Sex-Ratio Drive in *Drosophila simulans*: Variation in Segregation Ratio of *X* Chromosomes From a Natural Population

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ABSTRACT

The *sex-ratio* trait that exists in a dozen Drosophila species is a case of naturally occurring *X* chromosome drive that causes males to produce female-biased progeny. Autosomal and *Y* polymorphism for suppressors are known to cause variation in drive expression, but the *X* chromosome polymorphism has never been thoroughly investigated. We characterized 41 *X* chromosomes from a natural population of *Drosophila simulans* that had been transferred to a suppressor-free genetic background. We found two clear-cut groups of chromosomes, *sex-ratio* and *standard*. The *sex-ratio X* chromosomes differed in their segregation ratio (81–96% females in the progeny), the less powerful drivers being less stable in their expression. A sib analysis, using a moderate driver, indicated that within-*X* variation in drive expression depended on genetic (autosomal) or epigenetic factors and that the age of the males also affected the trait. The other *X* chromosomes produced equal or roughly equal sex ratios, but again with significant variation. The continuous pattern of variation observed within both groups suggested that, in addition to a major *sex-ratio* gene, many *X*-linked loci of small effect modify the segregation ratio of this chromosome and are maintained in a polymorphic state. This was also supported by the frequency distribution of sex ratios produced by recombinant *X* chromosomes.

SEGREGATION distorters are chromosomal factors that promote their inheritance by preventing the production of functional gametes carrying their allelic alternative in heterozygous individuals. Because of their advantage in segregation, distorters can spread throughout populations and eventually become fixed without providing any advantage in fitness to their carriers. They thus represent an example of nonadaptative selection. When located on a sex chromosome and expressed in the heterogametic sex, segregation distorters induce a sex-ratio bias in the progeny. This is the case with *X*-linked distorters that are responsible for the *sex-ratio* phenotype found in males of several Drosophila species (reviewed in Jaenike 2001). The drive of the *X* chromosome against the *Y* causes males to produce female-biased progeny and, in extreme cases, all-female progeny. *Sex-ratio* meotic drive can be considered to be the expression of a permanent struggle over segregation that occurs between the sex chromosomes. Because the *X* and *Y* chromosomes do not usually recombine in Drosophila, any driving allele located on the *X* chromosome that favors its own transmission at the expense of the *Y* chromosome will be selected for. Once an initial *sex-ratio X* chromosome is present in a population, selection will favor the accumulation of alleles on this chromosome that increases its transmission ratio. In response to the spread of an *X*-linked distorter in a population, drive suppressors arising on the *Y* chromosome and on the autosomes also will be favored (Fisher 1930; Hamilton 1967; Wu 1983). The spread of suppressors in turn will provide the conditions for the evolution of new *X*-linked distorters that are resistant to suppression or able to restore the driving capacity of the previous driver. This process theoretically can lead to an endless accumulation of drivers, modifiers, and suppressors. For this reason it has been proposed that meiotic drive systems evolve rapidly across species and play a role in reproductive isolation (Frank 1991; Hurst and Pomiankowski 1991). This hypothesis has recently been supported by Dermitzakis *et al.* (2000) and Tao *et al.* (2001) who reported cryptic *sex-ratio* distortion in Drosophila. In both cases the driving factor became unsuppressed after introgression of heterospecific chromosomal regions and in the former case was associated with a reduction in hybrid male fertility.

In all Drosophila species where *sex-ratio* drive has been described, drivers do not spread to fixation and appear in balanced polymorphism because their segregation advantage is counterbalanced by deleterious fitness effects (discussed in Carvalho and Vaz 1999). However, there are signs of an accumulation process on the *X* chromosome. In most species the *sex-ratio* phenotype is associated with one or more *X* chromosome inversions, sometimes spanning a large part of this chromosome. Studies of recombinant chromosomes in *Drosophila para-*

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melanica, *D. affinis*, and *D. persimilis* have shown that the loss of any of these inversions alters the *sex-ratio* characteristics, suggesting that they each contain at least one locus implicated in drive (Stalker 1961; Voelker 1972; Wu and Beckenbach 1983). Some of these loci are thought to have a major effect because they are necessary for drive to occur, while others probably have no drive ability on their own but act as enhancers and/or neutralize some suppressors. In *D. simulans*, a species that does not exhibit inversion polymorphism, several *X*-linked loci also seem to be involved in the *sex-ratio* trait. A major role is played by factors located within a region spanning at most 16 cM. The loss of a part of this region by recombination sometimes produces *X* chromosomes whose drive ability is lowered (Cazemajor *et al.* 1997). In several species, a large variation in the expression of *sex-ratio X* chromosomes among wild-caught males, which could be caused by genetic or environmental factors, has been described (Sturtevant and Dobzhansky 1936; Voelker 1972; Policansky and Dempsey 1978; Hauschteck-Jungen 1990). Autosomal and *Y* chromosome suppressor polymorphisms are known to play a role in this variation, but *X* chromosome polymorphism has never been thoroughly investigated, although knowledge of this effect is essential if we are to understand the evolution of *sex-ratio* drive in natural populations.

The aim of the present study is to determine whether variation exists among *D. simulans sex-ratio X* chromosomes with regard to their drive ability. It complements a recent study of *Y*-linked suppression in this species, which revealed a large diversity of *Y* phenotypes, from nonsuppressor to total suppressor, between and within natural populations. These data imply the existence of an accumulation process in which increasingly powerful suppressors arise and are maintained in a balanced polymorphism with less powerful ones (Montchamp-Moreau *et al.* 2001). Similarly, more or less powerful driving *X* chromosomes might coexist in populations. One of the *D. simulans* samples found to be polymorphic for *Y*-linked suppression was from Réunion island in the Indian Ocean. In this population, as is regularly observed in *D. simulans*, drive expression is prevented by the joint effects of *Y*-linked and autosomal suppression. This population was particularly appropriate for a study of *X* drive variation because in a previous worldwide survey it was found to harbor the highest frequency of *sex-ratio X* chromosomes (60%). It could then represent an advanced stage in the hypothetical accumulation process outlined above (Atlan *et al.* 1997).

Through crosses with laboratory stocks of *D. simulans* whose females carried compound *X* chromosomes, 41 *X* chromosomes sampled in Réunion were transferred into a standard background (assumed to be free of drive suppressors) and maintained in a paternal lineage, *i.e.*, without recombination. This allowed us to analyze thoroughly the pattern of between- and within-*X* variation in segregation ratio.

MATERIALS AND METHODS

Drosophila stocks: ST is our standard reference stock, free of distorters and drive suppressors. ST8 is a standard inbred line, derived from ST and maintained by single-sib mating (Montchamp-Moreau *et al.* 2001). C(1)RM (ST8 background) is a line in which females carry the compound *X* chromosomes from the *lz*[sp]/C(1)RM, *y, w* stock (Bloomington Stock Center, Indiana University) in a standard ST8 background. To obtain this line, C(1)RM, *y, w* females were mated with ST8 males and backcrosses with ST8 males were then performed for 10 generations. The line was then kept *en masse* in 20 replicate vials. At each generation, emerging flies were mixed before being evenly distributed into new vials to ensure a homogenous background across vials. *Cy;Ubx/Dl* is a stock obtained by combination of the stocks Cy^{NC} and *In(3R)Ubx,* Ubx^{m}*/Dl* from the National Drosophila Species Resource Center (Bowling Green State University); it carries the visible dominant marker *Curly* on the second chromosome and *Delta* and *Ultrabithorax* on the third chromosome.

Extraction of *X* chromosomes from Réunion: Forty-one *X* chromosomes, obtained from males randomly sampled in Réunion in December 1996, were introduced into the standard ST8 genome according to the crossing scheme shown in Figure 1. A preliminary cross was performed between C(1)RM (ST8 background) females and *Cy*; *Ubx/Dl* males to obtain attached *X*;*Cy*/+; *Ubx*/+ females. Each of the 41 wild-caught males was individually crossed with these females (parental cross). Crosses of [Cy Ubx] F_1 males with C(1)RM (ST8 background) females, followed by crosses of [Cy Ubx] F_2 males with C(1)RM (ST8 background) females, produced wild-type F_3 males whose autosomes II and III, as well as the *Y* chromosome, were of the standard type (ST8). One wild-type F_3 male, mated with C(1)RM (ST8 background) females, was the founder of each *X* line. In such a cross, with females carrying compound *X* chromosomes, the wild *X* chromosome was paternally transmitted. If it carried driving factors, it produced male-biased progeny. Each *X* line was maintained by repeated backcrosses of six males to six C(1)RM (ST8 background) females. This procedure avoided the selection of newly arising drive suppressors and ensured that the cytoplasmic, *Y*, and autosomal backgrounds remained constant across lines.

Characterization of *X* chromosomes from Réunion: *Segregation ratio test:* The segregation ratio of each *X* chromosome was measured in three replicate experiments, performed one, three, and five generations, respectively, after the foundation of the *X* lines. At each generation, 10 2- to 4-day-old males from each line were separately placed into a vial containing standard medium with a virgin ST female. Three days later, the pair was transferred into a new vial for another 3 days. The progeny emerging from the first vial were counted and sexed until no more flies emerged. If <50 offspring were produced, the progeny from the second vial were also examined. Only tests producing ≥ 50 flies were considered. All experiments were carried out at 25°.

A total of 114,945 flies were scored from 1200 individual tests, yielding an average of 28.5 tests per chromosome and 95.8 flies per progeny. The statistical analysis of the segregation ratios was performed on transformed data (arc sin $\sqrt{p}$) with SAS software. An analysis without this transformation gave the same qualitative results.

Cytological study: The *sex-ratio* trait in *D. simulans* is associated with spermiogenic failure. *Sex-ratio* males frequently have spermatid heads in an abnormal position, *i.e.*, in the tail region of

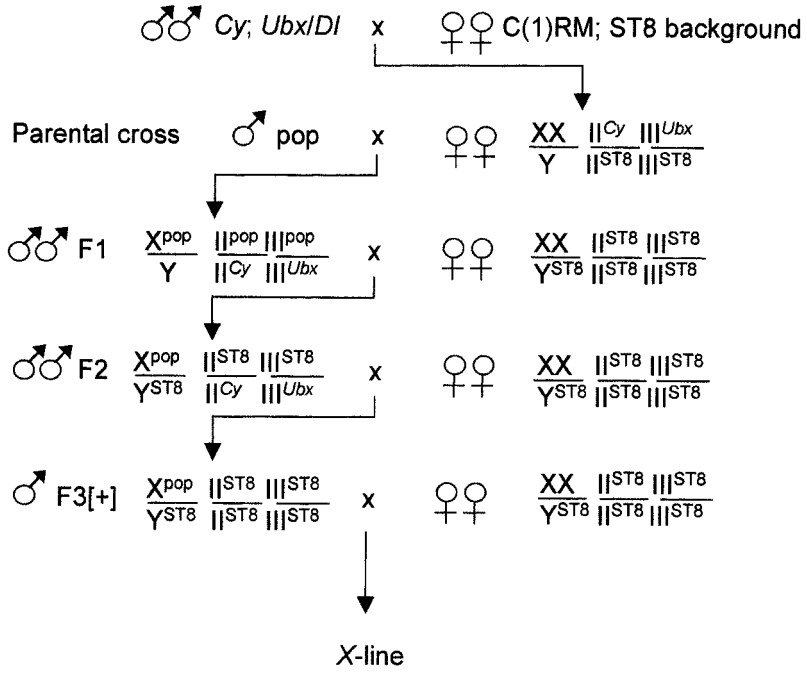

FIGURE 1.—Crossing scheme used to extract *X* chromosomes from the Réunion population and maintain them in the ST8 genetic background through a paternal lineage.

the cysts, which rarely occurs in wild-type males (MONTCHAMP-MOREAU and JOLY 1997). We looked for these defects in males from the *X* lines. For each line, we examined a total of 35 fully elongated cysts from seven different males (5 cysts per male), following the protocol outlined in MONTCHAMP-MOREAU and JOLY (1997).

Viability test: The male progeny scored in the segregation tests had the same genetic constitution across all *X* lines, but the female progeny differed for the *X* chromosome that they had received from their father. The variation in segregation ratio observed among *X* chromosomes from the wild could therefore be due either to differences in drive ability or to differential viability effects on the female progeny that carried them. Since the viability of the male progeny must be the same whatever the *X* chromosome tested, any difference in the total progeny viability between the *X* lines can be ascribed to differences in female viability. We applied the following procedure to measure the egg-to-adult survival of the total progeny for five *sex-ratio X* chromosomes (R21, R51, R10, R07, and R17) and two *standard X* chromosomes (R5 and R20). First, five replicates of each *X* line were set up in separate vials. From each replicate, 30 3-day-old males were collected and mass-mated with 15 4-day-old virgin ST females in a vial containing standard medium. Mating was allowed to occur for 24 hr; flies were then transferred into a half-pint bottle with fresh medium, and egg laying took place for 10 hr. A sample of 100 eggs was collected and placed in a vial with standard medium. This was close to the mean density over the segregation tests. Thus, over the five replicates, a total of 500 eggs were surveyed per *X* line. Eclosing flies were checked and sexed daily until no more flies appeared. The whole experiment was carried out at 25°.

Recovery and characterization of recombinant *sex-ratio*/*standard X* chromosomes: Recombinant chromosomes between the R17 *sex-ratio X* chromosome from Réunion and a *standard* ST8 chromosome were obtained as follows: a male from the *X* line R17 was mated with a ST8 female and a F_1 female was mated with a ST8 male. Then, 80 F_2 males, each possessing a different recombinant R17/ST8 *X* chromosome, were individually mated with five C(1)RM (ST8 background) females to settle 80 recombinant *X* lines. The lines were then kept through repeated backcrosses of males with C(1)RM (ST8 background) females.

Each recombinant *X* chromosome was tested for its segregation ratio, according to the procedure already used with the *X* chromosomes from Réunion, except that only five males were tested at each generation. Further tests were performed to characterize some of the recombinant chromosomes. In this case, four replicates of the corresponding *X* line were made seven generations after the foundation of the line, and four males emerging from each replicate were individually and simultaneously tested for the sex ratio of their progeny.

Source of variation in within-*X* segregation ratio: Among the 41 *X* chromosomes characterized, we chose R21, which was the least powerful driver and showed a relatively high level of segregation ratio variation, to explore the basis of this variation by sib-pair analysis. The experiment was performed 12 generations after the foundation of the *X* line. Thirty R21 parental males were individually mated with ST females to test the segregation ratio of this *X* chromosome. The general procedure outlined above was used, except that the males were 2 days old at the beginning of the test and were removed from the culture vials after 3 days (see Figure 2). Each male was then crossed to a 6-day-old C(1)RM (ST8 background) female for 2 days in a new vial. This procedure was repeated twice with other C(1)RM (ST8 background) females of the same age, 2 days apart. The male was finally mated with a ST female for 3 days as a second measure of the segregation ratio. The eggs laid by each of the three C(1)RM (ST8 background) females were divided into three sets and placed in different culture vials. Four F_1 males emerging from each vial were individually tested for segregation ratio.

RESULTS

Characterization of *X* chromosomes from Réunion: *Segregation ratio:* The mean percentages of females produced by each of the 41 *X* chromosomes over the three generations are given in Figure 3. Two clearly separated groups that were identified by Duncan's test can be

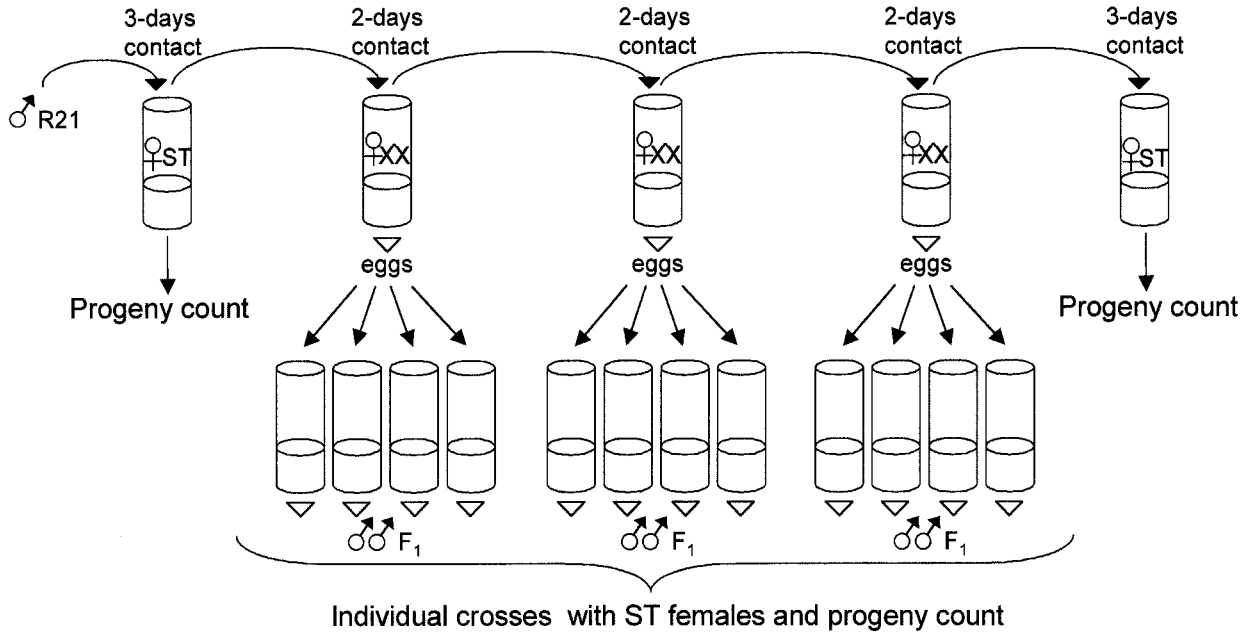

FIGURE 2.—Crossing scheme used to test the variation in segregation ratio among males carrying the R21 chromosome. XX females are C(1)RM (ST8 background) females.

seen. The first group consists of 21 chromosomes that we call *sex-ratio X*, which all produced strongly biased sex ratios. The second group consists of 20 chromosomes that we call *standard X*, which produced equal or slightly biased sex ratios (the mean percentages of females ranged from 49.5 to 58.7). A nested ANOVA, nesting generations within *X* chromosomes, was performed separately on the two groups.

The 21 *X* chromosomes of the *sex-ratio* group showed substantial variation in segregation ratio. The mean per-

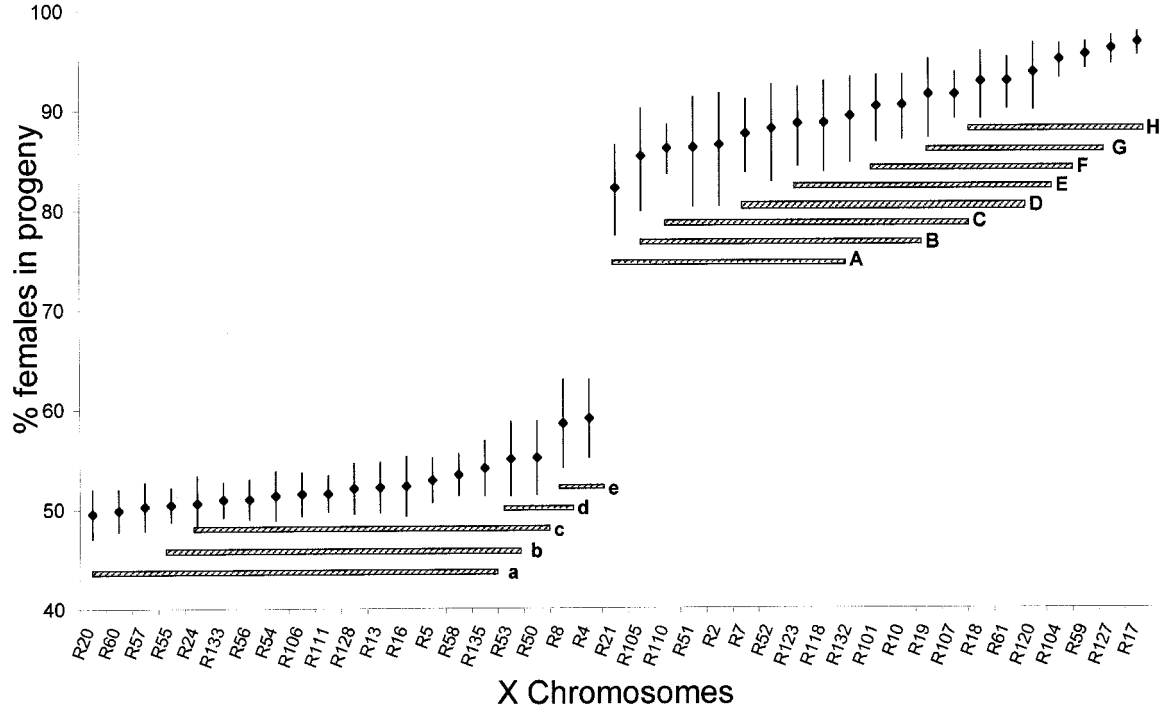

FIGURE 3.—Segregation ratios of the 41 *X* chromosomes from Réunion in the ST8 genetic background. For each chromosome the mean percentage of female offspring (unweighted mean over individual progenies) and the 95% confidence interval were recalculated after angular transformation of the data. Horizontal bars link means that are not significantly different (0.05 level) according to Duncan's test (performed separately on *standard* and *sex-ratio X* chromosomes).

TABLE 1

Analysis of variance of offspring sex ratios among *X* lines

	Sex-ratio X chromosomes				*Standard X* chromosomes			
Source	d.f.	MS	*F*	*P*	d.f.	MS	*F*	*P*
X	20	0.571	5.916	3×10^{-14}	19	0.084	4.047	3×10^{-8}
Generation (*X*)	42	0.145	1.503	0.024	40	0.027	1.291	0.114
Error	555	0.097			535	0.021		

MS, mean square.

centages of females over the three generations ranged from 80.6 to 96.0%, depending on the *X* chromosome; the statistical analysis by nested ANOVA revealed a highly significant *X* effect and a significant generation effect (Table 1). When each line was considered separately, only four showed a significant between-generation difference by a Kruskal-Wallis test: R104 ($P = 0.030$), R107 ($P = 0.015$), R110 ($P = 0.007$), and R17 ($P = 0.006$). It was not possible to classify the 21 *sex-ratio X* chromosomes into distinct homogenous classes: a Duncan's multiple-range test identified eight widely overlapping blocks of means within this group (Figure 3). The chromosomes differed in their average segregation ratio and also in their male-to-male variance in the progeny sex ratio, which seemed negatively correlated with average drive strength. The percentages of females observed among 40 individual progenies produced by males bearing R21, the weakest driving *X* chromosome, ranged from 56.6 to 95.74%, while the range was 73.5–98.6% for the intermediate driver R10 and 89.4–100% for the strongest driver R17 (Figure 4). This was not merely due to an effect of the binomial distribution of the segregation ratio that causes the sampling variance to be maximum for an equal sex ratio and to decrease as the sex-ratio bias increases. The correlation remained significant after the proportions of females were transformed to arc sin $\sqrt{p}$, thus making the variance independent of the sex-ratio bias (Spearman's rank correlation coefficient: $r_s = -0.571$, $P = 0.011$). The moderate driving *X* chromosomes were characterized by a highly variable expression, but there was an extrabinomial variance in all the lines, indicated by significant departures from homogeneity for progeny sex ratio between males of a given generation (chi-square or Fisher exact test), even in the case of the strongest driver, R17 (data not shown).

A nested ANOVA on the group of 20 *standard X* chromosomes also showed a highly significant effect of the *X* chromosome but no significant generation effect (Table 1). Duncan's test identified five blocks of means (Figure 3), among which the "a" block grouped 16 *standard X* chromosomes (from R20 to R135) and did not overlap with blocks "d" and "e," which together grouped the remaining chromosomes R53, R50, R8, and R4. These four chromosomes produced the highest mean percentages of females (from 54.7 to 58.8%) and also the highest male-to-male variances observed among the whole set of *standard X* chromosomes. This was because they sporadically induced female-biased sex ratios in individual progenies, as seen on Figure 4 for the R4 chromosome, when compared with a chromosome of the a block (R24). We used cytological criteria to further characterize these chromosomes.

Cytological study: This was performed for the control standard ST8 line, seven *standard* lines grouped in block a (R20, R24, R106, R13, R16, R5, and R135), three lines in group d or e (R53, R8, and R4), and six *sex-ratio* lines (R21, R51, R2, R7, R10, and R107). Results are shown in Figure 5. Among males bearing a *standard X* chromosome belonging to the a block, the maximum number of misplaced spermatid heads per cyst was 6, close to the maximum number (4) observed in control males; the mean number of misplaced heads did not differ significantly from the control (Mann-Whitney *U*-test). In contrast, when compared with control males, misplaced spermatid heads were found significantly more frequently in males bearing each of the six *sex-ratio X* chromosomes, the maximum number per cyst ranging from 17 to 40. With regard to the three lines that may have carried poorly expressed drive factors, a significant excess of abnormal spermatids was found in males with the R4 and R8 *X* chromosomes that showed a maximum number of misplaced heads (24 and 23, respectively) within the range observed for the *sex-ratio X* group. In males with the R53 *X* chromosome, the mean number of misplaced heads was higher than that in lines of the a block, but it did not differ significantly from that in the control ($P = 0.06$). These results led us to conclude that at least R4 and R8 were also driving *X* chromosomes.

Viability test: To test whether the differences observed between the *sex-ratio X* chromosomes in the segregation ratio test could be related to differential viability of the female progeny, we compared egg-to-adult viability among five *X* lines that covered the whole range of observed variations in segregation ratio (R21, R51, R10, R107, and R17; Table 2). The eclosion rates ranged from 79.0% (R17) to 82.4% (R10) and the differences were not significant (ANOVA: $F_{6,28} = 1.871$, $P = 0.12$). It can thus be concluded that most of the variation in segregation ratio between *sex-ratio X* lines is caused by

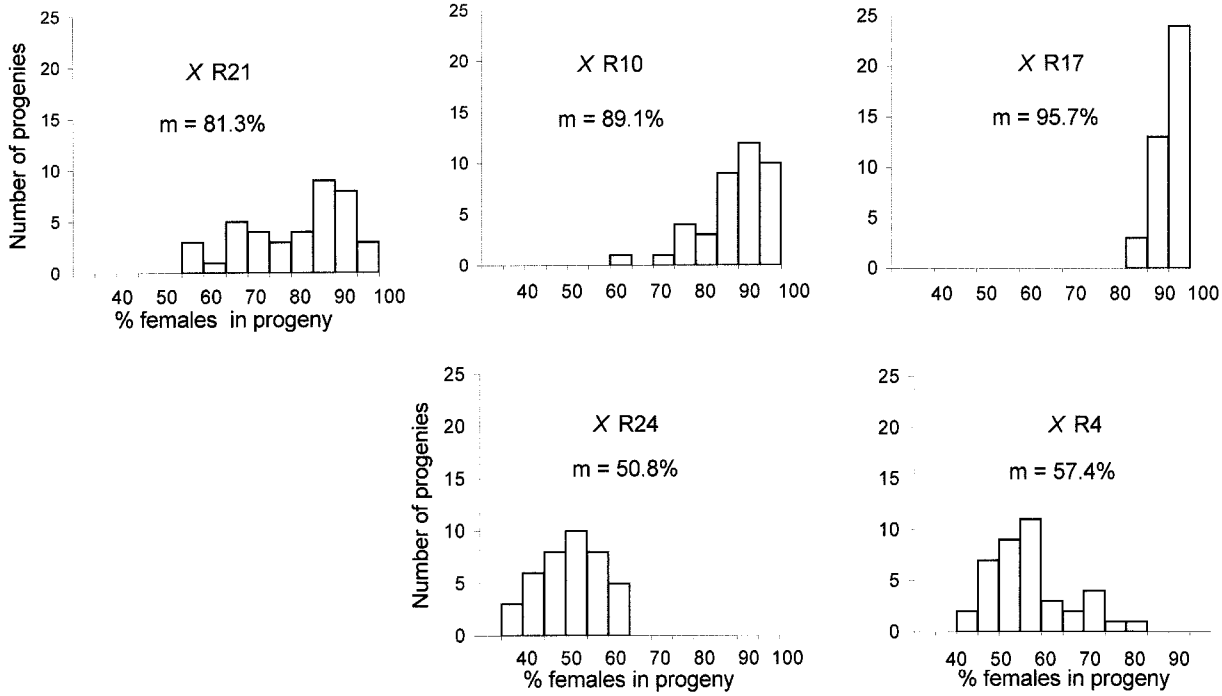

FIGURE 4.—Distribution frequencies of the percentage of females among 40 individual progenies of males carrying the *X* chromosomes R21, R10, R17, R24, and R4. m, mean percentage over progenies.

meiotic drive factors. In addition, a comparison was made with two *standard* lines, R5 and R20. The mean eclosion rate of the five *sex-ratio* lines was significantly lower than the mean of the two *standard* lines (80.3 and 86.4%, respectively; ANOVA, $F_{1,5} = 9.31$, $P = 4.9 \times 10^{-3}$), indicating that *sex-ratio* may lower slightly egg-to-adult female viability when it is present in a heterozygous state.

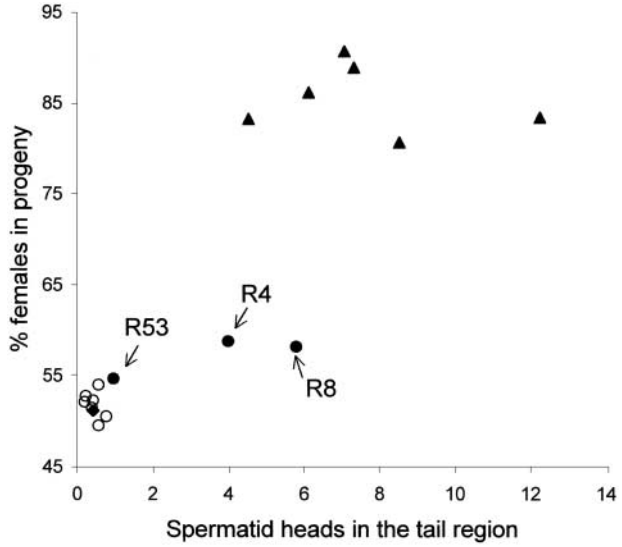

FIGURE 5.—Percentage of females in the progeny of males per tested *X* chromosome, plotted against the mean number of spermatid heads per cyst: (◆) control ST8; (○) *standard X* (seven from group a in Figure 3); (●) *standard X* (three from groups c and d in Figure 3); and (▲) *sex-ratio X* (six from groups A to H in Figure 3).

Segregation ratios in recombinant R17/ST8 *X* chromosomes: The *X* chromosomes obtained after recombination of the strong distorter R17 (96% females) and the *standard* ST8 fell into two equal and clearly separated groups: *sex-ratio* (43) and *standard* (37; Figure 6). This would be expected if a unique locus, or a group of tightly linked loci, were responsible for the *sex-ratio* trait. A continuous variation in segregation ratios was found in the group of *sex-ratio* chromosomes that produced, on average, 86.9–96.1% females over the three generations of males tested. A nested ANOVA showed no generation effect ($F_{86,487} = 1.186$, $P = 0.138$) but a strong *X* effect ($F_{42,86} = 2.746$, $P = 1.13 \times 10^{-7}$) and Duncan's test identified seven widely overlapping blocks of means (data not shown). Some recombination events therefore produced *sex-ratio* chromosomes whose drive ability was lower than that of the parental distorter R17. The range of variation was narrower than that in the *sex-ratio X* chromosomes from the wild. With regard to the other half of recombinant chromosomes (*standard*), the mean percentages of females ranged from 47.8 to 56.9%. A nested ANOVA showed no significant generation or *X* chromosome effects ($F_{74,427} = 0.978$, $P = 0.532$ and $F_{36,74} = 1.358$, $P = 0.086$, respectively). However, one of these chromosomes, R17.18, which produced 56.9% female progeny, appeared to be similar to the very unstable distorters detected in *X* chromosomes from the wild. We conducted additional segregation tests with this chromosome and two other *standard* recombinants (R17.13 and R17.15), which had produced 54.4 and 49.4% female progeny, respectively (marked with arrows on Figure 6). A slight female bias was again found for

TABLE 2

Segregation ratio and egg-to-adult viability in the progeny of males carrying different *X* chromosomes from the Réunion population

	Sex-ratio					*Standard*	
X chromosome	R21	R51	R10	R107	R17	R20	R5
Female percentage in progeny (SE)	77.0 (7.7)	80.6 (4.0)	89.6 (4.4)	90.4 (3.1)	96.8 (2.5)	47.3 (3.8)	52.8 (2.9)
Egg-to-adult viability (SE)	79.6 (7.5)	81 (2.7)	82.4 (5.3)	79.6 (7.2)	79.0 (2.9)	85.4 (1.5)	87.4 (5.0)

A total of 500 eggs were surveyed per *X* chromosome; standard errors are within parentheses.

R17.18 (60.8%) while the others produced equal sex ratios (50.4 and 50.6% female progeny, respectively). The three chromosomes significantly differed in their segregation ratio (nested ANOVA, $F_{2,9} = 11.688$, $P = 1.3 \times 10^{-4}$).

Sources of variation in within-line segregation ratio: *Sib analysis:* Only nine R21 parental males could be used for this analysis. A total of 3201 flies were scored from the two individual tests of these parents (356 flies per male on average). A total of 23,132 F_2 flies were scored from 206 individual tests on R21 F_1 males, corresponding to an average of 112 flies per progeny and 7.6 F_1 males tested per ST8/C(1)RM (ST8 background) female parent. The individual percentages of females produced by the nine parental males in each test are given in Table 3, together with the mean percentage produced by the corresponding groups of full-sib sons. The percentages observed in the individual progenies of the 206 sons ranged from 43.0 to 97.6% (mean = 80.3%). The proportion of females in the individual progenies of F_1 males varied substantially within parental males and also within female parents. A nested ANOVA on transformed data, nesting vials within female parents and female parents within male parents, showed a significant male effect and a highly significant female effect but no vial effect (Table 4). A major part of the variation must thus depend on genetic or epigenetic factors. This is consistent with our finding of a significant father-son correlation for the percentage of female offspring ($r = 0.604$, $P < 0.05$) . It should be pointed out that the value of the female mean square was higher than that of the male mean square (Table 4), showing that a large part of the variation came from maternal effects.

Age effects: An examination of Table 3 reveals a tendency for sex ratios in the progeny of the nine parental males to be more biased toward females in the second series of tests (parental male aged 11–14 days) than in the first series (parental male aged 2–5 days). These data, together with those on 11 other parental males not included in the sib study, were analyzed by a Wilcoxon's test, which showed a significant increase in the percentage of females in the second test (Wilcoxon's test, $P = 0.025$). When the parental males were considered individually, either the percentage of females was significantly higher in the second test (10 males) or the difference was not significant (10 males).

Given that variation between females for a given parental male could be due either to the females themselves or to the age of the male when mated with them, we reanalyzed the difference in sex ratios with a cross-

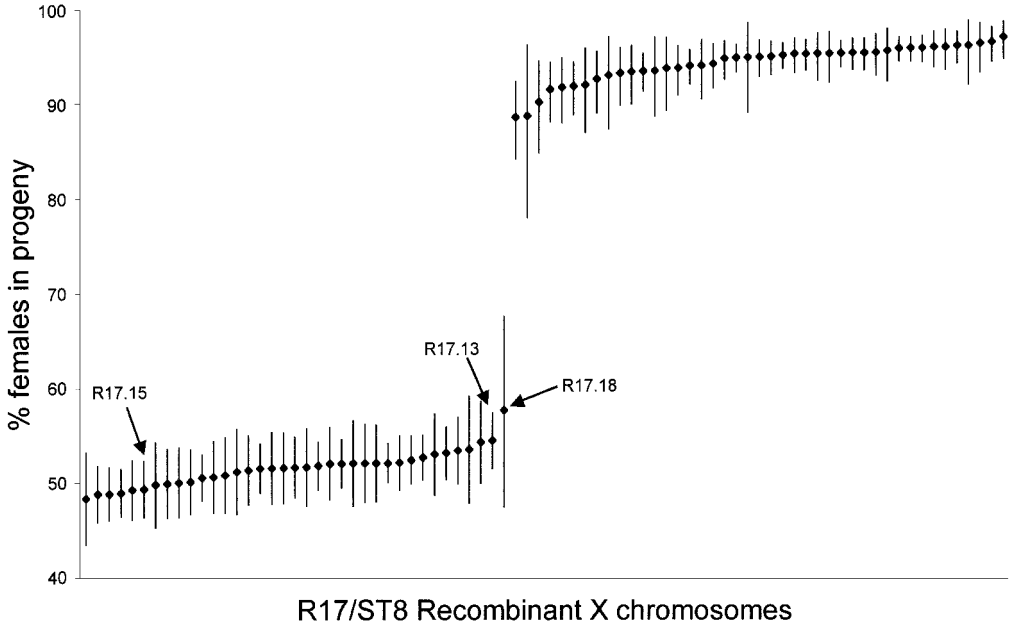

FIGURE 6.—Segregation ratios of the recombinant R17/ST8 *X* chromosomes. For each chromosome the mean percentage of female offspring (unweighted mean over individual progenies) and the 95% confidence interval were recalculated after angular transformation of the data.

TABLE 3

Variation in segregation ratio among individual progenies of males carrying the *sex-ratio X* chromosome R21

	% of females in progeny of				
	Father		Sons[a] from		
Father	2- to 5-day-old	11- to 14-day-old	1st mother	2nd mother	3rd mother
A	82.6	93.8	87.8 (5.4)	81.3 (6.0)	76.5 (6.2)
C	55.4	60.2	67.7 (5.5)	85.7 (3.1)	65.2 (5.4)
D	79.6	93	84.3 (3.7)	77.8 (4.1)	73.9 (3.9)
I	69.6	59.2	86.3 (5.0)	78 (6.0)	77.1 (4.2)
N	92.1	93.6	94.2 (0.8)	85.1 (4.3)	77.9 (5.1)
P	50	70.9	94 (1.1)	78.5 (6.4)	70.1 (5.3)
Q	68	93.8	88.8 (5.0)	87.6 (5.2)	77.4 (8.2)
S	89.9	93.4	77.7 (5.4)	82.6 (3.9)	88.6 (1.2)
T	48.4	56.6	68.4 (5.3)	92.5 (1.5)	67.9 (6.7)
Mean	70.6	79.3	83.2	83.2	74.9

[a] Means are over individual progenies of full-sib sons; standard errors are within parentheses.

classification of the factors "parental male" and "rank of the mother" (Table 5). We found a significant effect of the rank of the mother. This corresponds to a lower sex-ratio bias in the progeny when F_1 males are sons of the third mother (unweighted mean 74.9% females) than when they are sons of the first or second mother (unweighted mean 83.2%).

DISCUSSION

The genetic determinism of *sex-ratio* drive: We found two clear-cut groups, *sex-ratio* and *standard*, among the *X* chromosomes from Réunion. This pattern seems to be general in *D. simulans* populations: it has also been observed among *X* chromosomes from Zimbabwe, Mayotte, and Madagascar (C. Montchamp-Moreau, unpublished data). The *sex-ratio* and *standard* groups apparently differ for gene(s) responsible for a major drive effect, which are known to lie within a tiny part of the *X* chromosome (Cazemajor *et al.* 1997). The continuous within-group variation may be accounted for by allelic variation of the major gene(s) or by variation at several modifier loci scattered along the chromosome. Current population genetics theory suggests that the former possibility is unlikely, given the lack of intermediate phenotypes. On the contrary, the fact that *sex-ratio* chromosomes of different drive ability were obtained by recombination between a *standard X* chromosome and the *sex-ratio X* chromosome R17 strongly argues for a polygenic control of the trait. This also agrees with data from other Drosophila species suggesting that *X* chromosome segregation is controlled by many genes of small effect (Curtsinger 1981, 1984, 1991). The chromosomes with markedly lowered drive ability that we detected in the "standard" groups in both experiments could be of the standard type for the major gene(s) but carry combinations of modifier alleles that can distort independently. Such an independent distorting ability of modifier genes has been reported for the autosomal meiotic drive system segregation distortion (SD) in *D. melanogaster* (Temin 1991).

Within-*X* variation in segregation ratio: Among the *sex-ratio X* chromosomes, the less powerful drivers produced the highest variances in progeny sex ratio; *i.e.*, they had a less stable drive expression. Similar results have been found for recombinant SD chromosomes that had lost

TABLE 4

Nested ANOVA for sex ratio in the progeny of males bearing the *X* chromosome R21, using parental male, mother, and vial as the factors

Source	d.f.	MS	*F*	*P*
Parental male	8	0.293	2.489	1.5×10^{-2}
Mother (male)	18	0.400	3.400	2.8×10^{-5}
Vial (mother)	54	0.109	0.923	0.62
Error	125	0.118		

MS, mean square.

TABLE 5

ANOVA table for sex ratio in the progeny of males bearing the *X* chromosome R21, using parental male and rank of the mother as the factors

Source	d.f.	MS	*F*	*P*
Parental male	8	0.293	2.548	1.2×10^{-2}
Rank of the mother	2	1.095	9.540	1.2×10^{-4}
Male-rank interaction	16	0.313	2.724	6.6×10^{-4}
Error	179	0.148		

MS, mean square.

modifiers (Sandler and Hiraizumi 1960). Miklos and Smith-White (1971) proposed a threshold model for sperm dysfunction that accounted for such observations. In this model, the production of a functional gamete containing the target of the driver depends on a continuous variable: the total amount of all the systems responsible for drive present in the primary spermatocytes ("make"). The values of "make" are normally distributed across the spermatocytes of a given male. If its variance is constant, irrespective of driver strength, the between-male variance in segregation bias is maximum when the mean value of the bias is intermediate. For *sex-ratio* drive, the variance is expected to decrease as the percentage of females in progeny increases from 77 to 100%. This agrees with our observations.

The sib analysis showed that sex ratio was heritable in the R21 *X* line. The between-male variation thus cannot be ascribed to random processes alone. Several nonexclusive hypotheses can be proposed. First, the *X*-linked factors involved in drive may be unstable, leading to a polymorphism within the line. This seems unlikely: the mean percentage of females over the individual progenies of the 207 F_1 males (80.3%) was close to that obtained in the previous experiment (81.3%, Figure 4), showing that the *X*- and *Y*-linked factors that control drive in the R21 *X* line were stable over time. A second possibility is an effect of autosomal genes, which would be consistent with our finding that both parents have an effect. The crossing procedure used to maintain the *X* lines and perform these tests—backcrosses with the C(1)RM (ST8 background) females—should have minimized autosomal polymorphism because the ST8 background came from a highly inbred line, originating from one of the natural populations that is most sensitive to *X* drive and in which no *sex-ratio X* chromosome has been detected (Atlan *et al.* 1997; C. Montchamp-Moreau, unpublished data). However, the C(1)RM (ST8 background) stock was mass-reared at the beginning of the experiment, and relaxing inbreeding could have led to alterations in its genetic makeup. Selection experiments in *D. melanogaster* suggest that variation for polygenic drive suppressors of small effect exists in laboratory stocks that have not been challenged by a driver, even when they have an isogenic past (Lyttle 1979). Whether such variation occurs in standard stocks of *D. simulans* could be resolved by such experiments. Third, the variation in drive expression may have epigenetic causes. Heritable epigenetic changes induced by aging have been reported for autosomal SD (Sandler and Hiraizumi 1961). The age effect observed here strongly suggests that similar heritable changes occur for *sex-ratio* in *D. simulans*. In addition, although the crossing procedure ensured a similar level of autosomal polymorphism in female and male parents, their respective contribution to the total variation was unequal, most being attributable to the mother. This also argues in favor of a large contribution of nongenetic factors to the phenotypic variance of the *sex-ratio* trait. Either possible cause of variation (autosomal polymorphism or epigenetic factors) may be responsible for the moderate but significant differences in segregation ratio observed between generations in a minority of *X* lines in our survey of the Réunion sample.

The maintenance of *sex-ratio* polymorphism: One of the major challenges in understanding the evolution of *sex-ratio* in Drosophila species that are polymorphic for this trait is to identify the selective forces that are decisive in nature. Many unsuccessful attempts have been made in *D. pseudoobscura*. Both laboratory and field studies have revealed pleiotropic effects of *sex-ratio* in both sexes of this species, but they have not satisfactorily explained the stable polymorphism observed in natural populations (Beckenbach 1996). The high level of polymorphism in the Réunion population of *D. simulans*, where *sex-ratio X* chromosomes with widely differing drive ability coexist with nondriving chromosomes and with *Y* chromosomes of various suppression strengths (Montchamp-Moreau *et al.* 2001), suggests the existence of a series of complex selective interactions.

The polymorphism of both sex chromosomes was revealed under laboratory conditions, using a standardized and therefore artificial genetic background. It is possible that the rank order in drive ability of *sex-ratio X* chromosomes depends on the *Y* chromosome and autosomes. It is possible that only subtle differences in drive ability, and therefore in segregation advantage, occur between *sex-ratio X* chromosomes in the natural genome or even that no differences occur, given that drive expression is largely suppressed in the Réunion population. In a sample of 64 males collected at the same time as those used to extract the *X* chromosomes characterized here, only 4 produced progeny with a significant excess of females (72–89%) when crossed with standard (ST) females, although about one-half of the males probably carried a *sex-ratio X* chromosome. Whether this occasional expression of drive is due to *sex-ratio X* chromosomes that are unsuppressible or to *Y* autosome combinations with low or no suppression ability has yet to be determined. Both probably exist in the Réunion population given the results of studies on various populations and stocks of *D. simulans* (Cazemajor 1999; Montchamp-Moreau *et al.* 2001). In addition, variation in drive expression can also be caused by nongenetic factors. As we have shown, drive expression is age dependent. It is also temperature dependent (C. Montchamp-Moreau, unpublished data). Such features have been reported for other meiotic drive systems in Drosophila (Faulhaber 1967; Hiraizumi and Watanabe 1969; Carvalho and Klaczko 1992) and probably reduce the contribution of genetic variation to phenotypic variance in the wild.

Deleterious effects of *sex-ratio X* chromosomes are required to counterbalance their segregation advantage and to maintain their polymorphic state. Under con-

stant selection coefficients, differences in fitness among female genotypes are required (Edwards 1961; Clark 1987; Carvalho *et al.* 1997). We lack data on female fitness in *D. simulans*, apart from the results obtained here in the viability tests. These data suggest that females heterozygous for a *sex-ratio X* chromosome may have a lower viability than that of standard females. However, we did not detect any difference between *sex-ratio X* chromosomes of different strengths. This deserves further study, although it is unlikely that effects on females are a key factor in the maintenance of polymorphism of genes acting on *Y* chromosome behavior during male meiosis. An alternative possibility is frequency-dependent selection. In this case, fitness effects restricted to males can be sufficient. Under multiple mating or sperm competition conditions, *D. simulans sex-ratio* males suffer a strong disadvantage in fertility compared to standard males (Capillon 2000). There are strong arguments to support the idea that both phenomena depend on the sex ratio of the population, and thus on the frequency of driving *X* chromosomes, and that both phenomena can stabilize polymorphism under certain conditions (Jaenike 1996; Taylor and Jaenike 2002). Since the fertility loss is likely due to sperm depletion related to the elimination of *Y*-bearing sperm (Montchamp-Moreau and Joly 1997), it can be assumed to correlate with the strength of drive. This may explain why the deleterious effects of *sex-ratio* are rescued by drive suppressors (Capillon and Atlan 1999). Theoretical investigations of conditions for the simultaneous maintenance of drivers and drive suppressor polymorphisms observed in *D. simulans* will have to take account of the existence of *sex-ratio X* chromosomes of various drive strengths and the fact that their fitness values depend on the genetic background.

In addition, *sex-ratio* in *D. simulans* cannot be treated as a monogenic character, a simplification that is appropriate in species where the different factors implied in drive are bound together by chromosomal inversions. The possibility of obtaining easily recombinant *X* chromosomes with lowered drive ability suggests that at least some of these loci are not tightly linked. Their fates may thus be relatively independent. While it seems reasonable to assume that the polymorphism at the major drive locus (i) might be maintained by a balance between segregation advantage and a deleterious fitness effect, this is less obvious for loci of small effect. In particular because of drive suppression, allelic variants at these loci may be selectively neutral or nearly neutral with respect to the drive phenomenon itself. The polymorphism at minor loci, except for those loci that are involved in suppressibility, is probably maintained by widespread pleiotropy, as is often observed for quantitative traits. This could result in an apparent stabilizing selection, as suggested by the frequency distribution of the segregation ratios among the *sex-ratio* chromosomes.

Finally, a striking and original feature of *sex-ratio* in *D. simulans* is its strong geographical variation. It may reflect differences in local conditions, leading to a variety of balanced polymorphisms that are stable in the long term, or it may represent the present state of a recent invasive process. The molecular analysis of sequences tightly linked to the trait on the *X* chromosome should help us to identify the right scenario.

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