Male Sterility and Meiotic Drive Associated With Sex Chromosome Rearrangements in Drosophila: Role of *X-Y* Pairing

Bruce D. McKee,*,† Kathy Wilhelm,* Cynthia Merrill* and Xiao-jia Ren†

**Department of Biology, University of Wisconsin, Eau Claire, Wisconsin 54702 and †Departments of Zoology, and Biochemistry, Cellular and Molecular Biology, University of Tennessee, Knoxville, Tennessee 37996*

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ABSTRACT

In *Drosophila melanogaster*, deletions of the pericentromeric *X* heterochromatin cause *X-Y* nondisjunction, reduced male fertility and distorted sperm recovery ratios (meiotic drive) in combination with a normal *Y* chromosome and interact with *Y*-autosome translocations (*T(Y;A)*) to cause complete male sterility. The pericentromeric heterochromatin has been shown to contain the male-specific *X-Y* meiotic pairing sites, which consist mostly of a 240-bp repeated sequence in the intergenic spacers (IGS) of the rDNA repeats. The experiments in this paper address the relationship between *X-Y* pairing failure and the meiotic drive and sterility effects of *Xh* deletions. *X*-linked insertions either of complete rDNA repeats or of rDNA fragments that contain the IGS were found to suppress *X-Y* nondisjunction and meiotic drive in Xh^{-}/Y males, and to restore fertility to $Xh^{-}/T(Y;A)$ males for eight of nine tested *Y*-autosome translocations. rDNA fragments devoid of IGS repeats proved incapable of suppressing either meiotic drive or chromosomal sterility. These results indicate that the various spermatogenic disruptions associated with *X* heterochromatic deletions are all consequences of *X-Y* pairing failure. We interpret these findings in terms of a novel model in which misalignment of chromosomes triggers a checkpoint that acts by disabling the spermatids that derive from affected spermatocytes.

ONE of the most intriguing aspects of meiosis in metazoans is the anomalous behavior of sex chromosomes, both with respect to pairing/recombination and gene expression. Heteromorphic sex chromosomes typically pair within very restricted regions of homology that exhibit unusually high pairing/recombination frequencies, the nonhomologous regions being inert with respect to meiotic pairing and recombination. In addition, *X* and *Y* chromosomes of many species are rendered heterochromatic and transcriptionally inert during meiotic prophase when autosomal chromosomes are transcriptionally active (McKee and Handel 1993). Moreover, rearrangements involving the sex chromosomes are often highly disruptive to spermatogenesis in both mammals and Drosophila, leading to reduced fertility, distorted sperm recovery ratios and, in some cases, complete sterility (Lifschytz 1972; Lifschytz and Lindsley 1972; Handel 1987; McKee 1997). The significance of these effects is not known, although it has been suggested that such rearrangements may disrupt a chromosomal-level regulatory process that is essential for the differential behavior of sex chromosomes in spermatogenesis (Lifschytz and Lindsley 1972).

One class of rearrangements that disrupt spermatogenesis consists of deletions that encompass the pairing region of the *X* chromosome. Male mice carrying a deletion of the *X* chromosomal pseudoautosomal region are sterile and exhibit arrest of meiosis (Gabriel-Robez *et al.* 1990). In Drosophila, deletions that encompass most of the centric heterochromatin of the *X* chromosome (*Xh*), which is where the pairing sites are located (McKee 1996), cause *X-Y* pairing failure and nondisjunction at the first meiotic division, non-Mendelian recovery of reciprocal sperm classes in the progeny (meiotic drive), and low fertility (Gershenson 1933; Sandler and Braver 1954; Cooper 1964; Peacock 1965; Peacock *et al.* 1975; McKee and Lindsley 1987).

Previous findings have pointed to a connection between *X-Y* pairing failure and spermiogenic failure in *Xh*-deficient Drosophila males. The levels of meiotic drive and infertility are correlated with the frequency of nondisjunction among males that carry the same *Xh* deficiency but are raised at different temperatures or carry different genetic backgrounds (Peacock *et al.* 1975) and among males that carry different *Xh* deficiencies with different nondisjunction frequencies (McKee and Lindsley 1987). Other than this connection with pairing, the mechanism of sex chromosome meiotic drive has remained obscure. Meiotic drive is not due to any bias in meiosis itself, reciprocal products (*X vs. Y* and *XY vs.* nullo-*XY*) being present in equal frequency at the conclusion of both meiotic divisions (Peacock 1965; McKee and Lindsley 1987). Cytological studies point to both elimination of a fraction of

Corresponding author: Bruce D. McKee, Department of Biochemistry, Cellular and Molecular Biology, F235 Walters Life Sciences Bldg., University of Tennessee, Knoxville, TN 37996.
E-mail: bdmckee@utk.edu

developing spermatids at the individualization stage and failure to function of a fraction of sperm transferred to inseminated females (Peacock *et al.* 1975). Otherwise, sperm development appears reasonably normal in electron microscopic studies, with no gross abnormalities in head development such as are seen in *X*-autosome translocation males or in meiotic drive associated with the *Sd* gene (Tokuyasu *et al.* 1977). Nevertheless, the genetic data indicate selection against relatively chromatin-rich sperm classes, because recovery fractions decrease in the order $O > X > Y > XY$, and presence of other chromosome fragments reduces sperm viability in relation to fragment size (McKee 1984). Presumably, then, relatively chromatin-rich sperm classes are somehow selected against during individualization and/or after transfer to the female.

Xh deletions in Drosophila are also associated with a synthetic male sterility phenotype that is seen when such deletions are combined with otherwise fertile *Y*-autosome translocations (*T(Y;A)*s) (Besmertnaia 1934; Lindsley *et al.* 1979; Lindsley and Tokuyasu 1980) or with the y^+Ymal^+ chromosome, a *Y* chromosome containing a large insertion of proximal *X* chromosome material (Rahman and Lindsley 1981). This synthetic sterility cannot be suppressed by addition of either a free *X* duplication carrying the *X* heterochromatin (Besmertnaia 1934; Lindsley and Tokuyasu 1980; Rahman and Lindsley 1981) or, in the case of the $Xh^-/T(Y;A)$ genotypes, an extra intact *Y* chromosome (Lindsley and Tokuyasu 1980), which indicates that it does not result from insuffiency of any ordinary spermatogenesis genes, but rather from some feature of the rearranged karyotype. *X*-autosome translocations, which also cause male-specific sterility, exhibit a similar dominance, (duplications that cover the breakpoints do not suppress the sterility, Lindsley and Tokuyasu 1980), suggesting that these various cases of "chromosomal sterility" may be mechanistically related. This interpretation is buttressed by the observation that at least some cases of $Xh^-/T(Y;A)$ sterility are associated cytologically with a failure of spermatid nuclear elongation, a phenotype characteristic also of *X*-autosome translocation sterility (Lindsley and Tokuyasu 1980). Despite several concerted genetic attacks (Lifschytz and Lindsley 1972; Rahman and Lindsley 1981; Lyttle 1984; Stone 1984), the underlying mechanism of chromosomal sterility has remained obscure.

Previous work in our laboratory has focused on the role of sex chromosome pairing in the phenotypes associated with *Xh* deletions. We have mapped the *X-Y* pairing site to a small (240-bp) repeated sequence located in the intergenic spacers (IGS) of the rDNA repeats in central *Xh*. Transgenic insertions of either complete rDNA repeat units (McKee and Karpen 1990) or fragments containing only arrays of IGS repeats (McKee *et al.* 1992; Merrill *et al.* 1992; Ren *et al.* 1997) on a heterochromatically deficient *X* chromosome partially restore pairing competence and substantially reduce nondisjunction. These effects are largely independent of location within the euchromatin; comparable levels of rescue are seen with insertions of the same construct at a variety of sites (McKee and Karpen 1990; McKee *et al.* 1992; Merrill *et al.* 1992). Insertions of complete rDNA repeats on a heterochromatically deficient *X* also ameliorate meiotic drive (McKee and Karpen 1990), consistent with the idea that meiotic drive results from failure of *X-Y* pairing. However, complete rDNA repeats have other capabilities besides promoting *X-Y* pairing, most notably mediating nucleolus formation and contributing to the pool of rRNAs (Karpen *et al.* 1988). It is not known whether the suppression of meiotic drive by complete rDNA insertions is related to the ability of such insertions to suppress *X-Y* pairing failure or to other properties of rDNA. It is also not known what effect, if any, rDNA insertions have on synthetic sterility in males carrying both *Xh* deficiencies and *Y*-autosome translocations, although we have shown that rDNA transgenes suppress the chromosomal sterility resulting from combining an *Xh* deletion with the y^+Ymal^+ chromosome (McKee 1991).

The purpose of the experiments reported below is to address these unanswered questions concerning the apparent link between *X-Y* pairing and normal spermiogenesis. Transgenic insertions containing fragments of rRNA genes are tested for their ability to rescue meiotic drive and improve fertility in Xh^- males carrying a normal *Y*. Inserts that contain only IGS repeats cannot form nucleoli (McKee *et al.* 1992) or generate functional rRNAs but can mediate *X-Y* pairing, so this comparison tests for a direct association between *X-Y* pairing and normal spermiogenesis. In addition, rDNA insertions, both complete and fragmentary, are tested for rescue of the synthetic sterility associated with combining an *Xh* deletion with a variety of *Y*-autosome translocations. The results of these tests establish conclusively that the spermiogenic disruptions associated with deletion of *Xh* are indeed consequences of *X-Y* pairing failure.

These data suggest an intriguing link between prophase/metaphase of meiosis I, when the pairing phenotype is manifested, and the late stages of spermiogenesis, when many normal-appearing spermatids are eliminated. The possible nature of this link is discussed in terms of a novel model for a metaphase checkpoint that monitors chromosome alignment and that responds to misaligned chromosomes by disabling the spermatids that derive from the affected spermatocytes.

MATERIALS AND METHODS

Chromosomes: *Df(1)X-1* is a large heterochromatic deficiency with breakpoints proximal to the nucleolus-organizer (NO) and in the proximal euchromatin distal to or in *l(1)20Cb* in 20F (Lindsley and Zimm 1992). It is deficient for all of the rDNA and for all of the *X* chromosomal pairing sites

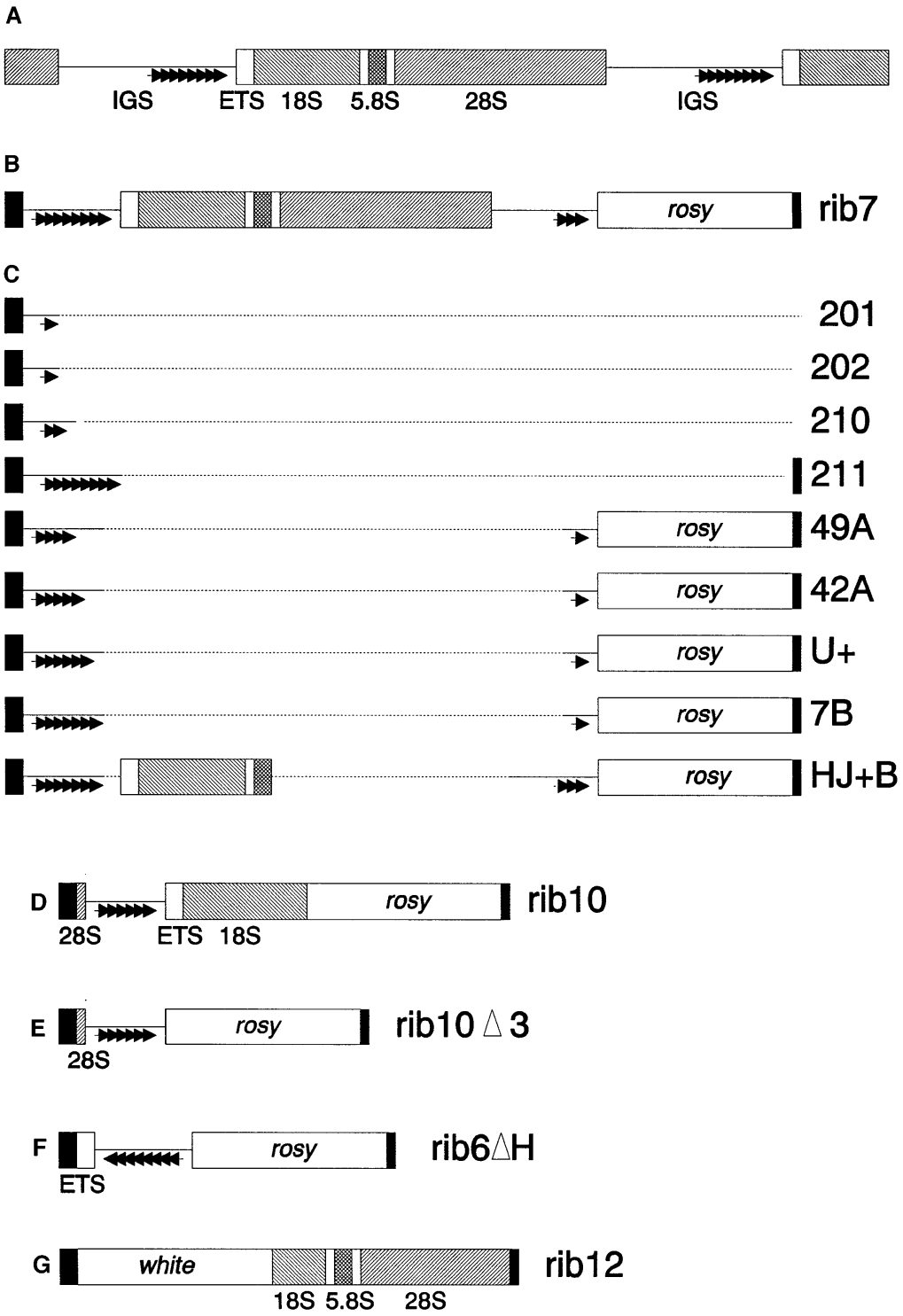

Figure 1.—Structure of rDNA repeat and insertions of rDNA fragments. (A) rDNA repeat. Transcription unit represented by rectangles and intergenic spacer (IGS) by a line. Arrowheads represent 240-bp repeats in IGS. ETS, external transcribed spacer. (B) Structure of [rib7]. Filled rectangles, *P* element sequences; open rectangle, *rosy*$^+$ eye-color marker; dotted lines represent deletions. Other symbols as in A. (C) Structures of *in vitro* deletions made from [rib7](1A1-4). (D–G) Structures of rDNA fragment vectors. Symbols as in A and B.

(McKee and Lindsley 1987). B^SYy^+ is a *Y* chromosome containing two small duplications of *X* material—the 1A1-B1 region of the *X* is appended to the tip of *YS*, and a fragment of proximal *X* is appended to the tip of *YL* (Lindsley and Zimm 1992). The *Y*-autosome translocations used in this study were all generated in a stock containing B^SYy^+. Details of their construction are in Lindsley *et al.* (1972). The breakpoints of the sample used in this study are given in the text and in Table 2. The structures of the rDNA insertions used in this study are summarized in Figure 1.

Progeny ratio and fertility tests: Males were placed singly with two virgin females from the *y w* stock in shell vials containing standard cornmeal-molasses agar. They were incubated at 23° for five days, then transferred to a fresh vial for seven more days, at which point they were discarded. All of the F_1 progeny in both vials were counted and (in the case of progeny ratio tests) scored for sex and eye shape.

Parameters and statistics: Meiotic drive levels are quantified by the parameters R_X and R_Y which measure the viability of *X*-bearing sperm (or *Y*-bearing sperm) relative to otherwise iden-

tical sperm that lack the *X* (or *Y*). The formulas are: $R_X = (O_X O_{XY}/O_Y O_O)^{1/2}$ and $R_Y = (O_Y O_{XY}/O_X O_O)^{1/2}$ where O_X, O_Y, O_{XY} and O_O are the numbers of *X*-, *Y*-, *XY*- and nullo-*XY*-bearing sperm (respectively) recovered among the progeny. The disjunction frequency (the frequency with which the *X* and *Y* go to opposite poles at anaphase I) is measured by the parameter *P*; the formula is $P = 1/(1 + (O_{XY}O_O/O_X O_Y)^{1/2})$. See McKee and Lindsley (1987) and McKee and Karpen (1990) for derivations. *P*, R_X and R_Y all equal 1 in chromosomally normal males. *P* takes a minimum value of 0.5 in males in which the *X* and *Y* fail to pair and disjoin randomly. R_X and R_Y normally range between 0 and 1. Parameters were compared by means of *z*-tests as described (McKee and Karpen 1990). Male fertility (*F*) was calculated as the number of progeny divided by the number of tested males (sterile as well as fertile).

RESULTS

Rescue of partial sterility and of sperm ratio distortion by insertion of pairing site sequences: The data in Table 1, line A1 illustrate the meiotic and spermiogenic abnormalities associated with *Xh* deletions. The males carried *Df(1)X-1*, a large *Xh* deletion with breakpoints proximal to the nucleolus organizer and in the proximal euchromatin, and completely deficient for the rDNA, and $B^S Yy^+$, a *Y* chromosome marked with two translocated segments from the *X*, including the dominant *Bar-eye* mutation. These males were crossed to chromosomally normal females and their progeny scored for Bar and Bar^+ males and females. Three abnormalities are evident. First, there are relatively few progeny, 13.7 per male compared to an average of 121.1 progeny per wild-type male (line A2) under these test conditions. Second, there is a great deal of *X-Y* nondisjunction, reflected in recovery of substantial numbers of *XY* and nullo-*XY* sperm. This results from failure of *X-Y* pairing in most or all primary spermatocytes and subsequent random assortment of the unpaired univalents (McKee and Lindsley 1987). When meiotic products are scored cytologically, regular and nondisjunctional products are equally frequent, indicating that pairing failure is virtually complete and that the *X* and *Y* segregate at random. Third, reciprocal meiotic products are recovered unequally. The nullo-*XY* nondisjunctional sperm are recovered approximately 50-fold more frequently than the *XY* nondisjunctional sperm and the *X* sperm are recovered fourfold more frequently than the *Y* sperm.

A partial restoration of both *X-Y* pairing/disjunction and normal sperm recovery ratios results from single (Table 1B, 1 and 2) and double (Table 1B, 3 and 4) insertions of complete rDNA repeats on the Xh^- chromosome (as reported in McKee and Karpen 1990). Disjunction percentages improve to 60–64% for the single insertion lines and to 77% for the double insertion lines (measured cytologically). Accompanying these increases in meiotic disjunction are marked improvements in male fertility (39.7–44.4 progeny per male for the single insertion lines and 59.9–74.7 progeny per male for the double insertion lines), and in recovery of reciprocal meiotic products. The latter phenotype is conveniently quantified by the parameters R_X and R_Y, which measure the viability of *X*-bearing and *Y*-bearing sperm classes, respectively, relative to otherwise identical sperm lacking those chromosomes. The underlying model and calculations are described in materials and methods and in McKee and Lindsley (1987). *Y* chromosome recovery is particularly depressed in *Df(1)X-1* males, reflected in an R_Y value of only 0.076. This value increases to 0.44–0.47 in the presence of one rDNA repeat and to 0.63–0.79 in the presence of two. Similar changes in *X* chromosome recovery are also evident.

If the improvements in male fertility and in recovery of reciprocal meiotic products are due to restoration of *X-Y* pairing, then rDNA fragments that stimulate pairing should also ameliorate these spermiogenic abnormalities while rDNA fragments that do not stimulate *X-Y* pairing should not. *X-Y* pairing is stimulated by rDNA insertions that contain intact IGS regions, even if none of the rDNA transcription unit is present (McKee *et al.* 1992; Merrill *et al.* 1992). Conversely, a large fragment of the transcription unit with no IGS is devoid of pairing ability (Ren *et al.* 1997). Most of the IGS-containing fragments, *e.g.*, lines 211 and 7B, Table 1C, 4 and 5, show clear-cut stimulation of recovery of both the *X* and *Y* chromosomes as well as enhanced fertility. The only exceptions are those with very small IGS fragments, those with fewer than six 240bp IGS repeats, *e.g.*, lines 201, 202, and 210, Table 1C, 1–3. These same fragments also fail to stimulate pairing (McKee *et al.* 1992; Merrill *et al.* 1992). The insertions of the transcription unit fragment without IGS (Table 1G, 1 and 2) have no detectable effect on recovery of either the *X* or *Y* and do not improve male fertility. Thus there is a very strong correlation between the ability to stimulate *X-Y* pairing, on the one hand, and the ability to normalize *X* and *Y* recovery ratios and improve male fertility on the other.

These correlations are evident from Figure 2, which shows male fertility (Figure 2A) and *Y* chromosome recovery (Figure 2B) as a function of the probability of *X-Y* disjunction (which in turn is a direct function of *X-Y* pairing), using the data from Table 1. This plot clearly shows that the pairing and spermiogenesis variables are quantitatively as well as qualitatively related; *i.e.*, the level of rescue of *Y* chromosome recovery is dependent on the degree to which a fragment rescues *X-Y* pairing. In addition, both male fertility and sperm recovery ratios are correlated with copy number of 240-bp IGS repeats present in the insertion (Figure 2, C and D). These correlations are expected since the *X-Y* pairing frequency is a function of *X* chromosomal copy number of 240bp repeats (McKee *et al.* 1992; Merrill *et al.* 1992) and since *X-Y* pairing/disjunction is correlated with both male fertility and sperm recovery ratios.

Although it is not possible to rule out position effects completely, it is evident that IGS insertions at a variety of *X* chromosomal sites are competent to suppress both the pairing and sperm recovery defects of *Xh* deficiencies. Some of the quantitative variations, such as the rela-

TABLE 1

Progeny ratios and fertility of *Df(1)X-1/B^{S}Yy^{+}* males carrying rDNA insertions

Insertion name[a]	Ref.[b]	No. of 240-bp repeats	No. of males	Sperm classes				F[c]	P[d]	R_X[e]	R_Y[f]
				X	*Y*	*XY*	*O*				
A. Controls											
1. No insertion	1	0	102	2713	717	168	7630	13.7	0.51	0.29	0.076
2. Normal *X*		>1000	27	1651	1612	2	6	121.1	0.997	1.02	0.98
B. [rib7] insertions											
1. 1A1-4	2	11	148	2572	1693	523	1771	44.4	0.64	0.67	0.44
2. 1A5-8	2	11	196	3138	1930	712	1992	39.7	0.60	0.76	0.47
3. (1A1-4)×2	2	22	102	2823	1745	776	767	59.9	0.77	1.30	0.79
4. 1A+2EF	2	22	35	1137	729	285	463	74.7	0.77	0.98	0.63
C. Deletions derived from [rib7](1A1-4)											
1. 201	3	1	165	1031	345	76	2534	24.2	0.51	0.30	0.10
2. 202	3	1	194	1649	534	151	4335	34.4	0.48	0.34	0.11
3. 210	3	2	141	1650	648	212	2969	38.8	0.52	0.43	0.17
4. 211	3	8	59	1092	473	117	883	43.5	0.67	0.55	0.24
5. 7B	3	8	166	2330	813	188	3073	38.6	0.62	0.42	0.15
6. 42A	3	5–6	131	1543	697	210	3053	42.0	0.53	0.39	0.18
7. U+	3	7	168	1119	331	69	2318	22.8	0.56	0.32	0.09
8. 49A	3	5	114	556	197	38	1926	23.8	0.51	0.24	0.08
9. HJ+B	3	10	38	1596	514	115	804	79.7	0.80	0.67	0.34
D. [rib10] insertions											
1. 12F-1	4	6	217	2487	757	150	3544	32.0	0.57	0.37	0.11
2. 12F-2	4	12	194	7929	3537	756	5020	88.9	0.73	0.58	0.26
3. 16AB	4	6	212	2787	1164	312	3876	38.4	0.53	0.44	0.18
E. [rib10Δ3] insertions											
1. 14D	4	6	49	856	352	119	1078	49.1	0.59	0.52	0.21
2. 1A-1	4	1	58	514	136	29	1138	31.3	0.55	0.31	0.082
3. 1A-2	4	11	39	834	603	204	558	56.4	0.72	0.71	0.51
F. [rib6ΔH] insertions											
1. 1F	4	8	47	476	134	26	1011	35.0	0.48	0.30	0.085
2. 5CD	4	8	53	412	100	16	1207	32.7	0.51	0.23	0.056
3. 18AB	4	8	48	102	19	7	535	13.8	0.51	0.26	0.049
4. 6E+12B	4	16	51	1275	490	121	1545	67.3	0.64	0.45	0.17
G. [rib12] insertions											
1. 6F	5	0	NK[g]	798	203	64	2163	NK[g]	0.55	0.34	0.087
2. 7C	5	0	NK[g]	1115	269	46	2971	NK[g]	0.50	0.25	0.061

[a] Cytological locations of insertions are given.

[b] References: 1. McKee and Lindsley 1987; 2. McKee and Karpen 1990; 3. McKee *et al*. 1992; 4. Merrill *et al*. 1992; 5. Ren *et al.* 1997.

[c] Fertility (F) = no. of progeny per male.

[d] *X-Y* disjunction (P) = fraction of meiosis I segregations with *X* and *Y* to opposite poles, calculated from cytological data. See materials and methods for formula.

[e] *X* chromosome recovery (R_X) = viability of *X*-bearing sperm relative to genotypically identical sperm without the *X*. See materials and methods for formula.

[f] *Y* chromosome recovery (R_Y) = viability of *Y*-bearing sperm relative to genotypically identical sperm without the *Y*. See materials and methods for formula.

[g] Not known.

tively weak effect of the double insertion of [rib6ΔH] on *X* and *Y* recovery (Table 1F, 4) despite a relatively high IGS repeat copy number, could result from position effects. Nevertheless, Figure 2 shows that such effects do not obscure the relationship between IGS repeat copy number and sperm viability.

Rescue of *Xh*$^{-}$*/T(Y;A)* sterility by rDNA insertions: Most *Y*-autosome translocations that are male-fertile in an otherwise normal genotype have proven to be male-sterile in combination with *X* heterochromatic deficiencies (Lindsley *et al.* 1979; Lindsley and Tokuyasu 1980). To determine if this synthetic sterility is also sensitive to the presence of pairing sites on the *X*, we compared the fertility of males carrying a *Y-2* or *Y-3* translocation along with the *Xh* deficiency *Df(1)X-1* with or without an insertion of the [rib7] transposon which

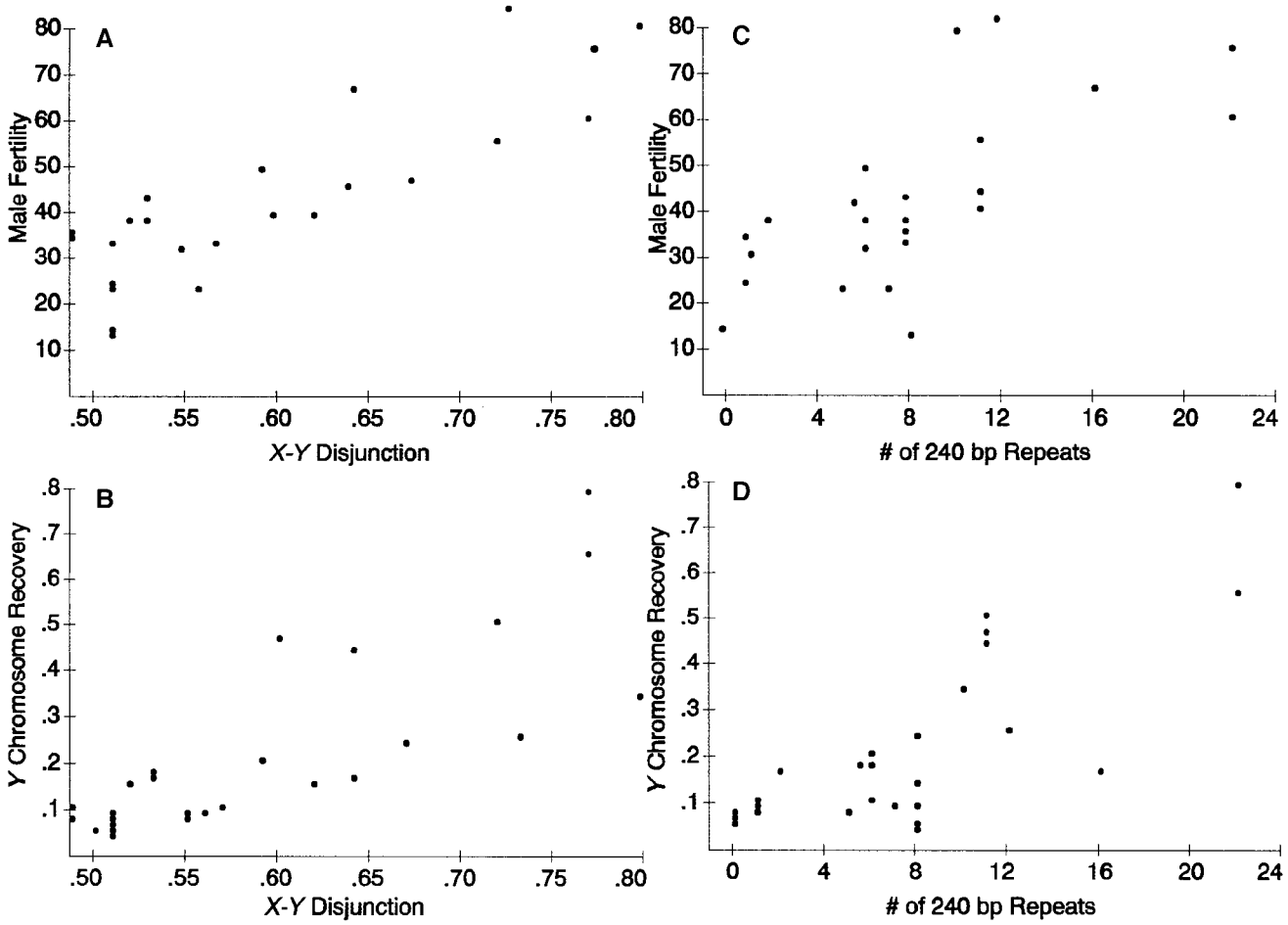

Figure 2.—Male fertility (A and C) and *Y* chromosome recovery (B and D) as a function of *X-Y* disjunction fraction (A and B) or copy number of 240-bp IGS repeats (C and D). Males carried *Df(1)X-1* with the ribosomal insertions listed in Table 1 and illustrated in Figure 1, and $B^{S}Yy^{+}$. The disjunction fraction (*P*), fertility (*F*) and *Y* chromosome recovery (R_Y) are from Table 1.

carries a single complete rDNA repeat. Four *Y-3* and five *Y-2* translocations were tested this way. Most of the extant *Y-2* and *Y-3* translocations could not be used in this test either because they have one or more mutations in essential fertility genes or because the B^S marker has been lost from the Y, rendering the test genotype lethal (*Df(1)X-1* is deficient for several essential loci from the proximal *X* that are present in the B^S duplication on the *Y* chromosome ($B^{S}Yy^{+}$) used to make most of these translocations).

For seven of the nine tested translocations, the presence of a single rDNA repeat on the Xh^{-} chromosome provided substantial fertility rescue (Table 2 and Figure 3). For all nine translocations, the combination of the translocation and the X heterochromatic deficiency without an rDNA insertion (open bars in Figure 3) was associated with complete or nearly complete sterility—the majority of males in all cases were completely sterile and total fertility amounted to less than one offspring per tested male. In the presence of a single copy of [rib7] (cross-hatched bars in Figure 3), fertility improved for seven of the nine translocations to between 9.86 and 46.8 progeny per male and the percent fertile males improved to between 65.2% and 92.6%. The exceptions were *T(Y;2)B177* which remained completely sterile in the presence of [rib7](1A1-4), and *T(Y;2)H158*, which exhibited only marginal improvement in fertility.

The effect of a double insertion of [rib7] on fertility was determined for the *Y-2* translocations (filled bars in Figure 3). Only in one case, that of *T(Y;2)H158*, was there a stronger response to two than to one insertion. This translocation responded only weakly to one insertion, but dramatically to two—fertility improved to 1.35 progeny per male with one insertion but to 35.8 progeny per male with two insertions. In the other four cases, the effect of a double insertion was indistinguishable from that of a single insertion. *T(Y;2)B177* remained completely sterile in the presence of one or two copies of [rib7]. The three *Y-2* translocations that responded dramatically to a single [rib7] insertion (*P59*, *H143*, and *B80*) responded approximately equally to two [rib7] insertions; in these cases, a single insertion is as effective as two. Thus a second rDNA insertion can improve fertility but apparently only in cases in which the effect of a single insertion is marginal.

Thus only one of the nine *Y*-autosome translocations,

TABLE 2

Fertility of males carrying *Xh* deletion and a *Y*-autosome translocation with and without rDNA repeats

T(Y;A) (breakpoint)	rDNA insertion	No. of males	Percent fertile	Fertility
B177 (*YL*;41)	None	47	0	0
	[rib7]	48	0	0
	[rib7]×2	50	0	0
H158 (*Xhy*$^+$;58D)	None	52	0	0
	[rib7]	17	35.3	1.35
	[rib7]×2	28	85.7	35.8
P59 (*Xhy*$^+$;59B)	None	39	30.8	0.38
	[rib7]	46	65.2	31.1
	[rib7]×2	40	80.0	32.5
H143 (*BSXh*;h14;59F)	None	58	13.8	0.15
	[rib7]	44	93.2	46.8
	[rib7]×2	42	100	36.5
B80 (*Xhy*$^+$;h21;60F)	None	44	0	0
	[rib7]	35	71.4	16.7
	[rib7]×2	35	74.2	19.9
B240 (*Xhy*$^+$;94B)	None	50	2.0	0.02
	[rib7]	57	91.2	12.7
H173 (*Xhy*$^+$;95E)	None	64	10.9	0.20
	[rib7]	50	76.0	9.86
H163 (*YL*;98B)	None	62	14.5	0.31
	[rib7]	26	88.5	31.0
R133 (*BSXh*;99E)	None	49	0	0
	[rib7]	27	92.6	18.8

All males carried *Df(1)X-1*. [rib7] is [rib7](1A1-4); [rib7]×2 is [rib7](1A)×2. Males were crossed singly with two *y w* females. Fertility is the number of progeny per male (counting both fertile and sterile males).

T(Y;2)B177, proved completely recalcitrant to fertility rescue by inserted rDNA genes. *T(Y;2)B177* is also the only translocation among the nine tested that does not have a relatively distal autosomal breakpoint; its second chromosome breakpoint in the proximal heterochromatin of chromosome arm 2R is much more proximal than those of the other eight. The possible significance of this difference is discussed below.

Rescue of fertility of *Xh*$^-$ / *T(Y;A)* males by rDNA fragments containing IGS repeats: If the stimulatory effect of [rib7] insertions on fertility of *Xh*$^-$ / *T(Y;A)* males is due to improved pairing between the *Xh*$^-$ chromosome and the translocated *Y*, then other insertions that improve *X-Y* pairing should also restore fertility to these males. As noted above, rDNA fragments that contain six or more 240-bp IGS repeats have been found to promote pairing between the *Y* and a *Xh*$^-$ chromosome. Consequently, several IGS repeat-containing rDNA insertions were tested for ability to rescue fertility of males carrying the sterilizing combination of *Df(1)X-1* and *T(Y;2)B80*. Included in the sample were several *P*-induced deletions from [rib7](1A1-4) (Table 3C) and three insertions, two single and one double (12F-2), of the [rib10] transposon (Table 3D). All of the [rib7] deletions retain at least some 240-bp repeats, the copy number ranging from 2 (210) to 10 (HJ+B). All but HJ+B, which retains the majority of the rDNA transcription unit, contain 240-bp repeats only, the remainder of the rDNA having been deleted. The [rib10] construct includes an IGS with six 240-bp repeats plus about 2 kb from the 5′ end of the rDNA transcription unit. The two single insertions thus contain six 240-bp repeats and the double insertion has 12. Four of the fragments in Table 3C and D—the [rib7] deletions 7B, HJ+B and 211 and the [rib10] double insertion 12F-2—strongly stimulate *X-Y* pairing in nontranslocated genotypes (Table 1). Three others, the [rib7] deletion U$^+$ and the two single insertions of [rib10], stimulate *X-Y* pairing weakly, while the [rib7] deletions 49A and 210 are among those with no effect on *X-Y* pairing (Table 1). As discussed previously, these differences in pairing efficacy correlate well with copy numbers of 240-bp repeats.

The results of the fertility tests, which are reported in Table 3 and displayed graphically in Figure 4, show that IGS repeats can provide at least partial fertility rescue for *Xh*$^-$ males carrying *T(Y;2)B80*. The four fragments with strong effects on *X-Y* pairing also had the strongest effects on fertility. In all four cases, the percent fertile males improved substantially (to as high as 70 with HJ+B) and overall fertility improved to greater than

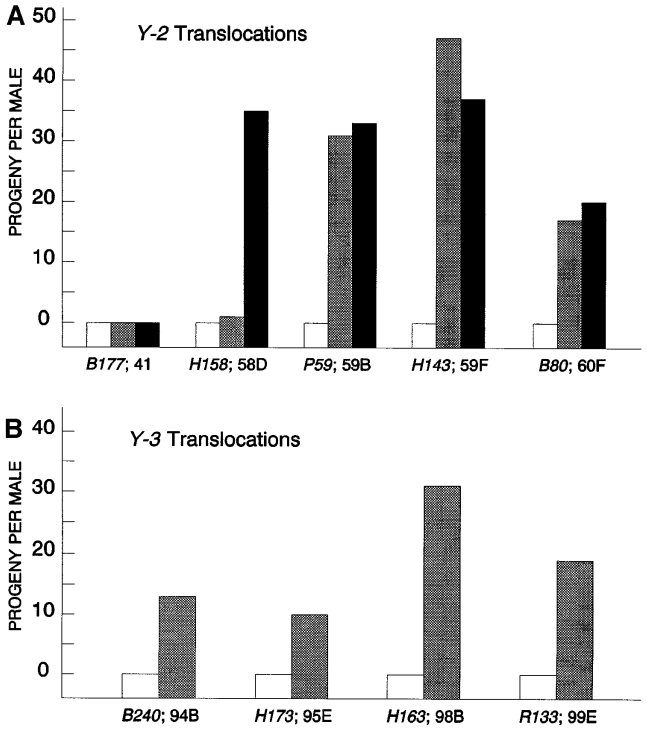

Figure 3.—Rescue of *Xh*$^{-}$ / *T(Y;A)* fertility by insertions of complete rDNA repeats. (A) *Y-2* translocations. (B) *Y-3* translocations. All males carry *Df(1)X-1.* Open bars, no rDNA insertion. Hatched bars, a single complete rDNA repeat ([rib7](1A1-4)). Filled bars, two complete rDNA repeats ([rib7](1A×2)).

one offspring per male, the highest, 6.7, again being HJ+B. The other five fragments had little effect on male fertility; in all cases total fertility remained below one offspring per male. A relationship between the effects of these insertions on fertility of *Xh*$^{-}$ / *T(Y;2)B80* males and the copy number of 240-bp repeats is clear from the graph in Figure 4A; a similar relationship between the fertility effect of the insertions and their effect on *X-Y* disjunction is evident from Figure 4B.

The effects of the rDNA fragments on fertility of *Df(1)X-1* / *T(Y;2)B80* males are not as dramatic as the effects of complete rDNA repeats. As shown in Table 3B, single or double insertions of [rib7] restore fertility to the range of 16–20 progeny per male, whereas the most effective rDNA fragment, HJ+B, stimulates fertility only to 6.7 progeny per male. It is not clear why complete repeats and fragments differ in their quantitative effects. rDNA fragments can be as effective (or even more effective in the case of HJ+B) as complete rDNA repeats in stimulating *X-Y* pairing and disjunction. Thus this result might imply that other segments of the rDNA besides the IGS contribute to the fertility-stimulating effect. Alternatively, since the complete rDNA repeats and the rDNA fragments were tested at different times, some uncontrolled background variable might account for the difference.

DISCUSSION

Pairing sites and sperm dysfunction: *X* heterochromatic deficiencies are associated with three different phenotypes related to male meiosis and spermatogenesis: elevated rates of *X-Y* pairing failure and nondisjunction, distorted sperm recovery ratios (meiotic drive) associated with reduced fertility, and male sterility when

TABLE 3

Fertility of males carrying *T(Y;2)B80* and *Df(1)X-1* with *X*-linked insertions of various rDNA fragments

rDNA insertion	No. of 240-bp repeats	No. of males	Percent fertile	Fertility
A. None	0	44	0	0
B. Complete rDNA repeats				
[rib7](1A1-4)	11	35	71.4	16.7
[rib7](1A)×2	22	35	74.2	19.9
C. [rib7](1A1-4) deletions				
49A	5	50	0	0
U+	7	53	22.6	0.64
7B	8	55	9.1	1.38
HJ+B	10	47	70.2	6.7
210	2	53	5.7	0.28
211	8	57	57.9	2.8
D. [rib10] insertions				
12F-1	6	14	7.1	0.07
12F-2	12	49	40.1	3.2
16AB	6	37	5.4	0.12

Males carrying *Df(1)X-1* and *T(Y;2)B80* along with the indicated rDNA insertions were crossed singly to two *y w* females. Fertility is the number of progeny per male, counting both fertile and sterile males.

combined with certain other types of rearrangements, such as *Y*-autosome translocations or the y^+Ymal^+ chromosome (McKee 1997). The meiotic drive and sterility phenotypes involve extensive spermatid lethality and sperm dysfunction at a variety of stages during spermiogenesis. Previous findings suggested that these phenotypes might be mechanistically interrelated. In males carrying the partial *Xh* deficiency *Df(1)sc4-sc8*, variations in *X-Y* nondisjunction due to background genotype or rearing temperature are correlated (positively) with the severity of distortion (Peacock 1965; Peacock *et al.* 1975). A similar correlation is evident across a sample of *Xh* deficiencies that vary in size (McKee and Lindsley 1987). Moreover, there is an excellent correspondence among *Xh* deficiencies between elevated levels of *X-Y* nondisjunction and meiotic drive in conjunction with a normal *Y* and sterility in conjunction with y^+Ymal^+

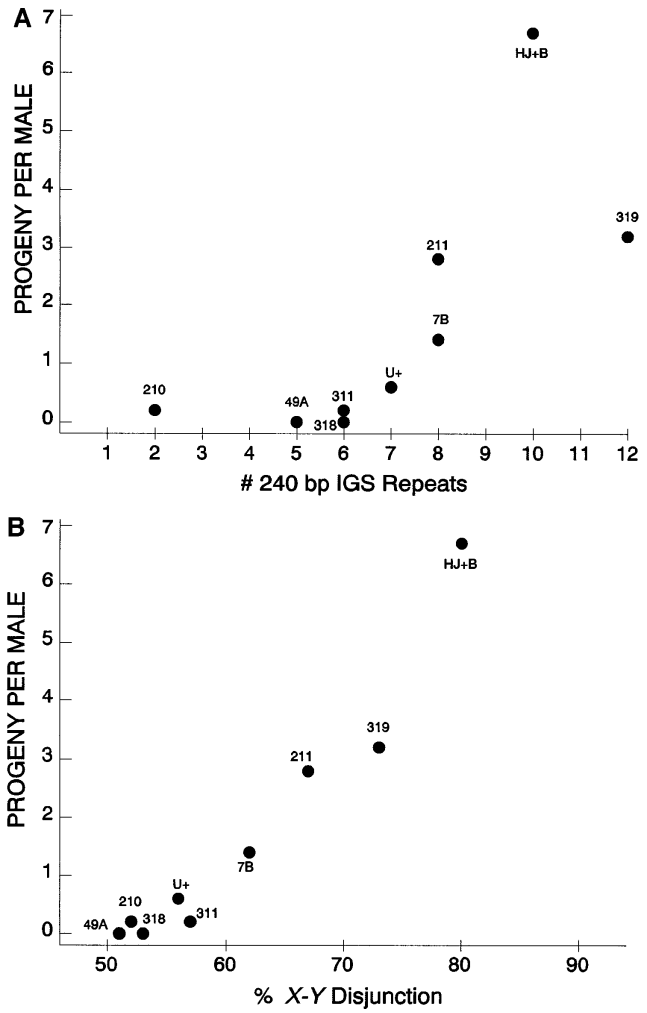

Figure 4.—Rescue of $Xh^-/T(Y;A)$ fertility by insertions of rDNA fragments. Males carry *Df(1)X-1* and *T(Y;2)B80* plus the indicated rDNA fragment. All fragments are deletion derivatives of [rib7](1A1-4) except for 311, 318, and 319 which are the [rib10] insertions 12F-1, 16AB, and 12F-2, respectively. (A) Progeny per male plotted against copy number of 240-bp IGS repeats. (B) Progeny per male plotted against %*X-Y* disjunction, measured cytologically.

(Rahman and Lindsley 1981; McKee and Lindsley 1987). The idea that both *X-Y* nondisjunction and meiotic drive result from deletion of the same locus received strong support from evidence that transgenic insertions of single, complete rDNA repeats on a heterochromatically deficient *X* chromosome partially suppressed both the nondisjunction and meiotic drive phenotypes (McKee and Karpen 1990). Moreover, the same insertions also suppressed sterility in $Df(1)X\text{-}1/y^+Ymal^+$ males (McKee 1991). These findings led to the suggestion that meiotic drive and chromosomal sterility result from deletion of the *X-Y* pairing sites, *i.e.*, that these phenotypes are consequences of *X-Y* pairing failure.

The present study addressed two unanswered questions related to this idea: whether the sterility associated with $Xh^-/T(Y;A)$ genotypes is, like that of Xh^-/y^+Ymal^+ males, suppressible by rDNA insertions; and whether the ability to suppress meiotic drive and sterility localizes to the pairing site region of rDNA repeats, which has been shown to correspond to the 240-bp IGS repeats (McKee *et al.* 1992; Merrill *et al.* 1992). The results presented herein answer both questions in the affirmative. First, complete rDNA repeats restored fertility to *Df(1)X-1*-bearing males carrying eight of nine tested *Y*-autosome translocations. Second, only rDNA fragments containing pairing sites (those with six or more copies of the 240-bp IGS repeats) were able to suppress meiotic drive and *T(Y;A)* sterility. Thus, these data indicate that all of the spermatogenic defects associated with *X* heterochromatic deficiencies can be at least partially reversed by insertions of defined sequences known to function as *X-Y* pairing sites. Since the IGS arrays are not competent by themselves to mediate other functions associated with complete rDNA genes, such as forming nucleoli (McKee *et al.* 1992) and contributing to the pool of ribosomal RNAs, the conclusion that meiotic drive and synthetic sterility are prevented by restoring *X-Y* pairing seems inescapable.

A sperm dysfunction "syndrome" related to chromosomal pairing failure: The results of this study strengthen the association between sex chromosome meiotic drive and chromosomal sterility. These phenomena have generally been treated separately, in part because of different cytological phenotypes: failure of sperm head elongation in *X-A* and *Y-A* translocation sterility (Shoup 1967; Lindsley and Tokuyasu 1980) *vs.* elimination of spermatids with elongated heads during individualization and reduced function of sperm in female storage organs in cases of meiotic drive (Peacock *et al.* 1975; Dernburg *et al.* 1996). However, the fact that the cases both of meiotic drive and of chromosomal sterility associated with *Xh* deletions can be rescued by insertions of pairing sites argues that they share a common mechanism. This finding is consistent with other genetic commonalities between the phenomena, especially the overlap in the types of responsible rearrangements (*e.g., X-2* and *X-3* translocations cause sterility, but *X-4* translocations

cause meiotic drive) and the fact that both phenotypes are cis-dominant (cannot be suppressed by transduplications of *Xh* or of material that covers *Y* or *X* breakpoints). (See McKee 1997, for more detailed review).

In addition, two phenotypic observations suggest a close relationship between *Xh*-deficiency-induced meiotic drive and chromosomal sterility. First, the time of appearance of the cytological abnormalities is correlated with severity of phenotype; failure of sperm head elongation, a relatively early defect, is associated with complete sterility, while failure of individualization and dysfunction of transferred sperm, both late effects, are associated with the less severe meiotic drive genotypes. Second, the severity of meiotic drive is inversely correlated with fertility. In this paper it was shown that *Df(1)X-1* / B^sYy^+ males average only 14 progeny each and a substantial fraction are completely sterile. Under the same conditions, in which each male is mated to two females and eggs are sampled over 20 days, wild-type males produce an average of more than 100 progeny. Single rDNA insertions increase the fertility of *Df(1)X-1* / B^sYy^+ males to more than 40 progeny each while also improving *Y* chromosome recovery from less than 10% to over 40%. A similar inverse correlation between fertility and severity of meiotic drive has been documented in *Df(1)sc4-sc8* males raised at different temperatures (Peacock *et al.* 1975).

In light of these similarities, we suggest that meiotic drive and sterility represent different levels of a common sperm dysfunction syndrome associated with a variety of sex chromosome rearrangements. According to this view, chromosomal sterility would be seen as an extreme case of the sperm dysfunction and infertility present in milder form in meiotic drive genotypes.

Meiotic errors and sperm dysfunction: A checkpoint hypothesis: *Sperm dysfunction as a regulatory response to meiotic misbehavior:* The most fundamental question with respect to the observations reported in this paper is, Why should sex chromosome pairing failure disrupt sperm development? Previous attempts to answer this question have treated meiotic drive as a *direct* consequence of pairing failure. Unsaturated pairing sites on the *Y* chromosome were postulated to function later in development as spermatid lethals (Baker and Carpenter 1972; Peacock and Miklos 1973). This idea was subsequently generalized to explain many cases of rearrangement-associated sterility (Miklos 1974). However, saturation of the *Y* chromosomal pairing sites by adding a heterochromatic free *X* duplication to an Xh^-/Y genotype does not suppress either meiotic drive (McKee 1984; McKee and Lindsley 1987) or chromosomal sterility (Besmertnaia 1934) so the relationship is unlikely to be so direct. Moreover, in some examples of chromosomal sterility, such as simple *X-A* and *Y-A* translocations (Lifschytz and Lindsley 1972; Kennison 1983), there is no evidence for and no reason to suspect unsaturated pairing sites as being the culprit.

We suggest instead that the sperm dysfunction associated with sex chromosome rearrangements in Drosophila is an outcome of a checkpoint concerned with proper chromosome segregation that selects against spermatocytes containing chromosomes that are misaligned in some way. Triggering of this postulated checkpoint would result in a general disabling of the spermatids that derive from the error-containing spermatocytes. Depending on degree, the disability could lead to abortion of spermatid development at an early or late stage or to production of mature but nonfunctional or subfunctional sperm.

A checkpoint concerned with chromosome alignment at metaphase has recently been documented in grasshopper and mantid spermatocytes as well as in mitotically dividing mammalian cells, which respond to the presence of univalents or other mono-oriented chromosomes by delaying the onset of anaphase until the alignment problem is fixed. In both insect spermatocytes and mammalian somatic cells, the delay is apparently triggered by a signal emitted from the kinetochores of misaligned chromosomes. The signal is associated with a phosphoepitope and its extinction is dependent on the tension associated with stable orientation of either sister kinetochores (in mitosis) or homologous kinetochores (in meiosis) to opposite poles (Li and Nicklas 1995; Nicklas *et al.* 1995; Rieder *et al.* 1995). Drosophila spermatocytes evidently lack the "wait anaphase" response since meiosis proceeds at least approximately on schedule in Xh^-/Y males despite the fact that the unpaired sex chromosome univalents typically fail to achieve bipolar orientation. However, a large fraction of the gametes in such males are either eliminated prior to maturity or fail to function. We suggest that this sperm dysfunction is an alternative mechanism to prevent transmission of aneuploid gametes.

We further suggest that the elimination of sperm derived from spermatocytes that suffer pairing failure or misalignment is at least partly the result of competition with normal sperm. Competitive viability is suggested by the fact that the *XY*/*O* survival ratio in various Xh^-/Y genotypes depends upon the *X-Y* nondisjunction frequency (McKee and Lindsley 1987); if inviability were absolute, the *XY*/*O* ratio should be constant. In addition, the failure to completely suppress transmission of aneuploid gametes in *Df(1)X-1* males (nullo-*XY* sperm are transmitted with reasonable efficiency) likely reflects the fact that all spermatocytes in such males are abnormal so that there are no normal sperm with which to compete. Under these abnormal conditions, nullo-*XY* sperm evidently have some advantage related to their low chromatin content that enables them to outcompete the other meiotic products. In chromosomally normal males, however, where this checkpoint must have evolved, nullo-*XY* sperm would be rare and would have to compete with the products of normal meioses.

Chromosome misalignment and sperm dysfunction: The checkpoint idea can account for sperm dysfunction in *Xh*-deficiency-bearing males because the unpaired sex

chromosomes behave as univalents and usually fail to achieve bipolar orientation. It also accounts for the amelioration of sperm dysfunction that results from insertion of pairing sites on the *X*, since these insertions enhance the frequency of bivalents in Xh^{-}/Y spermatocytes and thus, presumably, of properly oriented chromosomes. Moreover, the model has no difficulty with the failure of trans-heterochromatic duplications to suppress meiotic drive, because the Xh^{-} chromosome remains as a univalent in $Xh^{-}/Y/Dp$ males even though all pairing sites may be saturated.

But how can the suppression of $Xh^{-}/T(Y;A)$ sterility by *X* chromosomal pairing site insertions be explained in the context of a meiotic misalignment model? The expected chromosome configuration in $Xh^{-}/T(Y;A)$ males consists of a trivalent composed of the $Y^{P}A^{D}$ and $A^{P}Y^{D}$ translocation halves paired separately with the unrearranged autosome, plus the univalent *X*. The insertion of pairing sites on the *X* might be expected to accomplish nothing more than the transformation of this 3+1 configuration into a quadrivalent. It is not clear why this would be advantageous because sex chromosome-autosome quadrivalents are the expected configurations in most *X*-autosome translocations, as well as in many *Y*-autosome translocations, and most of these genotypes are sterile.

The answer to this question is not known, but the distribution of breakpoints among the suppressible *vs.* nonsuppressible translocations may provide an important clue. The results in Table 2 showed that pairing site insertions suppress sterility in the presence of translocations with distal autosomal breakpoints but not in the presence of a translocation broken in the centric heterochromatin of chromosome *2*. The relevant difference between the translocations for which sterility is suppressible or non-suppressible by pairing site insertions might involve the stability of the multivalent. Autosomal pairing sites are mostly weak, broadly distributed and additive (McKee *et al.* 1993), so that when the autosomal breakpoint is relatively distal, the pairing bond between the *A* and $Y^{P}A^{D}$ elements is expected to be weak. Thus for the eight translocations with distal autosomal breakpoints, all of which were rescued by the addition of pairing sites, the effect of the added *X* chromosome pairing sites might have been to disrupt the weak bond between $Y^{P}A^{D}$ and *A*, destroying the trivalent. The only translocation not subject to pairing site rescue has a centric autosomal breakpoint, so the trivalent should be much more resistant to disruption by competing pairing effects. In this case, fertility is restored by replacement of Xh^{-} with a normal *X*, so resolution into two bivalents might require a full dose of pairing sites. Thus, we suggest that the salutory effect of adding *X* pairing sites to an $Xh^{-}/T(Y;A)$ genotype may reflect replacement of the 3+1 configuration with two bivalents—$X{:}Y^{P}A^{D}$ and $A{:}A^{P}Y^{D}$—in a significant fraction of cells. Although speculative, this idea is testable, the prediction being that restoration of fertility in $Xh^{-}/T(Y;A)$ males associated with pairing site insertions should be accompanied by an increased frequency of meioses in which the rearranged chromosomes form two bivalents rather than a trivalent plus univalent. It also predicts that *Y*-autosome translocations in the unconditionally sterile class (those that are sterile even in the presence of a normal *X*) should form multivalents irrespective of the presence or absence of *X* pairing sites.

The underlying assumption of this argument is that multivalents involving sex chromosomes and autosomes, whether trivalents or quadrivalents, may often experience difficulty achieving bipolar alignment despite stable pairing. There is no direct evidence concerning the validity of this assumption. However, there is a precedent for the idea that rearrangements can interfere with the ability of paired chromosomes to achieve a bipolar orientation. The partial *Xh* deficiency *Df(1)sc4-sc8* and a normal *Y* form a bivalent in some spermatocytes and remain as univalents in others. When both the univalent frequency and the nondisjunction frequency have been measured in the same individuals, the latter has turned out to be somewhat higher than would be predicted from random assortment of univalents, suggesting that some of the nondisjunction may result from mal-orientation of the bivalents (Peacock 1965; Ault and Lin 1984; Lin *et al.* 1984). EM reconstructions of sectioned bivalents at metaphase or anaphase revealed that, unlike normal autosomal or sex bivalents at the same stage, the *Df(1)sc4-sc8/Y* bivalent was often not oriented properly. In some cases both kinetochores faced the same pole, while in others one or both kinetochores faced neither pole (Ault and Lin 1984). In light of these observations as well as the evidence that even wild-type bivalents have considerable difficulty achieving bipolar alignment in Drosophila spermatogenesis (Church and Lin 1985), the suggestion that certain types of multivalents may experience special difficulties achieving bipolar orientation is worth considering. If this idea is correct, it will be of considerable interest to explore the basis for differential meiotic behavior of sex chromosome-autosome multivalents *vs.* autosome-autosome multivalents, which do not typically cause sterility.

Summary and tests of the checkpoint model: To recapitulate briefly, we propose that the sperm dysfunction associated with rearrangements that involve the sex chromosomes in Drosophila results from action of a meiotic checkpoint that is sensitive to chromosome misalignment. The basic premise is that triggering of the checkpoint causes a general disabling of sperm that derive from the affected spermatocyte, and that this disability leads to developmental failure or elimination of spermatids or weakened functioning of sperm, depending on the degree of disability. This idea explains the effects of deletion of *X* pairing sites as due to meiotic instability of the resulting sex chromosome univalents, rather than to spermatid lethality of the unsaturated pairing sites.

It also accounts for the dysfunctional effects of various other sex chromosome rearrangements in terms of other types of disruptive misalignments associated with asymmetric bivalents and the special kinds of multivalents expected to form in translocations involving both sex chromosomes and autosomes. We have further suggested that the checkpoint "trigger" is lack of tension on kinetochores of chromosomes that have not achieved bipolar alignment. In other organisms, untense kinetochores have been shown to trigger a "wait anaphase" response, a response clearly absent in Drosophila. Our proposal is that the same signal triggers a pathway responsible for disabling spermatids in Drosophila, an alternative that may be more practical than cell-cycle delays in an organism selected for rapid development.

A major virtue of this model is that it generates testable predictions, many of which have been spelled out above. Four distinct predictions emerge from the hypothesis: (1) In wild-type males, the true nondisjunction frequency (that measured in the immediate products of meiosis) should be higher than the corresponding frequency gleaned from progeny counts, the difference reflecting the activity of the checkpoint in eliminating many of the products of defective meioses; (2) In males that experience high levels of meiotic drive, the surviving sperm should show evidence of disability, which may be reflected in poor competitive ability when challenged with sperm from wild-type males; (3) In males bearing sex chromosome-autosome translocations, sterile genotypes should exhibit mostly multivalents, whereas fertile genotypes should exhibit a significant fraction of spermatocytes in which all chromosomes pair as bivalents; and (4) Genotypes associated with sperm dysfunction should exhibit chromosomes that fail to achieve bipolar orientation in the available time and thus presumably have kinetochores that are not under tension. In addition to these specific predictions, the model also suggests that identification of genes required for sperm dysfunction via mutational analysis may prove useful in dissecting the genetic control of the metaphase checkpoint and its downstream effectors.

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