Non-Mendelian Segregation of Sex Chromosomes in Heterospecific Drosophila Males

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ABSTRACT

Interspecific hybrids and backcrossed organisms generally suffer from reduced viability and/or fertility. To identify and genetically map these defects, we introgressed regions of the *Drosophila sechellia* genome into the *D. simulans* genome. A female-biased sex ratio was observed in 24 of the 221 recombinant inbred lines, and subsequent tests attributed the skew to failure of Y-bearing sperm to fertilize the eggs. Apparently these introgressed lines fail to suppress a normally silent meiotic drive system. Using molecular markers we mapped two regions of the Drosophila genome that appear to exhibit differences between *D. simulans* and *D. sechellia* in their regulation of sex chromosome segregation distortion. The data indicate that the sex ratio phenotype results from an epistatic interaction between at least two factors. We discuss whether this observation is relevant to the meiotic drive theory of hybrid male sterility.

UNDER Fisher's principle, the sex ratio of a population is in evolutionary equilibrium when the parental expenditure is equalized for producing sons and daughters (Fisher 1958; Uyenoyama and Bengtsson 1982). With few exceptions (Hamilton 1967; Humphrey-Smith and Chastel 1990; Jaenike 1996), most populations maintain a 1:1 sex ratio (but see Carvalho and Vaz 1999), and large deviations may result in a population bottleneck and risk of extinction. The most common mechanism by which natural populations attain a skewed sex ratio is meiotic drive of the sex chromosomes. Many cases have been described in which males produce X- and Y-chromosome-bearing gametes far from the expected Mendelian proportions (Sturtevant and Dobzhansky 1936; Carvalho *et al.* 1989; Cazemajor *et al.* 1997; Lyttle 1991 for review). Autosomal meiotic drive systems have also been described, such as the *Segregation distorter* (SD) in *D. melanogaster* and the *t*-haplotype in mice (review in Lyttle 1991). According to some theoretical models, such meiotically driven factors are more likely to evolve and be maintained in a population when they are on the sex chromosomes rather than autosomes (Wu and Hammer 1990; Hurst and Pomiankowski 1991). On the other hand, Charlesworth *et al.* (1993) argue that a system of drivers and their target sequences are less likely to be maintained on the sex chromosomes due to lack of recombination between them. Deleterious fitness consequences of drive systems appear to be common and are the likely cause for the maintenance of polymorphism that is necessary to identify the phenomenon in the first place (Wu 1983a; Carvalho *et al.* 1997; Wilkinson *et al.* 1998; Carvalho and Vaz 1999).

Meiotic drive mechanisms have been implicated in the phenomena of unisexual inviability and sterility in the offspring of interspecific crosses known as Haldane's rule (Haldane 1922). Hurst and Pomiankowski (1991) and Frank (1991) have proposed that many of these cases can be explained by the loss of balance between drive factors and their modifiers. However, others have argued that experimental evidence does not support the role of meiotic drive in species isolation (Johnson and Wu 1992; Charlesworth *et al.* 1993; Coyne and Orr 1993).

Many systems of sex chromosome meiotic drive have been previously described in the Drosophila genus including the three most studied cases of *Drosophila pseudoobscura* (Sturtevant and Dobzhansky 1936; Policansky and Ellison 1970), *D. simulans* (Merçot *et al.* 1995; Atlan *et al.* 1997; Cazemajor *et al.* 1997), and *D. mediopunctata* (Carvalho *et al.* 1989). These systems involve a trait mapped on the X chromosome that drives the overrepresentation of itself in the sperm at the expense of the Y chromosome. For the cases of *D. simulans* and *D. mediopunctata*, autosomal or Y-linked suppressor loci have been identified that rescue the skewed sex ratio phenotype (Merçot *et al.* 1995; Atlan *et al.* 1997; Carvalho *et al.* 1997). Theory predicts that suppressors of such driving mechanisms would rapidly invade the population and restore the deficit of males (Hamilton 1967; Carvalho *et al.* 1997).

In the process of studying the effects of interspecific introgressions from *D. sechellia* to *D. simulans*, we identified lines that produced female-biased progeny. In this article we characterize the formal genetics of this sex ratio system, we map regions of the genome that seem

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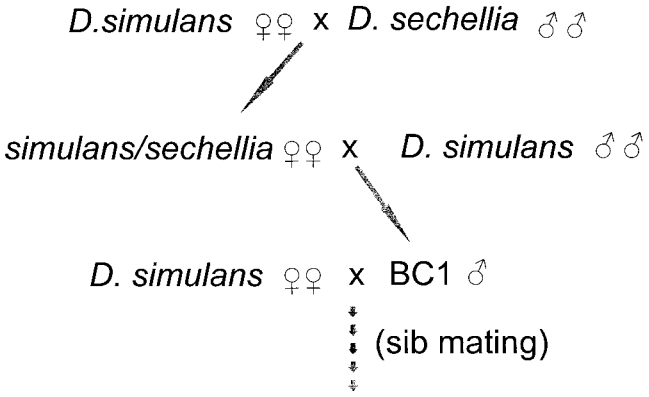

Figure 1.—Design of crosses performed to generate the introgression lines. *D. simulans* is isofemale line 2 (sim2) from Davis, CA, and *D. sechellia* is line 3588 from Drosophila Species Stock Center. At least 34 generations of inbreeding were done, including 14 generations of sib mating after the last cross of *D. simulans* × BC1. Female genotype is always given on the left.

to be important for the expression of this phenotype, and we discuss the possible models that explain our results.

MATERIALS AND METHODS

Construction of lines: Virgin females from the wild-caught "sim2" line of *D. simulans* (Winters, CA) were crossed to *D. sechellia* line 3588 (Drosophila Species Stock Center, 14021-0248.4), and F_1 females were backcrossed to *D. simulans* males. Progeny from 17.6% of these backcross male progeny were obtained in backcrosses with *D. simulans* females (Figure 1). Male and female backcross progeny were intercrossed, and 221 lines were made homozygous by single-pair matings for 14 generations. If the genes are strictly neutral in effects on viability and fertility, the expected proportion of the autosomal genome that should be *D. sechellia* in these lines is 0.125, and the expected proportion of the X chromosome that should be *D. sechellia* is 0.167. The Y chromosomes are entirely from *D. simulans.*

Sex ratio test crosses: The inbred introgression lines were tested for the transmission of sex ratio distortion by placing 10–15 virgin females with the same number of males in vials, allowing mating and oviposition for 7 days, removing adults and scoring progeny 10–15 days after egg laying began. For the crosses of F_2 males with sim2 females, single pair vials were set up, and after 4–5 days, parents were removed. Progeny were scored between days 10 and 15. For all the crosses described in this study only vials that produced >20 individuals were included in the analysis.

Viability test: Egg hatch was scored by allowing 6- to 10-day-old females to oviposit on standard cornmeal medium dyed with food coloring. Eggs were counted at 10–20 hr after introduction of the females into the vial. Females were removed from vials, and egg hatch was scored 10–30 hr later. Adult progeny were counted on day 15 after egg laying.

Markers: Oligonucleotide primers for PCR were designed to flank insertion/deletion or restriction fragment length polymorphism (RFLP) differences seen in alignments of genes whose sequences for both *D. simulans* and *D. sechellia* had been reported in GenBank. The markers are *white* (1-1.5, microsatellite repeat difference), *dec-1* (1-26, insertion/deletion difference), *Acp26* (2-20, restriction site difference), *Amy* (2-77, insertion/deletion difference), *ple* (3-22, microsatellite repeat difference), *cathpo* (map position 3-46, insertion/deletion difference), and *cpo17* (3-62, insertion/deletion difference). The oligonucleotides for each gene are as follows: *white*, Forward 5′-CAC ATA CAC AGA TTT ATT GAG CCC-3′, Reverse 5′-ACA CAC ACT TTT ATA CTC TCT CCG C-3′; *Amy*, Forward 5′-TAC GTG GAT GTG ATC TTC AA-3′, Reverse 5′-GAT GAC CTC CTG GAC GAT-3′; *cathpo*, Forward 5′-TTC GAC GGA TCA GAC TTG GTT TTT GGC-3′, Reverse 5′-GCG TTC GCC TTT CTT AGT CAA TTT CGG-3′; *cpo17*, Forward 5′-TCG CAC GAG TCC AAC TCC-3′, Reverse 5′-ACG GAG TCC ATG CTC TGC-3′; *dec-1*, Forward 5′-ATC CAA TGA TGA TGC AGC-3′, Reverse 5′-CAA AGG CAA TGG ACA GAG-3′; *Acp26*, Forward 5′-ATG AAC TAC TTC GCG GTG-3′, Reverse 5′-ATA GGG TTC TCA ACA TGC C-3′; *ple*, Forward 5′-TTC TGA GAG GGG CTT TTA-3′, Reverse 5′-GAG GCT AAC AAA TGA AG-3′. All amplifications were performed in an annealing temperature of 53° with 1.25 mm of $MgCl_2$ concentration.

Random amplification of polymorphic DNA (RAPD) markers were amplified by three oligonucleotides, g2 (5′-GGCACT GAGG-3′), g4 (5′-AGCGTGTCTG-3′), and g17 (5′-ACGACC GACA-3′), obtained from Operon Technologies (Alameda, CA). RAPD PCR amplifications were performed with annealing temperature of 36° and size of the products was resolved in 1.4% agarose gels. Mendelian segregation of RAPD bands was verified in crosses, and map locations were estimated relative to the other markers with MAPMAKER software (Lander *et al.* 1987).

RESULTS

Sex ratios differ among introgression lines: Most of the *simulans-sechellia* introgression lines appeared to exhibit a 1:1 sex ratio, but a subset of the lines exhibited a significant deficit of males (Figure 2), at about the levels of other previously described sex ratio phenotypes (0.01–0.30 male proportion). Counts of 130,721 offspring in 3374 vials (an average of 15.3 replicate vials per line) produced highly significant departures from the null hypothesis of equal sex ratio ($F_{220,3373} = 16.03$, $P < 0.0001$). In addition, the sex ratios were heterogeneous among those lines that significantly departed from 1:1 ($F_{23,325} = 3.98$, $P < 0.0001$), suggesting genetic

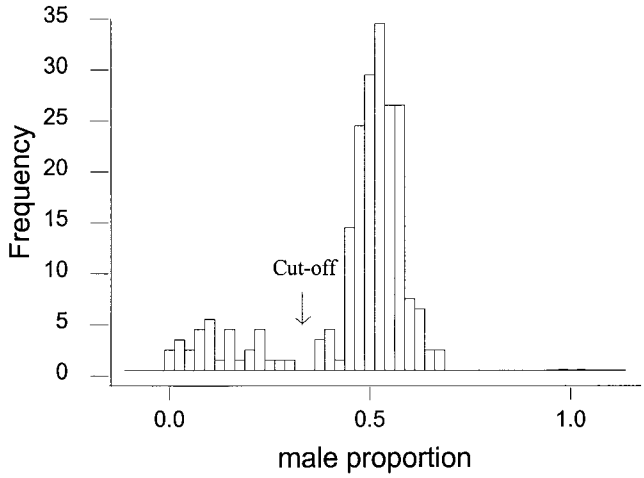

Figure 2.—Distribution of the mean male proportion for the 221 introgression lines. Note the two distinct clusters (SSR and NSR) and the cutoff at 0.30.

TABLE 1

SSR male proportion

Line	No. of vials scored	Male proportion[a]
IG12	23	0.220 ± 0.035
IG16	17	0.150 ± 0.021
IG19	4	0.070 ± 0.056
IG28	8	0.091 ± 0.033
IG33	10	0.213 ± 0.048
IG49	19	0.012 ± 0.007
IG54	21	0.190 ± 0.023
IG59	21	0.061 ± 0.012
IG62	20	0.154 ± 0.048
IG73	8	0.205 ± 0.045
IG112	28	0.102 ± 0.008
IG114	23	0.156 ± 0.033
IG125	21	0.172 ± 0.028
IG151	7	0.019 ± 0.013
IG160	9	0.020 ± 0.010
IG200	4	0.042 ± 0.011
Q3.1	10	0.091 ± 0.037
Q15.2	14	0.095 ± 0.036
Q15.3	6	0.268 ± 0.076
Q27.1	14	0.146 ± 0.021
Q28.3	6	0.022 ± 0.012
Q31.2	11	0.074 ± 0.023
Q40.2	8	0.107 ± 0.075
Q46	14	0.087 ± 0.027

[a] Mean male proportion ± 1 SE.

heterogeneity contributing to this variation (Table 1). For subsequent analysis we focus on 14 of the 24 lines that exhibited a significantly skewed sex ratio, and we refer to them as SSR (*s*kewed *s*ex *r*atio) lines, while we refer to normal sex ratio introgressed lines as NSR (*n*ormal *s*ex *r*atio). It is important to note here that both *D. simulans* and *D. sechellia* parental lines exhibit normal sex ratio.

Viability tests: To evaluate whether the deficit of males in these lines is due to male inviability, absolute egg-to-adult viability was scored. In a replicated design, egg hatch from each line was counted and the numbers of subsequently emerging adults were also scored. Egg-to-adult viability was found to be homogeneous across SSR and NSR lines (Table 2). The skewed sex ratio is evidently not caused by male inviability and must be caused by a deficit of eggs fertilized by Y-bearing sperm.

Formal genetics of SSR: Meiotic drive was suggested in crosses between males and females of the SSR lines with females and males of the original *D. simulans* line. Figure 3 and data in Table 3 show that only males from SSR lines can produce female-biased offspring sex ratio (the same results were observed when SSR males were crossed to females of other *D. simulans* or NSR lines). The reciprocal cross (SSR ♀ ♀ × *D. simulans* ♂ ♂) produced a normal offspring sex ratio. The sex ratios within the *D. simulans* and *D. sechellia* lines were normal. F_1 ♀♀ × F_1 ♂♂ crosses in both directions produce normal progeny sex ratios, suggesting that the critical introgression(s) is not on the X chromosome. When F_2 males from either direction were backcrossed to *D. simulans* females in single pair matings, ~25% of the vials produced biased sex ratio (Figure 3), while the rest produced normal sex ratio. The fact that only SSR males produced biased sex ratio and the $F_1 \times F_1$ crosses produced normal sex ratio suggests that there is non-Mendelian segregation of sex chromosomes in the gametes of SSR males and that the introgressions causing it have recessive effect. A cytoplasmic effect was rejected, because all lines derive from a *D. simulans* female and hence have the same cytoplasm.

Complementation tests: Complementation tests were performed to determine whether all 14 SSR lines had the same autosomal properties. Due to the large number of lines, not all pairwise tests were done, but rather each SSR line was crossed with at least two other SSR lines (Table 4). The F_1 males from each direction were backcrossed to sim2 females, and the sex ratio of their offspring was scored in an average of 13 vials per cross. In all cases the sex ratio was significantly female biased at about the levels of one or the other parental SSR line.

Test cross with *D. simulans* ST line and other *simulans* lines: The presence of an X-linked drive factor was tested

TABLE 2

Viability test

Line	No. of eggs scored	Hatching rate[a]	Emergence rate[a]
sim/sec SSR			
IG54	325	0.784 ± 0.0330	0.892 ± 0.0405
IG59	479	0.820 ± 0.0341	0.949 ± 0.0403
IG73	356	0.637 ± 0.0598	0.915 ± 0.0300
IG151	626	0.754 ± 0.0333	0.856 ± 0.0530
sim/sec non-SSR			
IG132	150	0.846 ± 0.0490	0.926 ± 0.0358
IG135	454	0.843 ± 0.0329	0.983 ± 0.0106
IG143	168	0.822 ± 0.0550	0.947 ± 0.0258

[a] Mean ± 1 SE. Emergence rate one-way ANOVA: $F_{6,55} = 1.43$, $P = 0.222$. Hatching rate one-way ANOVA including IG73: $F_{6,55} = 2.9$, $P = 0.017$. Hatching rate one-way ANOVA excluding IG73: $F_{5,47} = 0.83$, $P = 0.536$.

TABLE 3

Male proportion data for the *sim/sec* SSR lines test crosses

Line	SSR × SSR	SSR × sim2 (1)	sim2 × SSR (2)	(1) $F_1 \times F_1$	(2) $F_1 \times F_1$
IG12	0.220	0.499 ± 0.0166	0.258 ± 0.0194	0.495 ± 0.0114	0.475 ± 0.0479
IG49	0.012	0.509 ± 0.0186	0.063 ± 0.0240	0.496 ± 0.0189	0.469 ± 0.0502
IG54	0.190	0.510 ± 0.0214	0.183 ± 0.0162	0.474 ± 0.00272	0.454 ± 0.0970
IG59	0.061	0.531 ± 0.0276	0.296 ± 0.0401	0.390 ± 0.0383	0.314 ± 0.0729
IG62	0.154	0.527 ± 0.0198	0.189 ± 0.0212	0.461 ± 0.0288	0.432 ± 0.0473
IG73	0.205	0.493 ± 0.0247	0.269 ± 0.225	0.454 ± 0.0290	0.406 ± 0.0294
IG112	0.102	0.575 ± 0.0375	NA	0.492 ± 0.00262	0.457 ± 0.0556
IG114	0.156	0.492 ± 0.0179	0.195 ± 0.0296	0.503 ± 0.0123	0.465 ± 0.0406
IG125	0.172	0.498 ± 0.0374	0.263 ± 0.0235	0.492 ± 0.0180	NA
IG151	0.019	0.467 ± 0.0235	NA	0.503 ± 0.0363	0.421 ± 0.0323
Q15.2	0.095	0.439 ± 0.0185	NA	0.463 ± 0.0324	0.368 ± 0.0750
Q27.1	0.146	0.492 ± 0.0251	0.248 ± 0.0318	0.502 ± 0.0323	NA
Q31.2	0.074	0.503 ± 0.0165	0.153 ± 0.0297	0.376 ± 0.0656	NA
Q46	0.087	0.520 ± 0.0182	0.283 ± 0.0358	0.512 ± 0.0262	0.511 ± 0.0174

Each mean with one standard error represents data from a set of 8–15 independent replicate vials and 400–1000 flies per test cross. NA, data not available.

by crossing females from two of the introgression lines (IG49 and IG62) with the *D. simulans* ST strain described in Cazemajor *et al.* (1997) and Atlan *et al.* (1997).

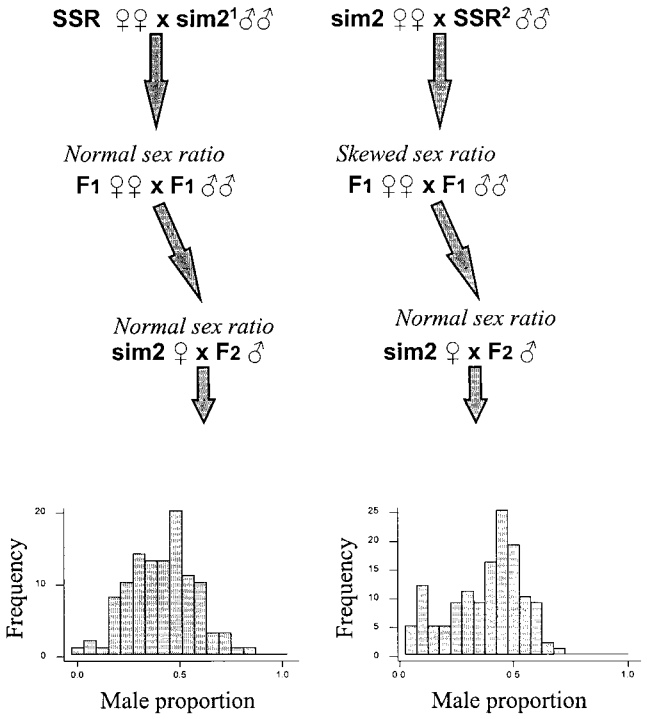

Figure 3.—Design of the test crosses performed to determine the characteristics of the SR phenotype. Both reciprocal crosses (1, 2) were performed with the sim2 line. We also did the $F_1 \times F_1$ and sim2 ♀ × F_2 ♂ (single pair matings) crosses for both directions. The distribution of male proportion of the vials from some lines is shown. The Kruskal-Wallis test indicated no significant differences in these distributions among lines ($P > 0.1$). The characteristics of the sex ratio (normal or skewed) are denoted at each generation. Female genotype is always given on the left.

This line is known to lack suppressors or drivers for the X-linked drive system described by Cazemajor *et al.* (1997). The sex ratio of the progeny from F_1 males from both reciprocal crosses with SSR lines IG49 and IG62 was normal (0.577 ± 0.0345 and 0.440 ± 0.0260). This result implies either that there is no X-linked driver or that the X-linked driver and autosomal suppressors are different from those of the SR strain described by Cazemajor *et al.* (1997).

To test the same effect with other wild-caught isofemale *D. simulans* lines, females from one of the sex ratio lines, IG49, were crossed to males from three different Davis *D. simulans* lines (sim1, sim3, sim4), and resulting F_1 males were then crossed to virgin sim2 females. In single pair matings replicated 11, 25, and 25 times, respectively, there was no significant deviation of the sex ratio from 50%. The mean and standard deviation of male proportion among offspring of sim2 ♀♀ × F_1 ♂♂ for sim1, sim3, and sim4 are (mean ± 1 SE) 0.449 ± 0.0290, 0.469 ± 0.0222, and 0.439 ± 0.0516, respectively.

QTL mapping of genes involved in the sex ratio phenotype: To map regions of the genome that are responsible for the skewed sex ratio, we scored all 221 lines for 13 molecular markers, including 7 whose map locations are known (*white*, *dec-1*, *Amy*, *Acp26*, *ple*, *cathpo*, and *cpo17*; see Figure 4). For the remaining 6 markers, RAPD was performed to generate random genetic markers for mapping (see Favia *et al.* 1994; Dimopoulos *et al.* 1996). Six of the polymorphic fragments were reliably mapped to one of the three chromosomes based on the known molecular markers described above and using the software MAPMAKER/Exp 3.0b (Lander *et al.* 1987).

The factors that affect sex chromosome segregation were mapped by treating the presence or absence of skewed sex ratio as a dichotomous trait and performing interval mapping (Lander and Botstein 1989) using

TABLE 4

Complementation test results

Line	Male IG12	IG49	IG54	IG59	IG62	IG73	IG112	IG114	IG125	IG151	Q15.2	Q27.1	Q31.2	Q46
Female														
IG12									0.17 ± 0.34			0.14 ± 0.019		
IG49			0.12 ± 0.021							0.22 ± 0.029				
IG54		0.14 ± 0.024												0.17 ± 0.039
IG59												0.04 ± 0.016	0.11 ± 0.042	
IG62			0.27 ± 0.019								0.19 ± 0.031			
IG73		0.07 ± 0.018							0.18 ± 0.024	0.29 ± 0.018				
IG112											0.14 ± 0.028			
IG114							0.33 ± 0.033							
IG125							0.19 ± 0.022							
IG151						0.24 ± 0.026		0.12 ± 0.027						
Q15.2					0.09 ± 0.021									
Q27.1					0.18 ± 0.034									0.17 ± 0.024
Q31.2				0.08 ± 0.018									0.27 ± 0.037	
Q46														

Entries of the mean male proportion ± 1 SE in offspring of sim2 females crossed to the indicated F_1 male genotype.

the QTL Cartographer software (Basten *et al.* 1999). Two quantitative trait loci (QTL) were located at map position 1-0.1 (near *white*) and at 3-0.1 (near g17.2). Likelihood ratios and permutation test significance lev-

Figure 4.—Likelihood ratio statistics along the three chromosomes of the introgressions lines for association with the SSR phenotype. The map positions are in centimorgans (cM). (**) Highly significant association ($P = 0.012$) and (*) marginally significant association ($P = 0.062$) as suggested by the permutation tests. The markers (inferred map position in cM) are as follows: for the X chromosome *white* (0), *dec-1* (24.9), g4.5 (70.6); for the second chromosome g2.1 (0), g4.3 (18.4), *Acp26* (24.6), *Amy* (67.7), g2.6 (81); and for the third chromosome g17.2 (0), *ple* (26.5), *cathpo* (50.1), *cpo17* (57.8), g17.3 (74.6).

els (Churchill and Doerge 1994) for the two QTL were $LR_w = 3.336$, $P_w = 0.062$ and $LR_{g17.2} = 6.62$, $P_{g17.2} = 0.013$ for the X and third chromosome QTL, respectively (Figure 4). The second chromosome appeared to have no significant QTL in any analysis. When Fisher's exact test was applied to test for association of individual markers with the sex ratio phenotype, only *white* and g17.2 showed marginally significant and significant results, respectively ($P_w = 0.063$ and $P_{g17.2} = 0.016$). Applying the same QTL analysis to the mean male proportion (instead of dichotomizing the sex ratio phenotype), the same intervals were identified, but the permutation tests indicated a lack of significance ($P_w = 0.086$ and $P_{g172} = 0.071$). The lack of significance for male proportions may be caused by segregating factors that affect relative viability of males *vs.* females, which would inflate the variation within the two clusters (SSR and NSR), essentially adding noise to the skewed sex ratio trait. Intriguingly, the two regions on the X and third chromosomes tend to have *D. simulans* alleles overrepresented in the SSR lines relative to the NSR lines. This is in contrast to the expectation that a QTL would flag the presence of an introgressed segment of the *D. sechellia* genome. The inability to detect introgressed segments of the *D. sechellia* genome that have an effect on the sex ratio phenotype was probably due to the low marker density, but it may also be due to complex interactions among introgressed segments. Additionally, some deleterious effects of the sex ratio phenotype could have contributed to the early loss of lines with strong sex ratio bias and decreased the ability to detect all the loci with significant effect on the phenotype.

DISCUSSION

In this article we describe the formal genetics and mapping of a sex ratio system that was unmasked by interspecific introgression. The main observations are as follows: (1) The sex ratio phenotype appears to be recessive and more than one factor is involved, (2) the sex ratio phenotype is distinct from the well-described *D. simulans* X-linked meiotic drive system (Merçot *et al.* 1995), (3) complementation test results are consistent with allelism in all the 24 SSR lines, (4) the sex ratio phenotype appears to be positively associated with the *D. simulans* alleles on the two identified QTL, rather than the *D. sechellia* alleles. The above observations suggest three possible models that could explain the data (Figure 5).

Model 1: A fixed, suppressed meiotic drive system may be present in *D. simulans*, and the introgressions replaced the suppressors with nonsuppressing alleles. The X and third chromosome QTL may be required for the meiotic drive system to be expressed under these nonsuppressed conditions. A prerequisite for this model is that the suppression system is dominant. This explains why F_1 males described in Figure 3 did not produce biased sex ratio. The simplest case of this model is an X-linked driver and a single fixed dominant suppressor.

Model 2: A polygenic *D. sechellia* driving system is transferred into the *D. simulans* genome. Two regions (or three), on the X and the third chromosome (and possibly the Y chromosome, which is entirely from *D. simulans*), may be mediators (and targets) of this complex system of drivers from *D. sechellia*. The relatively low marker density of our map would make it fairly likely that QTL corresponding to some components of this system would be missed.

Model 3: A system of genes interacts in a heterospecific background and produces a female biased sex ratio. Under this model, neither species had a segregating sex ratio phenotype before the introgression. Such a model has no experimental support from previous studies, but it cannot be completely disregarded.

For the last two models there is no indication of the nature of the hypothetical genetic interactions, so we

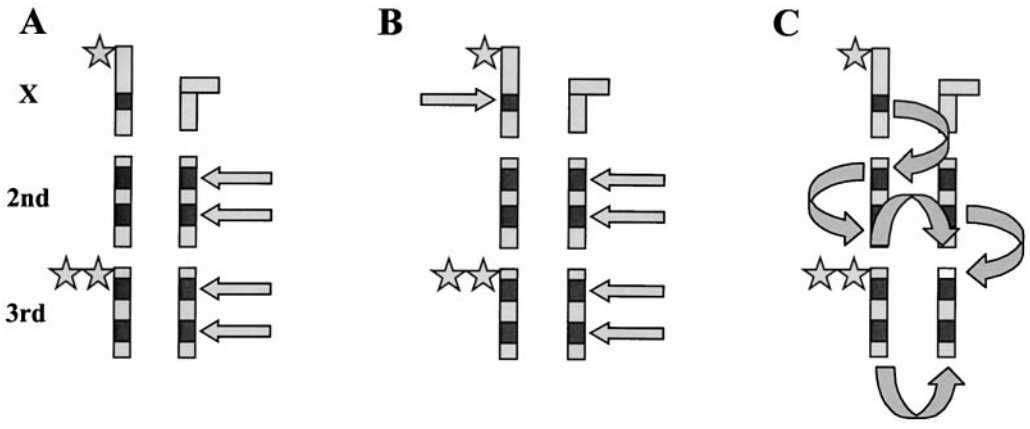

Figure 5.—The three models that could explain the sex ratio phenotype projected on the three sets of Drosophila chromosomes (dot chromosome not included). Light color indicates *D. simulans* DNA and dark color indicates *D. sechellia* DNA. Straight arrows indicate putative introgressions with effect, stars indicate detected QTL, and bent arrows indicate complex interactions. (A) Model 1: A suppressed sex ratio phenotype segregating in *D. simulans* is desuppressed by *D. sechellia* introgressions. (B) Model 2: A complex sex ratio system, segregating in *D. sechellia*, is introgressed into *D. simulans*; the arrow on the X indicates a putative *D. sechellia* X-linked driver. (C) Complex interactions in interspecific background cause the sex ratio phenotype, no sex ratio phenotype segregating in either species.

cannot speculate on their selective effects. Although we consider model 1 as the most plausible, models 2 and 3 cannot be formally rejected. However, in all three cases a simple model with one driver locus and one suppressor locus fails to explain the observations, and a more complicated genetic explanation has to be considered. Furthermore, a simple polygenic model with many loci having additive effects fails to fit the data. If the effects were additive we would expect to see a continuous distribution of male proportion, which was not the case (Figure 2). In summary, the detection of two *D. simulans* alleles associated with the SSR phenotype, the failure to detect significant *D. sechellia* introgressions associated with SSR, and the discontinuity of the distribution of male proportion is consistent with a model having several interacting genes, as proposed by Wu and Hammer (1990).

One issue that is raised by the models is the nature of selective effects of these genes responsible for the sex ratio phenotype. On the one hand, we observe autosomal enhancement of sex chromosome meiotic drive. This is in contrast with the typical selfish behavior of meiotic drivers and provides no explanation for the selective advantage of such genes (Wu 1983b; Wu and Hammer 1990). However, the same issue can be viewed as an advantage of dominant suppressors in a population having a skewed sex ratio (*D. simulans*). The skewed sex ratio does not exist in *D. sechellia* and therefore it is the advantage of the suppressors in the highly biased population that caused the divergence of the two species for these loci.

Although there is a strong selective disadvantage for a skewed sex ratio phenotype to be maintained in the sib-mated lines and the loci involved are many, we observed a high frequency of lines exhibiting a biased sex ratio. One could not easily explain this observation unless there is another force that favors the alleles that cause skewed sex ratio. One possible explanation is that antagonistic pleiotropy serves to balance the negative selective effects of a sex ratio phenotype, but we have no evidence at present for such pleiotropic effects.

Our results provide an example of cryptic meiotic drive revealed in heterospecific crosses. The results are consistent with a meiotic drive system that was unobserved due to fixation of suppressor alleles that were unmasked by interspecific introgression. Such effects may not have been observed in previous experiments (Johnson and Wu 1992; Coyne and Orr 1993), because whole chromosomes were being tested, or introgressed genomic segments were tested in heterozygotes. Our results have implications for the meiotic drive theory of hybrid male sterility (Frank 1991; Hurst and Pomiankowski 1991) and Haldane's rule (Haldane 1922). This theory states that reciprocal meiotic drive systems from two species may result in sperm failure in their F_1 male hybrids. The introgressions that seem to be causing the sex ratio phenotype are recessive, so we cannot directly say that we have uncovered a meiotic drive system that could cause sterility in F_1 hybrids. Dominant and reciprocal effects are required to provide direct support for the meiotic drive theory. However, we have shown that it is possible to have a breakdown of the suppression of a driver in a heterospecific environment, which is the main idea behind the meiotic drive theory. Further evidence is needed to show that such desuppression can occur in F_1 hybrids and to directly associate such meiotic drive phenomena with hybrid male sterility and speciation.

The exposure of meiotic drivers in a heterospecific genetic environment may reveal interesting patterns for the coevolution of meiotic drive systems with their suppressors, the evolutionary constraints that such loci face after fixation, and their possible association with species incompatibilities. The interaction between drivers and suppressors may not be as simple as has been described, and epistatic interaction may be an additional aspect of these systems that has not been sufficiently considered.

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