Sex-ratio Meiotic Drive in *Drosophila simulans* Is Related to Equational Nondisjunction of the *Y* Chromosome

Michel Cazemajor,* Dominique Joly† and Catherine Montchamp-Moreau*,†

**Institut J. Monod, Dynamique du Génome et Evolution, CNRS-Université Paris 7, 75251 Paris Cedex 05, France and †Populations, Génétique et Evolution, CNRS, 91198 Gif-sur-Yvette Cedex, France*

Manuscript received June 1, 1999
Accepted for publication September 13, 1999

ABSTRACT

The *sex-ratio* trait, an example of naturally occurring *X*-linked meiotic drive, has been reported in a dozen Drosophila species. Males carrying a *sex-ratio X* chromosome produce an excess of female offspring caused by a deficiency of *Y*-bearing sperm. In *Drosophila simulans*, such males produce ~70–90% female offspring, and 15–30% of the male offspring are sterile. Here, we investigate the cytological basis of the drive in this species. We show that the *sex-ratio* trait is associated with nondisjunction of *Y* chromatids in meiosis II. Fluorescence *in situ* hybridization (FISH) using sex-chromosome-specific probes provides direct evidence that the drive is caused by the failure of the resulting spermatids to develop into functional sperm. *XYY* progeny were not observed, indicating that few or no *YY* spermatids escape failure. The recovery of *X*O males among the progeny of *sex-ratio* males shows that some nullo-*XY* spermatids become functional sperm and likely explains the male sterility. A review of the cytological data in other species shows that aberrant behavior of the *Y* chromosome may be a common basis of *sex-ratio* meiotic drive in Drosophila and the signal that triggers differential spermiogenesis failure.

MEIOTIC drive is defined as an excess recovery of an allele or a chromosome, called the "distorter," among the functional gametes of a heterozygous parent. The *sex-ratio* trait, described in natural populations of a dozen Drosophila species (reviewed in Cazemajor *et al.* 1997), is a type of meiotic drive where the distorters are located on the *X* chromosome. Drosophila males that express the trait produce an excess of females in their progeny as a result of a deficiency of *Y*-bearing sperm. The events responsible for this differential failure of sperm are poorly understood. Here, we contribute to a better understanding of this process by tracking the sex chromosomes during spermatogenesis.

The diversity of cytological and functional features among the different cases of meiotic drive reported previously suggests a wide range of underlying biological mechanisms. In the three well-known cases of meiotic drive that correspond to autosomal distorters, *Segregation Distorter* (*SD*) in Drosophila, *t* haplotype in mouse, and *Spore killer* in fungi, meiosis was reported to unfold normally. The differential recovery of gametes appears to be related to postmeiotic defects (reviewed in Lyttle 1991). The distorter causes dysfunction or loss of the gametes carrying its homologue. However, the stage at which the distorter acts can precede the stage at which abnormalities are observed. For example, in heterozygous SD/SD^+ males of *Drosophila melanogaster*, the sensitive stage for *SD* is premeiotic (Mange 1968), but the first cytological aberrations appear during spermatid differentiation. The abnormal spermatids are eliminated in the testes during coiling, and only rare SD^+ sperm are released in the seminal vesicles (Peacock *et al.* 1972). In t/t^+ *Mus musculus* males, abnormalities are expressed late in gamete maturation: *t* and t^+ sperm are produced in equal quantities, but t^+ sperm are unable to fertilize ova (Silver and Odds-Clarcke 1984).

By contrast, in several cases of sex chromosome drive, the data suggest that the elimination of a fraction of the developing gametes is related to abnormal behavior of the sex chromosomes during meiosis. Male drive in mosquitoes is thought to be a consequence of breakage of the *X* chromosome at meiosis I (Newton *et al.* 1976; Sweeney and Barr 1978). Meiotic drive associated with sex chromosome rearrangements in *D. melanogaster* results from *X-Y* pairing failure and nondisjunction at meiosis I, followed by a differential failure of the spermatids derived from the affected spermatocytes (reviewed in McKee 1998). As for the *sex-ratio* trait, there has been little investigation about its cytology. In *D. pseudoobscura* and *D. athabasca*, the *Y* chromosome fails to undergo anaphase II (Novitsky *et al.* 1965; Cobbs *et al.* 1991). The resulting sperm is probably eliminated late in spermiogenesis (Policansky and Ellison 1970). The abnormal behavior of an unidentified sex chromosome has also been reported in *D. subobscura* (Hauschteck-Jungen *et al.* 1972). In this species and in *D. simulans*, some spermatids fail to develop properly and, therefore, are thought to be *Y* bearing (Hauschteck-Jungen and Maurer 1976; Montchamp-Moreau and Joly 1997).

Corresponding author: Catherine Montchamp-Moreau, Laboratoire Populations, Génétique et Evolution, CNRS, Avenue de la Terrasse, 91198 Gif-sur-Yvette, France. E-mail: montchamp@pge.cnrs-gif.fr

In *D. simulans*, the *sex-ratio* trait is rarely, if ever, expressed within strains or natural populations that contain *X*-linked distorters because drive resistance has evolved on the *Y* chromosome and on the autosomes (Atlan *et al.* 1997; Cazemajor *et al.* 1997). This local coevolution of genes ensures normal spermatogenesis (Montchamp-Moreau and Joly 1997) and equal sex ratio within populations, even when *X*-linked distorters are at high frequency (Atlan *et al.* 1997). The *sex-ratio* males described here are F_1 hybrids obtained by crosses between males from a reference standard strain (ST), which bring a drive-sensitive *Y* chromosome, and females from another reference strain (SR), which bring *X*-linked distorters. The progeny of such F_1 hybrid males are typically 70–90% female. They have been already used to describe the spermiogenic defects, *i.e.*, the failure of some spermatids to undergo normal elongation (Montchamp-Moreau and Joly 1997). Here, we use standard cytological methods and fluorescence *in situ* hybridization (FISH) with sex-chromosome-specific probes to determine the chromosome content of the spermatids that do not exhibit normal elongation in *sex-ratio* males and to look for related meiotic defects. We show that the abnormal spermatids originate from *Y*-bearing secondary spermatocytes in which the *Y* sister chromatids failed to disjoin. *X*O individuals have been observed among the progeny of *sex-ratio* males, indicating that some nullo-*XY* spermatids do become functional sperm.

MATERIALS AND METHODS

Strains: Standard males are from the ST strain, which is free of *X*-linked distorters and drive suppressors. *Sex-ratio* males are F_1 hybrids obtained by crosses between ST males and SR females raised at 25°. The ST and SR strains are the reference strains defined in Atlan *et al.* (1997). The SR strain contains *X*-linked distorters and both *Y*-linked and autosomal drive suppressors (Cazemajor *et al.* 1997). Therefore, a *sex-ratio* male carries a drive-sensitive *Y* chromosome brought by its father and *X*-linked distorters brought by its mother. Paternal autosomal suppressors are unable to prevent drive expression in such F_1 hybrid males.

Probes: The *X*-specific probe is a 3-kb *Sma*I fragment from p(rib, ry)[7] (Karpen *et al.* 1988), which contains a part of the rDNA transcribed sequence spanning the 18S and 28S genes from *D. melanogaster*. In *D. simulans*, this sequence is restricted to the *X* chromosome, where it is repeated ~250 times (Lohe and Roberts 1990). The plasmid A3235 (Lohe and Roberts 1990) was used as the *Y*-specific probe. It contains sequences spanning 4 units of the 240-base-pair repeat of the rDNA gene spacer of *D. simulans*. In *D. simulans*, this sequence occurs on both the *Y* and the *X*, but is six times more abundant on the *Y* (covering ~3 Mb; Lohe and Roberts 1990). The *X*-specific probe was biotin labeled using the BioNick kit from GIBCO BRL and detected using fluorescein Avidin DN (Vector Biosys, Compiègne, France). The *Y*-specific probe was labeled with rhodamine-dUTP (Amersham, Arlington Heights, IL), using terminal deoxynucleotidyl transferase as described in Dernburg and Sedat (1998). These probes allowed us to discriminate between *X* and *Y* chromosomes, as shown by *in situ* hybridization on mitotic chromosomes (Figure 1).

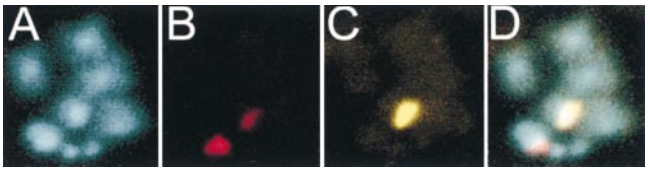

Figure 1.—FISH on mitotic chromosomes of ST male. (A) DAPI staining is shown in blue. (B) The rDNA spacer probe (red) colocalizes with both *X* and *Y* chromosomes, but the signal is stronger on the *Y*. (C) The rDNA transcribed sequences probe (yellow) colocalizes with the *X* chromosome. (D) Superimposition of the signals in A–C, showing that the discrimination between *X* and *Y* is unambiguous.

***In situ* hybridization:** Slides were coated with 3-aminopropyltriethoxysilane (TESPA) from Sigma (St. Louis). Testes were dissected in 10 mm dithiothreitol/buffer A (Belmont *et al.* 1989), fixed in 3.7% formaldehyde, and then squashed under a siliconized coverslip to properly separate the spermatid heads. Slides were frozen in liquid nitrogen to remove the coverslip, treated with 0.1% pepsine/10 mm HCl and 3:1 ethanol:acetic acid, and finally RNase treated. The hybridization-and-mounting procedure was a modification of the protocol of Rousseaux and Chevret (1995): after hybridization, the slides were washed three times in 50% formamide/2× SSC at 39°, three times in 2× SSC at 42°, and three times in 0.1× SSC at 55°. Pictures were obtained using a black and white CCD Photometrics CH250 camera mounted on an Axiovert 135 microscope (Zeiss, Thornwood, NY), and color pictures were then rebuilt using Adobe Photoshop 4.1.

Meiosis: Testes of 0- to 4-hr-old males were dissected. Cells were fixed and orcein stained according to Lifschytz and Hareven (1977).

Karyotypes: They were performed on larval neuroblasts, following steps 1–5 of protocol 2 in Gatti *et al.* (1994). Slides were then mounted in 1:1 glycerol:Ringer containing DAPI.

RESULTS

Fluorescence *in situ* hybridization: Spermatogenesis in *D. simulans* resembles that in *D. melanogaster*. A primary spermatogonium surrounded by a pair of cyst cells undergoes four mitotic divisions, forming a cyst of 16 primary spermatocytes. After the two meiotic divisions, the cyst contains a disk-shaped syncytium of 64 spermatids, half containing an *X* chromosome and half a *Y* chromosome. As the sperm tails expand, the cyst elongates while the spermatid nuclei remain at the anterior end of the cyst. After elongation and DNA condensation into needle-shaped nuclei, the spermatids individualize and become spermatozoa. In *sex-ratio* males, some spermatids fail to elongate normally and their nuclei are scattered caudally along the tail region of the cyst (Montchamp-Moreau and Joly 1997).

Our FISH procedure does not work for the highly condensed nuclei of mature sperm. Thus, we analyzed only unambiguously labeled cysts whose spermatid nuclei correspond to stages O–P described in Fuller (1993). Nuclei labeling was homogenous within a cyst,

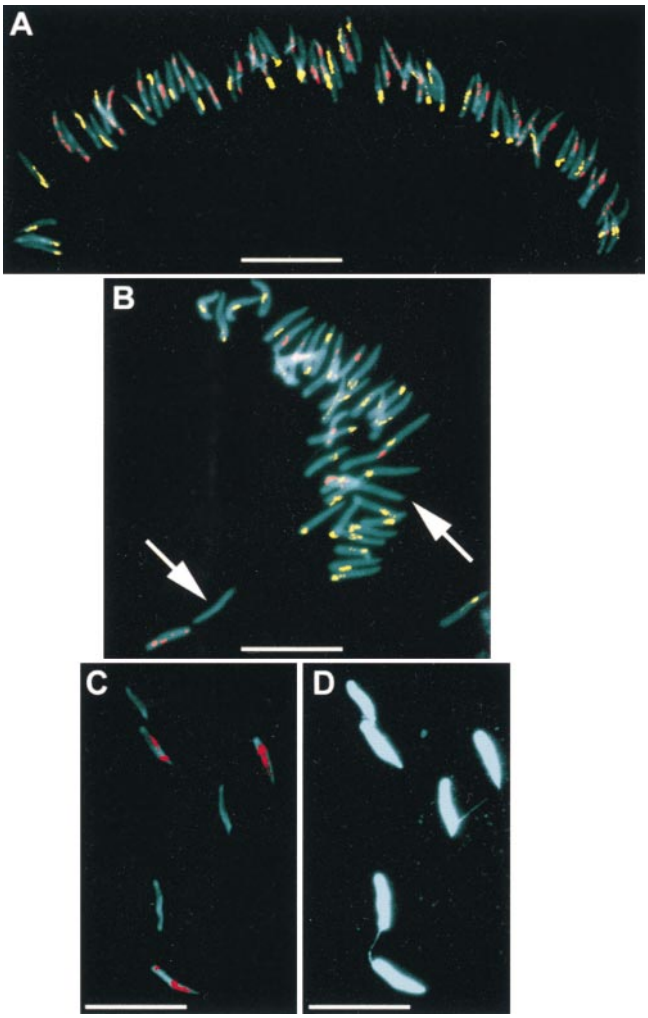

Figure 2.—Cysts from standard males and *sex-ratio* males, FISH-labeled and counterstained with DAPI. (A) Anterior end of a standard male cyst with 32 *X*-labeled nuclei (yellow) and 32 *Y*-labeled nuclei (red). (B) Anterior end of a *sex-ratio* male cyst with 32 *X*-labeled nuclei, 9 *Y*-labeled nuclei, and 2 unlabeled nuclei (arrows) marked only by DAPI. (C) Detail of the tail region of a *sex-ratio* male cyst showing pairs of *Y*-labeled and unlabeled nuclei. (D) Same as C, but with only DAPI staining. Long exposure time revealed a DNA junction between the *Y*-labeled and the unlabeled nuclei. Scale bars, 20 μm.

TABLE 1

Hybridization pattern of spermatid nuclei in 25 cysts of standard males

X	*Y*	Unlabeled	Total[a]
29	34	0	63
26	26	0	52
31	29	0	60
28	31	1	60
29	30	0	59
28	24	0	52
29	32	1	62
30	31	0	61
28	30	1	59
24	34	0	58
29	25	0	54
32	32	0	64
32	32	0	64
31	31	0	62
25	27	0	52
31	31	0	62
30	32	0	62
27	32	0	59
31	32	0	63
32	32	0	64
32	32	0	64
32	32	0	64
32	31	0	63
32	32	0	64
31	32	0	63
Total: 741	766	3	1510
Mean (SE): 29.6 (2.3)	30.6 (2.6)	0.12 (0.3)	60.4 (4.0)

[a] The treatment for FISH alters the cellular morphology and does not allow easy visualization of the limits of the cysts. For this reason, as well as possible loss of nuclei during the preparation of the slides, the total number of nuclei per cyst was often <64.

but labeling intensity varied between cysts, depending on the slide quality. Figure 2A shows an ST male cyst with 32 *Y*-labeled spermatid nuclei and 32 *X*-labeled nuclei, all located at the anterior end of the cyst. Table 1 presents results of FISH on 25 cysts from 6 ST males. Three nuclei among 1510 are devoid of any labeling, and the total number of *X*- and *Y*-labeled nuclei are not statistically different ($\chi^2 = 0.517$, 1 d.f., $P = 0.42$). This result confirms the specificity of the probes and demonstrates their equal signaling and the reliability of the method.

A typical FISH-labeled cyst from a *sex-ratio* male is shown in Figure 2, B and C. In the normal position, *i.e.*, at the anterior end of the cyst (Figure 2B), three types of nuclei were identified: *X*-labeled, *Y*-labeled, and unlabeled. The nuclei scattered in the tail region of the cyst (Figure 2C) were either *Y*-labeled or unlabeled. Table 2 summarizes data on the number and location of nuclei observed in 27 cysts of 12 *sex-ratio* males. The mean number of nuclei per cyst in the normal position was only 51.2, *vs.* 60.4 in ST males. The difference is not attributable to *X*-labeled spermatids, since their number was similar in *sex-ratio* and ST males (30.6 and 29.6, respectively). In accordance with this observation, we did not find any *X*-labeled nuclei in the tail region. This shows that neither the number nor the elongation process of *X*-bearing spermatids is affected by the *sex-ratio* trait. Unlabeled nuclei in *sex-ratio* cysts were much more frequent (12%) compared to ST cysts (0.2%). We therefore conclude that almost all unlabeled spermatid nuclei in *sex-ratio* males cysts come from *Y*-bearing secondary spermatocytes and must be devoid of all or part of this chromosome (including at least the tip of the

TABLE 2

Hybridization pattern of spermatid nuclei in 27 cysts of *sex-ratio* males

	Anterior end				Tail region		
	X	*Y*	Unlabeled	Total	*Y*	Unlabeled	Total[a]
	32	8	2	42	12	10	64
	29	6	0	35	9	9	53
	32	12	12	56	5	5	66
	32	3	2	37	11	9	57
	32	6	4	42	—	—	—
	32	26	2	60	2	2	64
	32	24	7	63	—	—	—
	32	21	9	62	—	—	—
	32	23	8	63	—	—	—
	32	32	0	64	—	—	—
	18	13	1	32	—	—	—
	32	23	0	55	2	2	59
	32	31	1	64	—	—	—
	30	31	0	61	—	—	—
	30	19	6	55	—	—	—
	32	32	0	64	—	—	—
	32	10	10	52	—	—	—
	29	24	4	57	—	—	—
	32	7	1	40	9	9	58
	30	13	6	49	5	5	59
	32	10	5	47	3	3	53
	30	15	10	55	4	2	61
	29	16	8	53	3	3	59
	28	9	6	43	—	—	—
	30	15	3	48	9	7	64
	32	12	2	46	5	5	56
	32	5	3	40	13	11	64
Total:	827	446	112	1385	92	82	
Mean (SE):	30.6 (2.8)	16.5 (9.0)	4.1 (3.6)	51.2 (9.9)			

[a] See Table 1.

—, Count was not done because it was impossible to visualize the limits of the cyst in the tail region.

YL arm that bears the target of the probe). The mean number of *Y*-labeled nuclei at the anterior end of the cyst was greatly reduced in *sex-ratio* males (16.5) when compared to ST males (30.6). On average, *Y*-labeled nuclei were about four times more numerous at the anterior end than were unlabeled nuclei (446 *vs.* 112 for all cysts examined). By contrast, among the nuclei scattered in the tail region, there was only a slight excess of *Y*-labeled over unlabeled nuclei (92 *vs.* 82, respectively). Overexposure of the tail region often revealed DNA bridges between pairs of nuclei of which one was *Y*-labeled and one unlabeled (Figure 2D). This strongly suggested that the pairs of nuclei were the product of the same second meiotic division in which the *Y* missegregated. Indeed, FISH on early anaphase II revealed three classes of daughter nuclei still paired: both *X* labeled, both *Y* labeled, and one *Y* labeled and the other unlabeled (data not shown).

Meiosis: Two types of meiosis II abnormalities can lead to pairs of *Y*-labeled and unlabeled spermatids: (i) the two *Y* sister chromatids remain attached and are therefore recovered in the same daughter cell; (ii) the *Y* chromatids separate but randomly segregate to either pole. The direct observations of orcein-stained spermatogonial cysts in different phases of the cell cycle showed that *sex-ratio* in *D. simulans* is related to the former case. Chromosomes behaved normally during meiosis I in *sex-ratio* males (data not shown), as did the chromosomes *X*, 2, and 3 during anaphase II (Figure 3, A compared to C). By contrast, abnormal behavior of *Y* sister chromatids was observed in all but 3 of the 74 anaphase IIs analyzed in *sex-ratio* males. The appearance of the *Y* chromosome during meiosis II was different from one cell to another. In 27 cases, the two *Y* chromatids did not separate, lagged behind the autosomes, and moved toward the same pole (as in Figure 3D). In 44 other plates, abnormal segregation occurred, following incomplete separation of the chromatids (see Figure 3E). In most cases, the pictures suggested that the two long arms (*YL*) and one short arm (*YS*) of the *Y* sister chromatids moved toward one pole, whereas the second short arm (*YS*) moved toward the opposite pole. A very thin

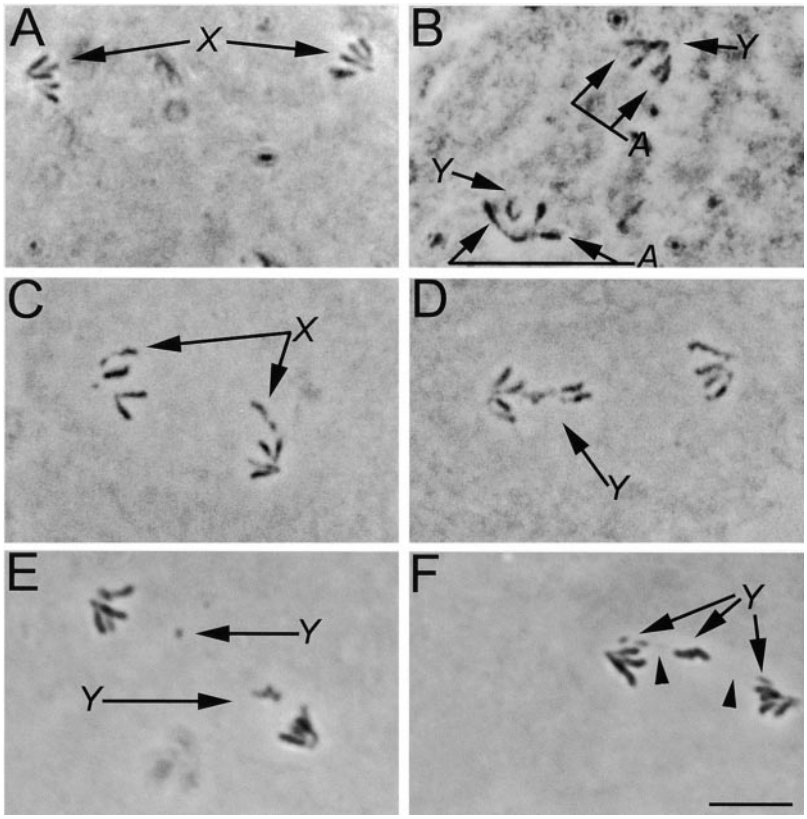

Figure 3.—Acetic-orcein-stained late anaphase IIs. (A and B) Normal segregation of *X* and *Y* in ST male. (C) Normal segregation of *X* and autosomes in SR males. (D–F) Abnormal segregation of *Y* chromosomes in SR male. (D) The two *Y* chromatids do not separate; they move toward the same pole. (E) A small part of one *Y* chromatid (*YL*?) migrates to one pole (upper arrow) as the remaining parts of the *Y*'s migrate to the other pole (lower arrow) with an appearance of broken chromosome. (F) Similar to E, but a thin chromatid fiber is visible between the different parts of the *Y*'s (arrowheads). Scale bars, 5 μm.

chromatin fiber could often be seen between the segregating *Y* parts, showing that the chromatid was not broken, but stretched (Figure 3F). Since the *X* and the autosomes were always found to segregate normally, the disjunction defect appears to be *Y*-chromosome specific (with the caveat that the dot chromosome 4 was not controlled).

Karyotypes of sons of *sex-ratio* males: To determine whether some of the spermatids resulting from abnormal *Y* segregation could become functional spermatozoa, we karyotyped the progeny of *sex-ratio* males. Because the *Y* chromosome carries only fertility genes, addition or loss of the *Y* chromosome is expected to have little effect on the viability of the resulting progeny (*XYY* and *X*O males, respectively), as is the case in *D. melanogaster* (Erickson 1965; Lyttle 1981). A total of 19 *sex-ratio* males were mated individually with a few ST females, and the flies were transferred every 3 days into new vials. The progeny of the first vial was used to measure the percentage of *X*-bearing sperm of the SR father, while the subsequent broods of progeny were karyotyped (Table 3). The karyotypes of 102 male larvae included 24 *X*O (23.5 ± 6.5%) and 78 *XY*, showing that some nullo-*XY* spermatozoa were fully functional. If we class arbitrarily the *sex-ratio* males in strong distorter (percentage of female in progeny above the mean of 90.9%) and moderate distorter (below the mean), as shown in Table 3, the fraction of *X*O male progeny is significantly higher in the former group ($\chi^2 = 4.83$, 1 d.f., $P = 0.02$). The fractions of *X*O males observed in the progeny of strong and moderate distorters (34 and 15%, respectively) are similar to the ratio of sterile males in the progeny of these two classes (30 and 15%, respectively) reported by Merçot *et al.* (1995). This shows that all or almost all the cases of sterility among the male progeny result from the lack of *Y* chromosome. No *XYY* male was observed in this experiment nor in an additional screening of 44 male larvae (6*X*O and 38*XY*), and we have not observed karyotypes with centric *Y* fragments, as expected from breakage of the chromosomal bridges. However, the protocol used produced highly condensed chromosomes, so that only large deletions of the *Y* could have been detected. Assuming equal viability of *XY*, *X*O, and *XYY* larvae, if any functional double-*Y* sperm is produced, their frequency must be <2% of the total ($\alpha = 0.05$).

DISCUSSION

This study has established that the excess of females among the progeny of *sex-ratio* males in *D. simulans* is related to meiotic defects, specifically to equational nondisjunction of the *Y* chromosome. FISH with chromosome-specific probes provides direct evidence of a relationship between this abnormal meiosis and subsequent spermiogenic failure: the spermatids that fail to elongate come from spermatocytes where the *Y* sister chromatids have failed to properly segregate during meiosis

II. However, the data raise numerous questions about the underlying mechanisms, the extent and the timing of the spermiogenic failure and the possibility that the *sex-ratio* trait in *D. simulans* may be representative of other cases reported among Drosophila species.

Extensive studies using sex chromosome rearrangements in *D. melanogaster* have demonstrated the relationship between *sex-ratio* meiotic drive and *X-Y* mispairing in meiosis I (McKee 1998). However, the distorter alleles carried by the *sex-ratio X* chromosome in *D. simulans* (Cazemajor *et al.* 1997) do not map to the rDNA region identified as the *X-Y* pairing site in this species (Ault and Rieder 1994). Our cytological observations lead to excluding the possibility of *XY* nondisjunction during meiosis I. The *X* and *Y* were found to pair and segregate normally in the first division. In addition, we never observed distinct *X* and *Y* labeling within the same spermatid nuclei. The *sex-ratio* trait seems, rather, to be related to the inability of the *Y* sister chromatids to separate at anaphase II. This may result either from structural abnormalities of the *Y* chromosome or from *Y*-specific disturbance in the mechanisms that normally release the cohesion of sister chromatids at this stage (reviewed in Bickel and Orr-Weaver 1996). We suspect a preferential segregation of the *YS* arm in cases where partial separation of the chromatids occurs. This relies on both the observation of meiotic figures and the results of FISH that showed, in the tail region of the cysts, pairs of *Y*-labeled and unlabeled spermatid nuclei, the latter therefore being devoid of the tip of the *YL* arm at least. There is a slight excess of *Y*-labeled over unlabeled nuclei in the tail region, which might correspond to rare cases with disjunction of the *YL*. Unfortunately, we did not have the opportunity to observe pairs of *Y*-labeled nuclei and to look for a DNA link between them. The assumption of a preferential segregation of *YS* must be considered with caution because it is inconsistent with the threefold excess of *Y*-labeled over unlabeled nuclei in the anterior end of the cysts (Table 2). Given the high frequency of abnormal *Y* meiosis II (96%), only a moderate excess of *Y*-labeled nuclei is expected. The discrepancy may also result from an overestimate of the frequency of abnormal meiosis through sampling bias because the *Y* chromosome is more easily distinguishable when lagging behind the autosomes. Another explanation rests on the observation that the total number of spermatid nuclei per cyst is significantly lower in *sex-ratio* males when compared to ST males (Montchamp-Moreau and Joly 1997). Here, we found similar numbers of *X*-labeled nuclei per cyst in both types of males (Kolmogorov-Smirnoff test, $P > 0.05$). Therefore, there could be a deficiency in spermatids produced from *Y*-bearing secondary spermatocytes in *sex-ratio* males. Unfortunately, it is not possible to obtain a reliable count of the total number of nuclei per cyst under FISH conditions. One potential source of reduced nuclei numbers and increase of the ratio of *Y*-labeled nuclei could be karyokinesis failure, leading to diploid spermatids with larger nuclei, but we did not detect any peculiar morphology heterogeneity between spermatid heads in normal position. However, the nuclei scattered in the tail region frequently showed variable degrees of condensation and elongation and did not allow inference about their ploidy.

The excess of females in the progeny of *sex-ratio* males had been found higher than expected given the mean number of normally elongated spermatids per cyst, suggesting that some of these spermatids were not becoming functional sperm (Montchamp-Moreau and Joly 1997). Here, we found this discrepancy again, but the presence of unlabeled nuclei at the anterior end (Table 2) indicates that among the normally elongated spermatids are many that are the product of abnormal meiosis. The defects in spermatid elongation may be restricted to the case where a stretch of DNA is maintained between the daughter nuclei. This could prevent the completion of karyokinesis and push the nuclei back toward the syncytial tail region of the cyst, perhaps for mechanical reasons. By contrast, karyokinesis could be completed successfully after absolute nondisjunction (Figure 3D) or breakage of the stretched chromatid, leading to spermatids that are able to elongate normally. Unfor-

TABLE 3

Sex ratios and karyotypes in the progeny of 19 *sex-ratio* males

	Adult progeny[a]		Male larvae in progeny[b]		
Sex-ratio male	Total	% Female	*X*O	*XY*	% *X*O
1	75	100.0	3	0	
2	78	98.7	0	4	
3	109	97.2	1	1	
4	105	97.1	1	4	
5	119	96.6	3	3	
6	96	95.8	0	11	
7	89	94.4	1	1	
8	88	94.3	2	1	
9	91	93.4	2	1	
10	102	92.2	1	2	
11	84	91.7	2	3	34.0[c]
12	98	90.8	0	1	
13	110	90.0	1	4	
14	124	88.7	1	7	
15	105	86.7	1	1	
16	81	85.2	1	1	
17	112	80.4	1	6	
18	120	79.2	0	7	
19	111	73.9	3	20	14.5[d]
Total	763	90.9	24	78	23.5

[a] Count of the first brood of progeny.
[b] Count of the subsequent broods.
[c,d] Mean over males 1–11 and 12–19, respectively.

tunately, we could not determine whether some nuclei in normal position were linked by a DNA stretch. Nuclei in the anterior end of the cyst were close to each other, often overlapping, so that a prolonged exposure gave a saturated signal of DAPI over the whole field. Considering the bias in sex ratio among the progeny of *sex-ratio* males (a mean of 90–91% females) and the ratio *X*O/*XY* among the male progeny (about one-quarter), one unlabeled and three *Y*-labeled spermatids per cyst are expected on average to become functional sperm. Therefore, most of the *Y*-labeled and unlabeled spermatids that are normally elongated (16.5 and 4.1 per cyst on average, respectively) must be eliminated at the subsequent stages. Are they all the product of defective meiosis II? We cannot answer this question because of the uncertainties about the ratio of abnormal meiosis and the preferential segregation of *YS* discussed above. Anyway, some spermatids resulting from achieved *Y* disjunction may still fail to function. If the abnormal behavior of *Y* chromosome in meiosis II is the event that triggers sperm dysfunction, perhaps a successful but delayed segregation could be sufficient to induce subsequent failure. Alternatively, nondisjunction may be only one possible symptom of the dysfunction of *Y*-bearing cells.

The probability of failure seems to depend on the chromosomal content of sperm nuclei. Among 146 male larvae progeny of *sex-ratio* males, 30 were *X*O and none were *XYY*. The *XYY* zygotes are not embryonic lethal in *D. simulans* (Yamamoto 1992), and it is unlikely that *YY* sperm are poor fertilizers, since in *D. melanogaster*, one finds recovery loss of only 0.33 for the *YY* gametes when compared to the *X* gametes in the progeny of *XYY* males (Lyttle 1981). A loss of the *Y* chromosome seems unlikely here because the cytology of meiosis II in SR males suggests that the *Y*'s are usually incorporated into the daughter nuclei. In other respects, the occurrence of a differential failure is consistent with the observations in *D. melanogaster* that the probability of recovering fully functional sperm after abnormal segregation of chromosomes is negatively correlated with their chromatin content (McKee 1984). The differential failure of spermatids that have achieved elongation may occur at various stages, from individualization up to their use by females. Hence, we have to develop alternative techniques to study the fate of late spermatids and mature sperm.

The *sex-ratio* trait has been described in a dozen Drosophila species belonging to different taxonomic groups, including two different subgenera (*i.e.*, Drosophila and Sophophora). One can raise the question about a common genetic basis of the *sex-ratio* trait among these species. Very few genetic data are available, but the general scheme is a polygenic control (Stalker 1960; Jungen 1968; Wu and Beckenbach 1983; Cazemajor *et al.* 1997). In the three documented cases for which cytological data are available, *i.e.*, *D. pseudoobscura*, *D. athabasca* (Novitsky *et al.* 1965; Cobbs *et al.* 1991), and here in *D. simulans*, a common feature emerges: abnormal behavior of the *Y* chromosome in meiosis II. In *D. pseudoobscura*, the *Y* chromosome was supposed to be lost because it shows no centromeric activity at anaphase II and sometimes at anaphase I, and the rare male progeny of *sex-ratio* males are *X*O. We have shown here that the production of *X*O males, but not *XYY* males, in relation to the nondisjunction of this chromosome can result as well from the selective failure of double-*Y* sperm. In other respects, cytological examination in *D. obscura* has suggested a disjunction defect of one of the sex chromosomes in meiosis II (Hauschteck-Jungen *et al.* 1972), but the authors could not discriminate between *X* and *Y*. The misbehavior of the *Y* chromosome may simply be an indirect consequence of general dysfunction in *Y*-bearing spermatocytes. Alternatively, it could be the signal that triggers the spermiogenic failure, under the hypothesis of a checkpoint sensitive to chromosome behavior at meiosis II, similar to that proposed for mitosis and meiosis I (McKee 1998). The schedule of the failure differs according to the species, since the first cytological defects are observed as early as spermatid elongation in *D. subobscura* (Hauschteck-Jungen and Maurer 1976) and *D. simulans* (Montchamp-Moreau and Joly 1997), but only later, after individualization and coiling, in *D. pseudoobscura* (Novitsky *et al.* 1965).

The *sex-ratio* trait in *D. simulans* was found to be under polygenic control involving both sex chromosomes and autosomes (Cazemajor *et al.* 1997). The genetic dissection of the system is possible because natural populations of *D. simulans* are polymorphic for these genes, which are not trapped within inversions. It should allow new insights into the study of meiosis control, especially regarding chromosome-specific sister chromatid segregation.

We thank A. R. Lohe and A. F. Dernburg for providing the plasmids; S. Rousseau, C. Wourc'h and A. Dernburg for technical advice; F. Lemeunier and P. Guillaud for use of equipment; D. Anxolabéhère, R. Karess, D. Lachaise, D. Poinsot, and S. Ronsseray for critical reading of the manuscript.

LITERATURE CITED

Atlan, A., H. Merçot, C. Landre and C. Montchamp-Moreau, 1997 The *sex-ratio* trait in *Drosophila simulans*: geographic distribution of distortion and resistance. Evolution **51:** 1884–1893.

Ault, J. G., and C. L. Rieder, 1994 Meiosis in *Drosophila* males I. The question of separate conjunctive mechanisms for the *XY* and autosomal bivalents. Chromosoma **103:** 352–356.

Bickel, S. E., and T. L. Orr-Weaver, 1996 Holding chromatids together to ensure they go their separate way. BioEssays **18:** 293–300.

Belmont, A. S., M. B. Braunfeld, J. W. Sedat and D. A. Agard, 1989 Large-scale chromatin structural domains within mitotic and interphase chromosomes in vivo and in vitro. Chromosoma **98:** 129–143.

Cazemajor, M., C. Landre and C. Montchamp-Moreau, 1997 The *sex-ratio* trait in *Drosophila simulans*: genetic analysis of distortion and suppression. Genetics **147:** 635–642.

Cobbs, G., L. Jewell and L. Gordon, 1991 Male-sex-ratio trait in

Drosophila pseudoobscura: frequency of autosomal aneuploid sperm. Genetics **127:** 381–390.

Dernburg, A. F., and J. W. Sedat, 1998 Mapping three-dimensional chromosome architecture *in situ*. Methods Cell Biol. **53:** 187–233.

Erickson, J., 1965 Meiotic drive in *Drosophila* involving chromosome breakage. Genetics **51:** 555–571.

Fuller, M. T., 1993 Spermatogenesis, pp. 71–148 in *The Development of Drosophila melanogaster*, edited by M. Bate and A. Martinez Arias. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Gatti, M., S. Bonnacorsi and S. Pimpinelli, 1994 Looking at *Drosophila* mitotic chromosomes. Methods Cell Biol. **44:** 371–391.

Hauschteck-Jungen, E., and B. Maurer, 1976 Sperm dysfunction in sex-ratio males of *Drosophila subobscura*. Genetica **46:** 459–477.

Hauschteck-Jungen, E., H. Jungen and M. Muller, 1972 Karyotyp und meiose bei wild- und sex ratio-mannchen von *Drosophila subobscura*. Revue Suisse Zool. **79:** 297–305.

Jungen, H., 1968 Sex-ratio in naturlishen populationen von *Drosophila subobscura*. Arch. Jul Klaus-Stift. **43:** 52–57.

Karpen, G. H., J. E. Schaeffer and C. C. Laird, 1988 A *Drosophila* rDNA gene located in heterochromatin is active in transcription and nucleolus formation. Genes Dev. **2:** 1745–1763.

Lifschytz, E., and D. Hareven, 1977 Gene expression and the control of spermatid morphogenesis in *Drosophila melanogaster*. Dev. Biol. **58:** 276–294.

Lohe, A., and P. A. Roberts, 1990 An unusual *Y* chromosome of *Drosophila simulans* carrying amplified rDNA spacer without rRNA genes. Genetics **125:** 399–406.

Lyttle, T. W., 1981 Segregation in XYY males and XXY females of *D. melanogaster*. Dros. Info. Serv. **56:** 87–90.

Lyttle, T. W., 1991 Segregation distorters. Annu. Rev. Genet. **25:** 511–557.

Mange, A. P., 1968 Temperature sensitivity of Segregation Distortion in *Drosophila melanogaster*. Genetics **58:** 399–413.

McKee, B. D., 1984 Sex chromosome meiotic drive in *Drosophila melanogaster* males. Genetics **106:** 403–422.

McKee, B. D., 1998 Pairing sites and the roles of chromosome pairing in meiosis and spermatogenesis in male *Drosophila*. Curr. Top. Dev. Biol. **37:** 77–115.

Merçot, H., A. Atlan, M. Jacques and C. Montchamp-Moreau, 1995 Sex-ratio distortion in *Drosophila simulans*: co-occurrence of a meiotic drive and a suppressor of drive. J. Evol. Biol. **8:** 283–300.

Montchamp-Moreau, C., and D. Joly, 1997 Abnormal spermiogenesis is associated with the X-linked *sex-ratio* trait in *Drosophila simulans*. Heredity **79:** 24–30.

Newton, M. E., R. J. Wood and D. I. Southern, 1976 A cytogenetic analysis of meiotic drive in the mosquito, *Aedes aegyptii* (L.). Genetica **46:** 297–318.

Novitsky, E., W. J. Peacock and J. Engel, 1965 Cytological basis of "sex ratio" in *Drosophila pseudoobscura*. Science **148:** 516–517.

Peacock, W. J., K. T. Tokuyasu and R. W. Hardy, 1972 Spermiogenesis and meiotic drive in Drosophila, pp. 247–268 in *The Genetics of the Spermatozoon* (Proceedings of the International Symposium, Edinburgh, Aug. 16–20), edited by R. A. Beatty and S. Gluecksohn-Waelsch. Departments of Genetics, University of Edinburgh and the Albert Einstein College of Medicine, Edinburgh / New York.

Policansky, D., and J. Ellison, 1970 "Sex ratio" in *Drosophila pseudoobscura*: spermiogenic failure. Science **169:** 888–889.

Rousseaux, S., and E. Chevret, 1995 In-vitro decondensation of human spermatozoa for fluorescence *in-situ* hybridization (FISH). Hum. Reprod. **10:** 2209–2213.

Silver, L. M., and P. Odds-Clarck, 1984 Transmission ratio distortion of mouse *t* haplotypes is not a consequence of wild type sperm degeneration. Dev. Biol. **105:** 250–252.

Stalker, H. D., 1960 The genetic systems modifying meiotic drive in *Drosophila paramelanica*. Genetics **46:** 177–202.

Sweeney, T. L., and A. R. Barr, 1978 Sex-ratio distortion caused by meiotic drive in a mosquito, *Culex pipiens* L. Genetics **88:** 427–446.

Wu, C. I., and A. T. Beckenbach, 1983 Evidence for extensive genetic differentiation between the sex-ratio and the standard arrangement of *Drosophila pseudoobscura* and *Drosophila persimilis* and identification of hybrid sterility factors. Genetics **105:** 71–89.

Yamamoto, M. T., 1992 Inviability of hybrids between *D. melanogaster* and *D. simulans* results from the absence of *simulans X*, not the presence of *simulans Y* chromosome. Genetica **87:** 151–158.

Communicating editor: C.-I Wu