons.

**Egg to adult lethality:** The frequency of lethality among offspring of hybrid males was measured by aging hybrid F₁ males for 8-9 days and then single-pair mating them to 3-4 day old virgin Bogota females. When a pair of flies began to produce first instar larvae, the pair was transferred to an egg-counting vial. This vial contained a small plastic spoon filled with standard media dyed purple to ease visualization of eggs. The adult pair were left in this vial for 24 hours and were then transferred to a new egg-counting vial for another 24 hours. Because hybrid males are almost completely sterile, almost all eggs are unfertilized. It is thus impractical to measure hatch rates among the (very rare) fertilized eggs. Instead, we simply counted the number of dead offspring. In particular, each egg-counting vial was scored for number of dead eggs and dead first instar larvae 24 and 48 hours after the parents were removed. (Dead eggs and larvae of *D. pseudoobscura* are necrotic and unmistakably brown.) The media from the egg-counting vials were transferred to fresh vials and reared at room temperature. Vials were scored for further larval lethality seven days after the parental pair was removed. Vials were later scored for number of emerging adults. After three consecutive days in which
no adults emerged, pupae were scored for lethality. (Empty pupal cases are easily
distinguished from those containing a dead individual.) Pupal cases containing dead
individuals were dissected to score sex; this is typically possible as *D. pseudoobscura*
males have bright orange testes.

**Statistics:** When comparing sex ratios produced by males of different genotypes,
we treat each father as a single data point, *i.e.*, each father produces a percentage
daughters. This is much more conservative than treating each offspring as a single data
point. The null hypothesis of no difference in sex ratio between genotypes was tested
with unpaired *t* statistics on arcsin square root transformed proportion daughters (SOKAL
and ROHLF 1981). Nonparametric tests (Mann-Whitney *U*) on untransformed proportions
almost always yielded similar results; we note the few cases in which this was not true.
In our large *X* chromosome mapping experiments, the effect of chromosome regions on
sex ratio was tested by comparing the sex ratios produced by all fathers that differ at a
marker; only fathers producing 10 or more offspring were included, to ensure some
accuracy in sex ratio measurements.

**RESULTS**

**A hybrid fertility rescue mutation:** Our study began as a search for a hybrid
fertility “rescue mutation.” It is well known that certain single mutations can rescue the
viability of normally-lethal hybrids in the *D. melanogaster* group (WATANABE 1979;
HUTTER and ASHBURNER 1987; HUTTER *et al.* 1990; SAWAMURA *et al.* 1993abc; ORR and
IRVING 2000; COYNE and ORR 2004, chapter 8). We hoped to recover a similar mutation
that would rescue the fertility of normally-sterile hybrid males produced in the cross of Bogota females to USA males. We screened 97 wild-type iso-female USA lines for fertility rescue. In particular, we mass mated wild-type Bogota-ER females to males from each of the 97 USA lines, establishing multiple vials of each cross. We scored the number of emerging hybrid F₁ females and males and transferred all F₁ hybrids to fresh vials, testing for the appearance of F₂ hybrids. As we expect F₁ males to be sterile, the appearance of F₂ hybrids shows that F₁ male fertility has been at least partially rescued.

The sex ratio among F₁ hybrids was close to even, although there was a slight excess of females (mean ± st. dev.: 55.1% ± 4.6%; Figure 1), suggesting mild hybrid male inviability in the F₁ generation. Surprisingly, however, 18 of 97 lines produced some F₂ hybrids. These cases did not reflect contamination as, in all instances, crosses were extremely difficult and very few F₂ hybrids appeared (often only a single fly). Several USA iso-female lines that produced F₂ hybrids were re-tested; most consistently produced a few F₂ offspring (results not shown). As the “Flagstaff-5” isofemale line produced the largest number of F₂ hybrids, this line was divided into sublines and each subline was tested further. We chose the subline that produced the most F₂ hybrids for further analysis. (The number of F₂ hybrids produced by this subline varied with the Bogota stock to which it was crossed. In the best case, an average of 1.7 F₂ progeny were produced per F₁ male; in the worst case, an average of 0.02 F₂ progeny were produced per F₁ male.) As the results presented below suggest that this subline carries a Mendelizing hybrid fertility rescue mutation, we refer to this stock as Hybrid male fertile (Hmf).

Hmf represents a true USA, not a Bogota, stock. This is confirmed by several lines of evidence. First, as expected, the cross of Hmf female X USA ct sd y se sp male
produces all fertile F1 males (sperm motility: 81 Many: 0 Few: 0 None). Second, also as expected, the cross of Bogota-ER female × Hmf male produces almost all sterile F1 males (4 Many: 96 Few: 101 None). Hmf thus only weakly rescues the fertility of F1 males.

We crossed hybrid F1 males carrying Hmf to many different genotypes of D. pseudoobscura females. The results are shown in Table 1. Remarkably, these normally-sterile hybrid F1 males produce almost all daughters (88%-99%). This is true regardless of the Bogota strain to which Hmf was initially crossed and regardless of whether the resulting F1 males were subsequently crossed to pure USA females, to pure Bogota females, or to hybrid F1 females (having USA or Bogota cytoplasm). The reciprocal class of Hmf F1 males — those having a USA mother — do not produce distorted sex ratios. Instead, these normally-fertile F1 males produce offspring having nearly even sex ratios (Table 2).

Because normally-sterile F1 males carrying Hmf do not receive their X chromosome or cytoplasm from USA, the gene(s) underlying fertility rescue must reside on the USA autosomes or Y. This was confirmed in a large analysis involving chromosome substitution between the (rescuing) Hmf stock and (non-rescuing) USA stocks containing balancer chromosomes on the second (Ba; 2-62.1, associated with an inversion) or third (L; 3, associated with medial Santa Cruz inversion chromosomes). Briefly, 130 single hybrid males that either did or did not carry a second or third chromosome from Hmf were crossed to Bogota-ER females. The results show that hybrid males must carry a second chromosome from Hmf to produce offspring (Table 3). The Hmf fertility rescue mutation thus appears to reside on the second chromosome.
Importantly, fertility-rescued F1 males again showed distorted sex ratios among their offspring; indeed only daughters appeared (79 females : 0 males; Table 3).

Finally, we tested whether Hmf also rescues hybrid male fertility between the more evolutionarily distant species pair, *D. pseudoobscura* and *D. persimilis*. It does not (Table 4).

**Evidence for hybrid segregation distortion:** The fact that hybrid males with Bogota mothers produce almost all daughters could be an artifact of Hmf: we know essentially nothing about this mutation except that it is zygotically-acting, dominant, and resides on the second chromosome. To our surprise, however, we discovered that “normal” hybrid males between arbitrary strains of Bogota and standard marker strains of USA become weakly fertile when aged for several weeks. Although these F1 males almost never produce offspring within the first two weeks of a cross, they often produce offspring after several weeks of repeated transfers to fresh vials (Table 5). Cytological examination of hybrid testes confirms an effect of age on hybrid sperm motility (Table 6): although “normal” hybrid F1 males sometimes produce a few motile sperm at day 2, they produce significantly more at day 14. This age-effect is also seen in hybrid males that carry the Hmf mutation (Table 6).

Remarkably, hybrid F1 males between normal stocks of Bogota and USA also produce almost all daughters. Indeed F1 males from all stock combinations showed sex ratio distortion among their offspring (Table 5). Once again, distortion occurs whether F1 males are crossed to pure Bogota females, to pure USA females, or to hybrid F1 females. The sex ratio distortion seen among the offspring of hybrid males is not, therefore, an artifact of Hmf—distortion appears to always occur among the offspring of hybrid males,
regardless of which stocks are used. (Additional examples involving other stock combinations appear below.) The key question is: Why do hybrid $F_1$ males produce almost all daughters?

There are at least three possibilities. First, sex transformation may occur among the progeny of hybrid males, with genetic males transformed into somatic females. Second, hybrid inviability may occur among the progeny of $F_1$ males, with most sons dying. Third, segregation distortion may occur in $F_1$ males, such that most zygotes derive from sperm that carry an $X$ chromosome. The sex transformation hypothesis is more plausible than it might first seem: partial or complete sex transformation has been observed in species hybrids both in Drosophila (STURTEVANT 1946) and in Caenorhabditis (BAIRD 2002). We were, however, able to rule out this possibility by using $X$-linked visible markers: we crossed $Hmf$-rescued hybrid $F_1$ males to USA females carrying the $X$-linked mutation, yellow ($I$-74.5). As expected, almost all offspring were again female (Table 1, line 5). These daughters were all phenotypically $y^+$, while the few emerging sons were phenotypically $y$. Sex transformation does not, therefore, occur among the offspring of $F_1$ males.

There are two ways to distinguish the hybrid inviability and segregation distortion hypotheses. The first is indirect: with hybrid inviability, we expect the fitness of the sons of $F_1$ males to depend on their genotype, e.g., on whether sons carry a Bogota or a USA $X$ chromosome. As emphasized, however, $F_1$ males produce almost all daughters regardless of whether $F_1$ males are crossed to pure Bogota, pure USA, or hybrid females (and so regardless of whether their sons carry a pure Bogota $X$, a pure USA $X$, or a
recombinant X chromosome). This pattern differs qualitatively from that expected with hybrid inviability.

The second approach is direct: if the absence of sons reflects hybrid inviability, massive lethality must occur among the offspring of hybrid F₁ males. To assess this, we measured egg, larval, and pupal lethality in a large cross involving hybrid F₁ males, as described in the Materials and Methods and in Table 7. Once again, we found that hybrid F₁ males produced almost all daughters (485 females : 29 males; Table 7). Not surprisingly, some lethality was seen in this cross (which does, after all, involve subspecific hybridization). This lethality was, however, far too rare to explain the near-absence of sons. Although ~456 sons are “missing” (485 – 29 = 456), we observed very few dead embryos or larvae and only 37 dead pupae (Table 7). Importantly, we were able to score the sex of 21 of these dead pupae and almost all were female (17 females : 4 males). Sex ratio distortion thus appears before the pupal stage— but there is almost no embryonic or larval lethality (Table 7).

Taking these facts together—i) hybrid males produce almost all daughters regardless of whom they are crossed to, and ii) hybrid males produce very few dead offspring— it appears that hybrid F₁ males show sex chromosome segregation distortion. We do not, however, yet know the precise functional basis of this distortion (see below).

**Genetic basis of hybrid segregation distortion: X chromosome mapping:** We would like to understand the genetic basis of hybrid segregation distortion. However, given that even “fertility-rescued” hybrid males are highly sterile, all genetic analyses proved extremely difficult. Nonetheless, we were able to establish several genetic facts, which we describe for the remainder of this paper.
Given that hybrid segregation distortion occurs in F1 males that carry a Bogota X and a USA Y and that almost all daughters result, it seems likely that the Bogota X chromosome carries gene(s) that cause segregation distortion. To confirm this and to roughly map these putative X-linked genes, we produced backcross hybrid males that carried recombinant X chromosomes, a USA Y chromosome, and mostly USA autosomes. In particular, we backcrossed F1 females from the cross of Bogota Toro-1 females X USA ct (1-22.5) sd (1-43.0) y (1-74.5) se (1-156.5) males to USA Hmf males. Recombinant backcross males of known X chromosome genotype were then singly mated to wild-type Bogota Toro-1 females and the sex ratio of the resulting progeny scored. The results are shown in Figure 2, which is arranged to match Figure 2 of ORR and IRVING (2001).

As expected, segregation distortion depends on X chromosome genotype. Males that have a USA-like X genotype (ct sd y se; genotype 1) produce nearly even sex ratios, while males that have a Bogota-like genotype (ct+ sd+ y+ se+; genotype 16) produce mostly daughters. Figure 2 also shows that gene(s) tightly linked to se are essential for segregation distortion: genotypes 1-8, which carry USA material at se, produce nearly even sex ratios, while genotypes 9-16, which carry Bogota material at se, often produce biased sex ratios (t = 8.08, P < 0.0001). Perhaps most remarkably, ct+ sd+ y+ se males (genotype 8)— which carry Bogota material at all markers except se— produce nearly even sex ratios, while ct+ sd+ y+ se+ males (genotype 16)— which also carry Bogota material at se— produce very biased sex ratios. Segregation distortion thus requires Bogota material near se. Restricting our attention to hybrids that carry se+, Figure 2 also shows that the y and sd regions have large effects on sex ratio (y: t = 5.02, P < 0.0001; sd: t = 2.65, P = 0.0099). The ct region has a lesser effect on sex ratio (t = 1.53, P = 0.13,
although this contrast is significant with a nonparametric test). Looking across Figure 2, it is clear that genes residing on both the left arm (XL: sd, y and perhaps ct) and the right arm (XR: se) of the X chromosome affect offspring sex ratio.

The X-linked genes causing segregation distortion also show strong conspecific epistasis: comparisons among genotypes 1-4 and 9 reveal that no single X chromosome region from Bogota has any effect on sex ratio by itself. Instead, sex ratio distortion appears only when several X chromosome regions from Bogota are jointly introgressed into a USA background. It also appears that at least one gene causing segregation distortion is loosely linked to our X-linked markers, since the most Bogota-like of our backcross genotypes (ct+ sd+ y+ se+; genotype 16) does not suffer full F1-male like levels of segregation distortion.

**Hybrid segregation distortion and hybrid male sterility:** Although crude, the above mapping results are similar to those from our previous work on hybrid male sterility between the Bogota and USA subspecies (ORR and IRVING 2001). Indeed the same regions of the Bogota X chromosome are involved in both hybrid male sterility and hybrid segregation distortion and these regions show a similar pattern of complex conspecific epistasis for both phenotypes. Moreover, the region near se plays a large—and necessary—role in both hybrid segregation distortion and hybrid male sterility. Our findings are, then, at least consistent with the idea that the same genes cause both phenomena. Indeed throughout many of the above crosses we noticed that hybrid males that show segregation distortion produce few progeny, while males that do not show segregation distortion produce many progeny.
To better assess this possible association between hybrid segregation distortion and hybrid male sterility, we scored the number of offspring produced by each of the 164 recombinant backcross males studied in the above X chromosome mapping experiment. The results are shown in Figure 3. There is a highly significant correlation between sex ratio among progeny and the number of offspring produced by a male ($r = -0.472, P < 0.0001$; KENDALL’S $\tau = -0.297, P < 0.0001$). This negative correlation is not an artifact of any inviability of sons, as sex ratio and number of daughters produced by a hybrid male are also strongly correlated ($r = -0.372, P < 0.0001$; KENDALL’S $\tau = -0.205, P < 0.0001$).

Although the evidence presented in this section does not prove that the same genes cause both hybrid segregation distortion and hybrid male sterility, we cannot exclude this possibility.

**Genetic basis of hybrid segregation distortion: autosomal suppressors:** The genes on the Bogota X chromosome that cause segregation distortion do so only in hybrids, not within the Bogota subspecies. The Bogota genome must therefore carry suppressors of segregation distortion. Moreover, these suppressors must be $Y$-linked and/or autosomal and must be incompletely dominant (as a single dose of Bogota autosomes does not fully suppress distortion in $F_1$ hybrid males).

The existence of Bogota suppressors of segregation distortion is confirmed in the top panel of Figure 4. Genotypes A and B both carry an unrecombined X chromosome from Bogota as well as pure Bogota cytoplasm. But genotype A carries a $Y$ chromosome from USA and only half of its autosomes from Bogota and shows strong segregation distortion; genotype B, on the other hand, carries a $Y$ chromosome from Bogota and
three-fourths of its autosomes (on average) from Bogota and shows little segregation distortion ($t = 4.58, P < 0.0001$). Replacing the $Y$ chromosome and/or autosomes from USA with those from Bogota thus suppresses distortion. The middle panel of Figure 4 confirms that at least some of the suppressors of segregation distortion reside on the Bogota autosomes. Genotypes C and D both carry an unrecombined $X$ chromosome from Bogota and a $Y$ chromosome from USA; they differ only in the fraction of the autosomes that, on average, derives from Bogota (one-half in genotype C, and three-fourths in genotype D). Genotype C shows strong segregation distortion, while genotype D shows weaker distortion (although this difference has borderline significance: $t = 1.92, P = 0.065$). While this contrast involved the $Hmf$ stock, the bottom panel in Figure 4 shows that these findings do not depend on $Hmf$. Instead, hybrid males that carry fewer autosomes from Bogota (genotype E) show significantly stronger segregation distortion than those that carry more autosomes from Bogota (genotype F), even when $Hmf$ is not used ($t = 2.63, P = 0.015$).

Further crosses involving repeated backcrosses to Bogota show that the severity of segregation distortion gradually decreases as the autosomes become more Bogota. In particular, we backcrossed hybrid males for three generations to pure Bogota females. All backcross males in each generation carried an unrecombined $X$ from Bogota, unrecombined autosomes (as backcrossing proceeded through males), a USA $Y$ chromosome, and Bogota cytoplasm. Table 8 shows that increasing the fraction of autosomes from Bogota causes the sex ratio among the progeny of backcross males to become progressively more even (eventually leveling off at ~60% females). While the
top half of Table 8 involves $Hmf$; the bottom half does not. In both cases, the autosomes affect the strength of hybrid segregation distortion.

While the results of this section show that the Bogota autosomes carry suppressors of hybrid segregation distortion, we have not yet succeeded in mapping these suppressors to particular autosomes. The necessary crosses involved passing dominantly-marked balancer chromosomes through $F_1$ males that carry a USA $Y$ chromosome and that are therefore nearly completely sterile; these crosses were extremely difficult and mostly failed.

**Genetic basis of hybrid segregation distortion: $Y$ chromosome:** Finally, it appears that segregation distortion is strongest when hybrid males carry a USA $Y$ chromosome. We performed a number of crosses which were essentially identical to many described above but in which hybrid males carried a Bogota $Y$ chromosome. In all cases, we observed little sex ratio distortion among offspring. One example is shown in Figure 5. The backcross males shown in Figure 5 are similar to those shown in Figure 2. The key difference is that the males in Figure 2 carry a USA $Y$ chromosome (and show strong sex ratio distortion), while the males in Figure 5 carry a Bogota $Y$ chromosome (and show little sex ratio distortion; indeed all sex ratios are within 7% of each other). It is especially interesting to note that males having a Bogota-like $X$ chromosome genotype ($ct^+ sd^+ y^+ se^+$) produce ~65% daughters when carrying a Bogota $Y$ chromosome (Figure 5); the same genotype produces ~85% daughters when carrying a USA $Y$ chromosome (Figure 2). Thus while some segregation distortion may occur on a Bogota $Y$ genetic background, it is weaker than on a USA $Y$ background.
**Connection to Sex Ratio rearrangement.** Finally, we tested whether the segregation distortion seen in Bogota-USA hybrids is connected to the meiotic drive caused by the *Sex Ratio* (*SR*) chromosome, an X-linked rearrangement that segregates in USA populations of *D. pseudoobscura*. (*SR* is associated with three inversions on *XR* and reaches 20% frequency in some populations in the Southwestern United States; see review in Jaenike [2001].) Dobzhansky *et al.* (1963) showed that the *SR* inversions are not present in Bogota populations of *D. pseudoobscura*; we confirmed this via salivary gland preparations of Bogota-USA F₁ hybrids (not shown). Nonetheless, hybrid segregation distortion and *SR* drive might still be connected as the segregation distortion seen in *SR* males is almost certainly due to genes within the *SR* inversions, not to the inversions per se (Wu and Beckenbach 1983; Jaenike 2001). We also know, however, that hybrid segregation distortion involves genes on both *XL* and *XR* (Figure 2), whereas *SR* drive involves gene on *XR* only. At best, then, the genetic bases of the two types of segregation distortion might be partly overlapping.

To test this, we asked whether the *SR* chromosome causes segregation distortion on a largely Bogota genetic background, *i.e.*, on a background that contains some suppressors of hybrid segregation distortion. In particular, we crossed USA *SR* females to Bogota Toro-1 males; the resulting F₁ males carry an *SR X* chromosome, but a *Y* chromosome and haploid complement of autosomes from Bogota. As a control, we crossed USA *SR* females to USA *Standard* arrangement males; the resulting F₁ males carry an *SR X* chromosome on an entirely USA genetic background. As expected, *SR* causes strong segregation distortion in control USA F₁ males: scoring the offspring of 52 F₁ fathers (singly mated to USA *Standard* females), the average sex ratio was 96.5%
daughters. The experimental hybrid F$_1$ males also showed segregation distortion, although not as strong: scoring the offspring of 75 F$_1$ fathers (singly mated to USA *Standard* females), the average sex ratio was 87.4% daughters. While highly statistically significant ($t = 7.26, P < 0.0001$), this difference is nonetheless fairly small and SR segregation distortion clearly still occurs in hybrid males. We also attempted to produce backcross hybrid males that carry an SR $X$ chromosome on a *homozygous* autosomal Bogota background. Unfortunately, these crosses proved extremely difficult and we could not recover progeny from a meaningful number of SR backcross males.

It thus appears that SR is slightly less effective on a Bogota genetic background. However, SR still causes strong segregation distortion when paired with a Bogota $Y$ chromosome, unlike the $X$-linked hybrid segregation distortion genes described above. The hybrid and SR meiotic drive systems thus appear mostly independent; at the least, we have no evidence that the two systems are closely connected.

**DISCUSSION**

We can draw three main conclusions from our study. First, male hybrids between the Bogota and USA subspecies of *Drosophila pseudoobscura* are not completely sterile. Although F$_1$ hybrid males having Bogota mothers have been described as sterile throughout several decades of study (Prakash 1972; Dobzhansky 1974; Orr 1989ab; Orr and Irving 2001), the present results reveal that this is incorrect. It is clear, however, why the fertility reported here went unnoticed by previous workers (including the present authors): hybrid fertility is very weak and hybrid males typically produce
offspring only after being aged for several weeks. Our results further show that the extent of hybrid male fertility varies somewhat with the particular parental strains used. In particular, we isolated a subline of *D. pseudoobscura* USA deriving from Flagstaff, AZ that allows the recovery of considerably more progeny from F1 males than is usually possible. Because our results suggest that the weak hybrid fertility rescue seen with this strain involves a factor(s) on the second chromosome, we refer to this strain as *Hybrid male fertile* (*Hmf*). The important point, however, is that—with enough effort—offspring can be obtained from hybrid F1 males between essentially any arbitrary stocks of the Bogota and USA subspecies.

Our second main conclusion is that Bogota-USA hybrid males show segregation distortion. More precisely, Bogota-USA hybrid males having Bogota mothers produce almost all daughters (typically > 90%). Several lines of evidence suggest that this sex ratio bias is caused not by male inviability or sex transformation, but by sex chromosome segregation distortion. This distortion occurs in crosses between all Bogota and USA strains tested. Reciprocal F1 hybrid males (those having a USA mother and that are highly fertile) do not produce distorted sex ratios. It is important to note that the present case of hybrid segregation distortion, unlike those recently described in the *D. simulans* clade, affects F1 hybrids, not only later-generation hybrids.

We do not yet know the precise functional basis of hybrid segregation distortion. The possibilities include “classic” meiotic drive in which X-bearing sperm inactivate Y-bearing sperm, which are not transferred to females (Lyttle 1991); failure of Y-bearing sperm to function properly in the female reproductive tract (e.g., failure to migrate to sperm storage organs); or failure of Y-bearing male pronuclei to fuse with X-bearing
female pronuclei following fertilization (yielding eggs that suffer no obvious necrosis). We currently know only that hybrid males produce few necrotic eggs or dead larvae; that sex ratio distortion appears before the pupal stage; and that hybrid segregation distortion is largely independent of the meiotic drive caused by the Sex Ratio (SR) X chromosomal arrangement. Because F₁ hybrid males are highly sterile — and show disrupted spermatogenesis — our preliminary work suggests that cytological approaches alone will not cleanly resolve the functional basis of segregation distortion. We are thus planning real-time PCR analyses to characterize the stage at which sex chromosome segregation distortion first appears (e.g., in hybrid males vs. in the uterus of females immediately after copulation vs. in sperm storage organs several hours after copulation).

There are at least two possible interpretations of the present— and other— cases of normally-cryptic hybrid segregation distortion. The first is that described in the Introduction: a mutation causing segregation distortion appears within one of the parental taxa, is subsequently suppressed, and becomes re-expressed upon hybridization of two taxa. The second is that segregation distortion never appeared in the evolutionary histories of either lineage leading to the present species and instead represents a hybrid pathology (Dermitzakis et al. 2000; Orr and Presgraves 2000). Under this second interpretation, hybrid segregation distortion is a consequence of the inappropriate interaction of genes from two taxa and represents a special case of Dobzhansky-Muller incompatibilities between taxa (Orr 1995b). Unfortunately, we currently know of no way to distinguish between these possibilities.

We also report the results of a preliminary genetic analysis of Bogota-USA hybrid segregation distortion. Although the near-complete sterility of
hybrid males showing segregation distortion rendered our genetic analysis extremely difficult, several facts seem clear. For one thing, segregation distortion requires genes from several regions of the Bogota X chromosome. Also, the effect of these genes is suppressed within the Bogota subspecies by (incompletely dominant) autosomal alleles (we have not yet succeeded in localizing these autosomal genes). Moreover, segregation distortion is more extreme when the Bogota X chromosome is paired with a USA Y chromosome than with a Bogota Y chromosome.

These mapping experiments lead to our third and final main conclusion: the genetic basis of segregation distortion in the Bogota-USA hybridization is similar to that of hybrid male sterility between the same taxa. To the resolution of our experiments, the genes causing both phenomena map to the same regions of the X chromosome. More remarkably, both hybrid phenotypes show the same pattern of conspecific epistasis: both hybrid male segregation distortion and hybrid male sterility appear only when hybrids carry the appropriate combination of X-linked alleles from the Bogota subspecies, and no single X-linked region can, by itself, cause any hybrid segregation distortion or hybrid sterility (ORR and IRVING 2001). Moreover, gene(s) tightly linked to sepia play a large—and necessary—role in both phenomena. Our results also show that there is a strong correlation between the fertility of individual backcross hybrid males and the sex ratio of their offspring. (The fact that this correlation is imperfect is unsurprising as individual males often produced very few offspring, causing sex ratio to be measured with considerable error.)
Although these findings are suggestive, we do not claim that hybrid segregation distortion causes Bogota-USA hybrid male sterility. Indeed there are some reasons for thinking that segregation distortion cannot be the sole cause of hybrid male sterility. For one thing, the segregation distortion discovered here— if involving classic meiotic drive— would inactivate only half of all sperm (i.e., those carrying a \( Y \) chromosome), which could not explain the near-complete sterility of \( F_1 \) males having a Bogota mother. Although additional meiotic drive systems might act within Bogota-USA hybrids— perhaps also inactivating many \( X^- \), as well as \( Y^- \), bearing sperm—we presently have no evidence for such systems.

We also do not claim, however, that segregation distortion plays no causal role in Bogota-USA hybrid sterility. Instead, while our results suggest an association between hybrid segregation distortion and hybrid male sterility, they do not currently allow us to either accept or reject the hypothesis that segregation distortion causes hybrid sterility. Indeed it is worth noting that all of our findings can be accommodated by the more moderate hypothesis that hybrid segregation distortion contributes to— but is not the sole cause of— hybrid male sterility. Interestingly, Tao et al. (2001) arrived at a similar conclusion in their analysis of \( D. \) simulans-\( D. \) mauritiana hybrids. Through an impressively fine-scale genetic analysis, Tao et al. showed that the same 80kb region that allows hybrid segregation distortion also causes partial hybrid male sterility. They further showed, however, that complete hybrid male sterility requires the action of at least one additional autosomal locus (which they mapped and named broadie). Indeed Tao et al. (2001) speculate that hybrid segregation distortion and hybrid male sterility may often involve partially (but not completely) overlapping sets of genes.
In conclusion, the most important unanswered question now confronting us is clear: Do the genes that cause hybrid segregation distortion between the Bogota and USA subspecies also contribute to hybrid male sterility? Fortunately, this question can be resolved in a straightforward way: one need only determine if, in some chromosome region of large effect on both phenotypes, the genes causing hybrid segregation distortion can be separated meiotically from those causing hybrid male sterility (e.g., Tao et al. 2001). We are now attempting to answer this question via a large introgression experiment in which the genes causing hybrid segregation distortion and hybrid male sterility in the sephia region of XR will be fine-mapped using molecular markers. This analysis will obviously be facilitated by the current sequencing of the complete D. pseudoobscura genome.
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<table>
<thead>
<tr>
<th>Hybrid father</th>
<th>Mother</th>
<th># daughters</th>
<th># sons</th>
<th>% females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bog-ER X <em>Hmf</em></td>
<td>Bog-ER</td>
<td>93</td>
<td>4</td>
<td>95.9</td>
</tr>
<tr>
<td>Bog-ER X <em>Hmf</em></td>
<td><em>Hmf</em></td>
<td>155</td>
<td>21</td>
<td>88.1</td>
</tr>
<tr>
<td>Bog-ER X <em>Hmf</em></td>
<td>Bog-ER X <em>Hmf</em></td>
<td>433</td>
<td>21</td>
<td>95.4</td>
</tr>
<tr>
<td>Bog-ER X <em>Hmf</em></td>
<td><em>Hmf</em> X Bog-ER</td>
<td>82</td>
<td>1</td>
<td>98.8</td>
</tr>
<tr>
<td>Bog-ER X <em>Hmf</em></td>
<td>USA y gl or inc</td>
<td>117</td>
<td>2</td>
<td>98.3</td>
</tr>
<tr>
<td>Bog Toro-1 X <em>Hmf</em></td>
<td>Bog Toro-1</td>
<td>464</td>
<td>26</td>
<td>94.7</td>
</tr>
<tr>
<td>Bog Toro-1 X <em>Hmf</em></td>
<td><em>Hmf</em></td>
<td>190</td>
<td>26</td>
<td>88.0</td>
</tr>
<tr>
<td>Bog Toro-1 X <em>Hmf</em></td>
<td>Bog Toro-1 X <em>Hmf</em></td>
<td>335</td>
<td>35</td>
<td>90.5</td>
</tr>
<tr>
<td>Bog Potosi-1 X <em>Hmf</em></td>
<td>Bog Potosi-1</td>
<td>26</td>
<td>2</td>
<td>92.9</td>
</tr>
<tr>
<td>Bog Potosi-3 X <em>Hmf</em></td>
<td>Bog Potosi-3</td>
<td>45</td>
<td>1</td>
<td>97.8</td>
</tr>
</tbody>
</table>
### Table 2

Reciprocal hybrid males carrying a USA X produce even sex ratios

<table>
<thead>
<tr>
<th>Hybrid father</th>
<th>Mother</th>
<th># daughters</th>
<th># sons</th>
<th>% females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hmf X Bog-ER</td>
<td>Bog-ER</td>
<td>223</td>
<td>195</td>
<td>53.3</td>
</tr>
<tr>
<td>Hmf X Bog-ER</td>
<td>Hmf</td>
<td>909</td>
<td>724</td>
<td>55.7</td>
</tr>
<tr>
<td>Hmf X Bog-ER</td>
<td>Hmf X Bog-ER</td>
<td>1685</td>
<td>1357</td>
<td>55.3</td>
</tr>
<tr>
<td>Hmf X Bog-ER</td>
<td>Bog-ER X Hmf</td>
<td>1245</td>
<td>1154</td>
<td>51.9</td>
</tr>
</tbody>
</table>
**TABLE 3**

Mapping of the autosomal hybrid fertility rescue mutation, *Hmf*

<table>
<thead>
<tr>
<th>Hybrid father</th>
<th>Vials set up</th>
<th>Vials with progeny</th>
<th>Total progeny (female : male)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$X_{Bog}/Y_{USA} \cdot 2Hmf/2Bog$</td>
<td>37</td>
<td>6</td>
<td>41 : 0</td>
</tr>
<tr>
<td>$X_{Bog}/Y_{USA} \cdot 2Ba/2Bog$</td>
<td>36</td>
<td>0</td>
<td>0 : 0</td>
</tr>
<tr>
<td>$X_{Bog}/Y_{USA} \cdot 3Hmf/3Bog$</td>
<td>27</td>
<td>4</td>
<td>28 : 0</td>
</tr>
<tr>
<td>$X_{Bog}/Y_{USA} \cdot 3L/3Bog$</td>
<td>30</td>
<td>4</td>
<td>10 : 0</td>
</tr>
</tbody>
</table>

Second chromosome experiment involved crossing *y; Ba/Delta; or* females $X Hmf$ males and crossing the resulting phenotypically *y Ba* males to Bogota-ER females, producing the above two genotypes of males (lines 1 and 2). Third chromosome experiment involved crossing *or L/or L*; *spa* females $X Hmf$ males and crossing the resulting phenotypically *L* males to Bogota-ER females, producing the above two genotypes of males (lines 3 and 4).
### Table 4

Test of whether *Hmf* rescues the fertility of *D. pseudoobscura-D. persimilis* hybrid males

<table>
<thead>
<tr>
<th>Female</th>
<th>Male</th>
<th>F1 male fertility</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Many</td>
</tr>
<tr>
<td><em>D. pseudo y; gl; or; inc</em></td>
<td><em>D. persimilis or</em></td>
<td>0</td>
</tr>
<tr>
<td><em>D. persimilis or</em></td>
<td><em>D. pseudo y; gl; or; inc</em></td>
<td>0</td>
</tr>
<tr>
<td><em>D. pseudo Hmf</em></td>
<td><em>D. persimilis or</em></td>
<td>0</td>
</tr>
<tr>
<td><em>D. persimilis or</em></td>
<td><em>D. pseudo Hmf</em></td>
<td>0</td>
</tr>
</tbody>
</table>

Top two lines confirm that “normal” F1 hybrid males between *D. pseudoobscura* and *D. persimilis* are sterile in both directions of the hybridization. The bottom two lines show that the *D. pseudoobscura Hmf* stock does not rescue this sterility. Hybrid male fertility was assessed cytologically in testis squash preparations.
### Table 5

“Normal” hybrid males also produce almost all daughters

<table>
<thead>
<tr>
<th>Hybrid father</th>
<th>Mother</th>
<th># daughters</th>
<th># sons</th>
<th>% females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bog Poto-1 X y gl or inc</td>
<td>Bog Potosi-1</td>
<td>32</td>
<td>0</td>
<td>100.0</td>
</tr>
<tr>
<td>Bog Poto-3 X y gl or inc</td>
<td>Bog Potosi-3</td>
<td>12</td>
<td>1</td>
<td>92.3</td>
</tr>
<tr>
<td>Bog Toro-1 X y gl or inc</td>
<td>Bog Toro-1</td>
<td>69</td>
<td>2</td>
<td>97.2</td>
</tr>
<tr>
<td>Bog Toro-1 X y gl or inc</td>
<td>y gl or inc</td>
<td>71</td>
<td>26</td>
<td>73.2</td>
</tr>
<tr>
<td>Bog Toro-1 X y gl or inc</td>
<td>Bog Toro-1X y gl or inc</td>
<td>27</td>
<td>6</td>
<td>81.8</td>
</tr>
<tr>
<td>Bog Toro-1 X USA Tempe-5 Bog Toro-1</td>
<td></td>
<td>348</td>
<td>34</td>
<td>91.1</td>
</tr>
<tr>
<td>Bog w X y gl or inc</td>
<td>Bog w X y gl or inc</td>
<td>83</td>
<td>9</td>
<td>90.2</td>
</tr>
</tbody>
</table>
TABLE 6

Effect of age on hybrid male fertility

<table>
<thead>
<tr>
<th>Mother</th>
<th>Father</th>
<th>Day</th>
<th>F1 male fertility</th>
<th>$\chi^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Many</td>
<td>Few</td>
</tr>
<tr>
<td>Bogota-ER</td>
<td>USA $y \ gl \ or \ inc$</td>
<td>2</td>
<td>0</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14</td>
<td>0</td>
<td>77</td>
</tr>
<tr>
<td>Bogota $w$</td>
<td>USA $y \ gl \ or \ inc$</td>
<td>2</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14</td>
<td>4</td>
<td>80</td>
</tr>
<tr>
<td>Bogota-ER</td>
<td>USA $Hmf$</td>
<td>2</td>
<td>4</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14</td>
<td>22</td>
<td>158</td>
</tr>
<tr>
<td>Bogota $w$</td>
<td>USA $Hmf$</td>
<td>2</td>
<td>7</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14</td>
<td>20</td>
<td>136</td>
</tr>
</tbody>
</table>

The $\chi^2$ values reflect comparing sperm motility within a genotype after males were aged 2 vs. 14 days. *** = $P < 0.0001$. In cases in which a cell value equaled 0, it was set to 0.1 to allow calculation of a $\chi^2$ statistic; other small values do not substantially change the above probabilities.
### Table 7

**Lethality among offspring of hybrid F₁ males**

<table>
<thead>
<tr>
<th>Stage</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dead eggs (24 hours)</td>
<td>15</td>
</tr>
<tr>
<td>Dead eggs (48 hours)</td>
<td>19</td>
</tr>
<tr>
<td>Dead larvae</td>
<td>6</td>
</tr>
<tr>
<td>Dead pupae (unemerged female)</td>
<td>17</td>
</tr>
<tr>
<td>Dead pupae (unemerged male)</td>
<td>4</td>
</tr>
<tr>
<td>Dead pupae (sex undetermined)</td>
<td>16</td>
</tr>
<tr>
<td>Total dead offspring</td>
<td>77</td>
</tr>
<tr>
<td>Emerged adult females</td>
<td>485</td>
</tr>
<tr>
<td>Emerged adult males</td>
<td>29</td>
</tr>
</tbody>
</table>

Offspring resulted from the cross of F₁ males (Bogota Toro-1 females X USA *Hmf* males) X Bogota Toro-1 females. A total of 50 F₁ males were single-pair mated to Bogota females. See Materials and Methods for detailed protocol.
**TABLE 8**

Sex ratio among the offspring of F₁ and repeated backcross males

<table>
<thead>
<tr>
<th>Cross</th>
<th>% daughters</th>
<th>N</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bogota Toro-1 X USA Hmf</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F₁</td>
<td>89.2</td>
<td>18</td>
<td>238</td>
</tr>
<tr>
<td>BC₁</td>
<td>78.4</td>
<td>14</td>
<td>5724</td>
</tr>
<tr>
<td>BC₂</td>
<td>65.0</td>
<td>22</td>
<td>3782</td>
</tr>
<tr>
<td>BC₃</td>
<td>62.3</td>
<td>15</td>
<td>2874</td>
</tr>
<tr>
<td>Bogota Toro-1 X USA Tempe-5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F₁</td>
<td>90.3</td>
<td>13</td>
<td>110</td>
</tr>
<tr>
<td>BC₁</td>
<td>71.3</td>
<td>13</td>
<td>5301</td>
</tr>
<tr>
<td>BC₂</td>
<td>60.4</td>
<td>23</td>
<td>4314</td>
</tr>
<tr>
<td>BC₃</td>
<td>60.1</td>
<td>17</td>
<td>1954</td>
</tr>
</tbody>
</table>

Hybrid males were backcrossed to Bogota Toro-1 females each generation. The sex ratios shown were calculated by averaging the percentage daughters produced by individual hybrid fathers. The number of fathers (N) producing offspring, as well as the total number of offspring scored each generation (n), are shown. Due to hybrid sterility, many more fathers were typically set up than produced offspring, especially among F₁ males. “BC₁” represents first-generation backcross hybrids, and so on.
FIGURE LEGENDS

FIGURE 1.— Percentage females among F₁ offspring from the cross Bogota-ER females X USA males. Each cross involved a different USA isofemale line; 97 lines were tested.

FIGURE 2.— Mapping of X chromosome segregation distortion genes. The X axis shows the average percentage daughters produced by hybrid backcross males of a given genotype; the Y axis shows the X chromosome genotypes that were produced. Backcross males resulted from the cross of F₁ females (Bogota Toro-1 females X USA ct sd y se males) X USA Hmf males. Data derive from 164 backcross males of known genotype that successfully produced offspring when singly mated to Bogota Toro-1 females (backcross males were nearly evenly distributed over the genotypes shown); a total of 59,632 offspring were scored for sex. All backcross hybrid males carry a recombinant X chromosome, a USA Y chromosome, and mostly USA autosomes. White chromosome regions represent USA material, while black chromosome regions represent Bogota material.

FIGURE 3.— Scatterplot of percentage daughters vs. number of offspring produced by the hybrid backcross males studied in Figure 2. Because these backcross males were produced through F₁ females (that undergo recombination), they are genetically heterogeneous. 164 backcross males were studied in these single-male crosses.
FIGURE 4.— Evidence for autosomal suppressors of segregation distortion in Bogota. Plot shows percentage females produced by hybrid males of various genotypes. The short chromosomes at the left represent the sex chromosomes (X on top, Y on bottom; Y shown with hook). The long chromosomes at the right represent haploid sets of autosomes. Black chromosomes derive from Bogota and white from USA. Genotype A resulted from the cross of Bogota Toro-1 females X USA Hmf males; genotype B resulted from Bogota Toro-1 female X F₁ male (Hmf female X Toro-1 male). Genotype C again resulted from Bogota Toro-1 females X USA Hmf males; genotype D resulted from Bogota Toro-1 female X F₁ male (Toro-1 female X Hmf male). Genotype E resulted from Bogota Toro-1 females X USA Tempe-5 males; genotype F resulted from Bogota Toro-1 female X F₁ male (Toro-1 female X Tempe-5 male). N is the number of fathers of a particular genotype that produced progeny; n is the total number of progeny produced.

FIGURE 5.— Segregation distortion is weaker on a Bogota Y genetic background. The backcross males shown are analogous to those in Figure 2 except that the present males carry a Bogota Y chromosome (genotypes are numbered to match those in Figure 2). Backcross males resulted from the cross of F₁ females (USA ct sd y se female X Bogota Toro-1 male) X Bogota Toro-1 males. Data derive from 60 backcross males of known genotype that successfully produced offspring when singly mated to Bogota Toro-1 females (backcross males were nearly evenly distributed over genotypes); a total of 16,512 offspring were scored. All other details as in Figure 2.
A 93.1% \( (N = 19, n = 101) \)

B 60.8% \( (N = 10, n = 4065) \)

C 89.2% \( (N = 18, n = 238) \)

D 78.4% \( (N = 14, n = 5724) \)

E 90.3% \( (N = 13, n = 110) \)

F 71.3% \( (N = 13, n = 5301) \)