Inseparability of X-Heterochromatic Functions Responsible for X:Y Pairing, Meiotic Drive, and Male Fertility in *Drosophila melanogaster*

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ABSTRACT

Deficiencies encompassing part or all of the *X* heterochromatin of *Drosophila melanogaster* have been linked to three abnormalities in male meiosis and spermatogenesis: *X-Y* nondisjunction, skewed sperm recovery ratios favoring sperm with reduced chromatin content, and sterility in males carrying either a *Y*-autosome translocation or mal^+Y. In this study, 18 *X* heterochromatic deficiencies of varying sizes were tested in *XY* males for their spermatogenic phenotypes. All 18 proved to be either mutant for all three phenotypes or wild type for all three. Although variable among mutant deficiencies, expression levels of all three phenotypes were strongly correlated. Deficiencies that cause high levels of nondisjunction also cause severe recovery ratio distortion and are completely sterile in conjunction with mal^+Y. Low nondisjunction deficiencies cause comparable mild effects for the other phenotypes. The same deficiencies were also tested in males carrying a large heterochromatic free *X* duplication *Dp(1;f)3*. For all deficiencies which induce nondisjunction in *XY* males, the *Y* and free duplication pair regularly and the *X* fails to pair in *XYDp* males. Drive levels are constant across deficiencies in these males. Thus elimination of variability in the pairing phenotype also eliminates variability in sperm recovery ratios.

DROSOPHILA melanogaster males deficient for most or all of the *X* heterochromatin exhibit both *X-Y* nondisjunction and skewed sex-chromosome recovery ratios (meiotic drive). *X*-bearing sperm are recovered in excess of *Y*-bearing sperm and nullo-*X*, nullo-*Y* sperm are recovered greatly in excess of *XY*-bearing sperm (GERSHENSON 1933; SANDLER and BRAVER 1954). Neither zygotic lethality (SANDLER and BRAVER 1954) nor meiotic chromosome loss (PEACOCK 1965; COOPER 1964) contribute to these inequalities. Sperm development is abnormal however; many of the sperm from these males fail to become individualized and are degraded (PEACOCK, MIKLOS and GOODCHILD 1975).

Despite the large amount of sperm death, males deficient for *X* heterochromatin (Xh^-) are fertile if they carry a normal *Y*. However, sterility results when some heterochromatically deficient *X* chromosomes are combined either with a *Y*-autosome translocation (LINDSLEY *et al.* 1979) or mal^+Y, a *Y* duplicated for a substantial piece of proximal *X* (LIFSCHYTZ and LINDSLEY 1972; SCHALET 1972; SCHALET and LEFEVRE 1973; RAHMAN and LINDSLEY 1981). The latter phenotype has been shown to depend both upon the amount of duplicated material in the *Y* and upon the amount of material missing from the *X* heterochromatin (RAHMAN and LINDSLEY 1981).

The relations, if any, among the various spermatogenic lesions associated with deficiency for *X* heterochromatin are unclear. COPPER (1964) has shown that the *X* sites involved in *X-Y* meiotic pairing (collochores) are exclusively heterochromatic and that deficiency for those sites disrupts *X-Y* meiotic pairing. So it is no surprise that deficiency for *X* heterochromatin leads to *X-Y* nondisjunction. It is not obvious, however, why a collochore deficiency should lead to sperm death or male sterility. Although many of the sperm from Xh^- males are aneuploid for sex chromosomes, aneuploidy does not generally impair sperm viability in Drosophila (MULLER and SETTLES 1927; LINDSLEY and GRELL 1969). *XY*-bearing sperm (the class with the poorest viability in Xh^- males) are recovered perfectly well from *XYY* males who carry a normal *X*. Furthermore, aneuploid sperm are not the only ones susceptible to elimination in Xh^- males; euploid sperm are also vulnerable (MCKEE 1984).

It is possible that these phenotypes are related only by linkage, that the *X* heterochromatin contains not only pairing functions but also other functions needed for normal sperm development and that large heterochromatic deficiencies delete both sets of functions. However, several observations suggest a causal connection between the failure of sex chromosome pairing and the skewed recovery ratios. First, *X-Y* pairing, nondisjunction, and meiotic drive (skewed recovery ratios due to gamete lethality) are all temperature

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We dedicate this article to the memory of L. SANDLER, whose untimely death on February 7 has deprived the field of Drosophila genetics of one of its most incisive investigators and dedicated teachers. Larry was a reviewer of both this paper and of its predecessor (MCKEE, 1984), and we gratefully acknowledge his careful and constructive reviews. His insights and enthusiasm will be sorely missed.

sensitive in the same direction (more normal at lower temperatures) (ZIMMERING 1963; PEACOCK, MIKLOS and GOODCHILD 1975). Second, data for several different X-heterochromatic deficiencies exhibit a correlation between the frequency of nondisjunction and the level of meiotic drive (PEACOCK and MIKLOS 1973). Third, data for individual Xh^- males carrying the same deficient X exhibit a similar correlation (PEACOCK, MIKLOS and GOODCHILD 1975). Finally, all 20 EMS-induced X-linked mutants isolated in a search for male meiotic mutants that cause X-Y nondisjunction also cause drive (BAKER and CARPENTER 1972).

These observations have led to the suggestion that unpaired chromosomes act as gametic lethals in Drosophila males (BAKER and CARPENTER 1972; PEACOCK and MIKLOS 1973). Factors that affect the probability of pairing in Xh^- males such as temperature or size of deficiency would affect the probability of X-Y pairing failure and thus the probability of sperm dysfunction. However, it has been found that both unpaired and paired chromosomes (sex chromosomes and autosomes, respectively) from Xh^- males exhibit depressed recovery (MCKEE 1984). Also, addition of a full dose of X pairing sites in the form of a heterochromatic free X duplication to an Xh^-/Y genotype provides a regular pairing partner for the Y but fails to improve its recovery (HAEMER 1978; MCKEE 1984). Thus gametic lethality of unpaired chromosomes is not the explanation for the skewed recovery ratios. In this regard, LINDSLEY and SANDLER (1958) demonstrated gametic lethality despite regular pairing in certain attached XY/free *Dp* males. The connection between X-Y pairing failure and sperm death remains obscure.

The work described below was undertaken in the hope of clarifying this relation. If the X-heterochromatic functions responsible for X-Y pairing and for sperm viability are different, then it ought to be possible to find deficiencies that separate those functions and exhibit one phenotype without exhibiting the other(s), *e.g.*, nondisjunction without drive or sterility. The strategy of the study is to determine the phenotypes with respect to nondisjunction, drive and (if not previously determined) mal^+Y-induced sterility of a large number of X-heterochromatic deficiencies of varying sizes and to compare those results with previously published results on mal^+Y-induced sterility of some of the deficiencies (RAHMAN and LINDSLEY 1981). The same deficiencies are also tested in the presence of a free X duplication to monitor the effect of the *Dp* on both Xh^-:Y pairing and meiotic drive.

MATERIALS AND METHODS

Some of the deficiency and inversion X chromosomes and duplicated Y chromosomes used in this study were obtained from the Bowling Green Stock Center. $Df(1)y^{X2}$ was a gift from S. ENDOW and $Df(1)w^{m4L}w^{m51bR}$ was obtained from J. SPOFFORD. *Dp(2;f)f29* was supplied by J. BRITTNACHER.

Nondisjucntion and meiotic drive were measured by crossing Xh^-/B^SY (Table 1) or $Xh^-/B^SY/Dp(1;f)3$ (Table 2) males to *y w/y w* females. Crosses were performed at 25° on standard cornmeal, molasses, agar medium supplemented with yeast. Single males were mated to one or two females. Adults were transferred to fresh vials on day 5 and discarded on day 12. Progeny in the first vial were counted on days 12, 15, and 17. Progeny in the transfer vial were counted on days 17, 20 and 22.

Crosses are compared by means of the parameters P, the probability of X-Y disjunction and R_i, the recovery probability of chromosome *i*. R_i is defined as the viability of sperm carrying chromosome *i* relative to otherwise identical sperm lacking chromosome *i*. P is 1 when the X and Y disjoin regularly; it is 0.5 when X-Y disjunction is random. R_i is 1 when the presence of chromosome *i* has no impact on sperm viability. It is less than 1 when chromosome *i* causes a reduction in sperm viability. The true probability associated with each sperm class generated by an Xh^-/Y male can be expressed in terms of these parameters as follows. $p(X) = 1/2\ PR_X$. $p(Y) = 1/2\ PR_Y$. $p(XY) = 1/2\ (1 - P)\ R_XR_Y$. $p(0) = 1/2\ (1 - P)$. Among the survivors the observed frequencies are given by $p'(X) = PR_X/(PR_X + PR_X + (1 - P)R_XR_Y + (1 - P))$ $p'(Y) = PR_Y/(PR_X + PR_Y + (1 - P)R_XR_Y + (1 - P))$. $p'(XY) = (1 - P)R_XR_Y/(PR_X + PR_Y + (1 - P)R_XR_Y + (1 - P))$. $p'(0) = (1 - P)/(PR_X + PR_Y + (1 - P)R_XR_Y + (1 - P))$. Since there are three parameters and four observations (*A*, the frequency of X-bearing sperm, *B*, the frequency of Y-bearing sperm, *C*, the frequency of XY-bearing sperm and *D*, the frequency of nullo-X, nullo-Y sperm) there must be a unique algebraic solution. It is apparent that the ratio

$$\frac{p'(X)\cdot p'(XY)}{p'(Y)\cdot p'(0)} = \frac{AC}{BD} = \frac{PR_X(1 - P)R_XR_Y}{PR_Y(1 - P)}$$

leads to the solution for R_X ($R_X = \sqrt{AC/BD}$)

Similarly the ratio

$$\frac{p'(Y)\cdot p'(XY)}{p'(X) \times p'(0)} = \frac{BC}{AD} = \frac{PR_Y(1 - P)R_XR_Y}{PR_X(1 - P)}$$

leads to the solution for R_Y ($R_Y = \sqrt{BC/AD}$). By similar reasoning P is found to be $1/(1 + \sqrt{CD/AB})$.

Although maximum likelihood estimation is unnecessary here, these can be shown to be the maximum likelihood solutions. These formulas are useful when nondisjunction is substantial. However, for very low nondisjunction deficiencies, drive is best measured among the disjunctional classes only since error is thereby reduced. A useful parameter under these conditions is $R_Y/R_X = \sqrt{(BC/AD)/(AC/BD)} = B/A$. This measures the recovery of the Y chromosome relative to that of the X. Since nondisjunction varies widely among the deficiencies in this study, R_Y/R_X is used as the universal statistic for comparison of results from Xh^-/Y crosses.

In $Xh^-/Y/Dp$ males the Y and *Dp* always disjoin from each other. This renders calculation of R_Y and R_{Dp} impossible since there are no sperm lacking either chromosome that are otherwise identical. All non-Y-bearing sperm carry the *Dp*; all non-*Dp*-bearing sperm carry the Y. What can be calculated is $R_{Y/Dp}$, the recovery probability of the Y relative to that of the *Dp*. The other parameters, P and R_X, are the same as in Xh^-/Y males. The calculation formulas are:

$$R_X = \sqrt{AC/BD}, R_{Y/Dp} = \sqrt{BC/AD} \text{ and } P = 1/1 + \sqrt{CD/AB}$$

where *A* is the number of *XDp*-bearing sperm, *B* is the number of Y-bearing sperm, *C* is the number of XY-bearing sperm and *D* is the number of *Dp*-bearing sperm.

It was shown in MCKEE (1984) that the recovery parameters R_X and $R_{Y/Dp}$ are independent. This was accomplished

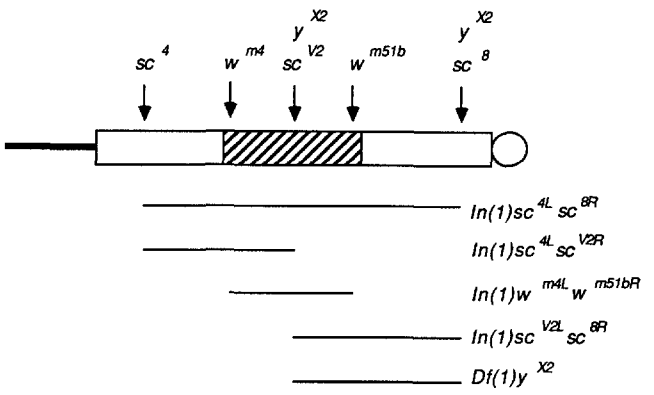

FIGURE 1.—Heterochromatic breakpoints of large *X* chromosome inversions. The rectangular region is the heterochromatin. The rDNA is indicated by cross-hatching. The extents of deficiencies generated by crossing over between inversions are indicated by the horizontal lines below.

by measuring *P* cytologically so that an extra degree of freedom was obtained for a test of homogeneity. This finding justifies the multiplicative treatment of recovery parameters in the model.

RESULTS

Nondisjunction, meiotic drive and sterility in Xh⁻/Y males

The first set of deficiencies tested (group A, Table 1) are derived from large inversions with one break near the tip of the *X* and one break in the proximal heterochromatin. The positions of the heterochromatic breaks are shown in Figure 1. When two deficiencies with similar euchromatic breakpoints but different heterochromatic breakpoints are made heterozygous, the single crossover products are reciprocal deficiencies and duplications for the heterochromatin between the breaks (GERSHENSON 1933). The three inversions with euchromatic breaks in the scute locus near the tip of the *X* ($In(1)sc^4$, $In(1)sc^{V2}$ and $In(1)sc^8$) have widely divergent heterochromatic breaks and can be combined to produce *Xh* deficiencies with common endpoints. $In(1)sc^4$ is broken in the distal heterochromatin, distal to the *rDNA* (RITOSSA and SPIEGELMAN 1965) [a block of about 250 tandemly repeated genes encoding the 28 S and 18 S *rRNAs* located in the middle of the *X* heterochromatin (RITOSSA 1976)]. $In(1)sc^{V2}$ is broken in the middle of *Xh* and splits the *rDNA* approximately in half (LINDSLEY, APPELS and HILLIKER 1982). $In(1)sc^8$ is broken in proximal *Xh*, proximal to the *rDNA* (RITOSSA and SPIEGELMAN 1965). $In(1)sc^{4L}sc^{8R}$ is deficient for approximately 90% of *Xh*, including all of the *rDNA*. $In(1)sc^{4L}sc^{8R}$ homozygotes are inviable but *XY* hemizygotes survive because the short arm of the *Y* carries an additional 200 or so copies of the *rDNA* (RITOSSA 1976). Deficiencies, such as $In(1)sc^{4L}sc^{8R}$, that delete enough *rDNA* copies to cause recessive lethality are called bobbed-minus (bb^-) in reference to a bristle phenotype associated with some partial *rDNA* deficiencies (LINDSLEY and GRELL 1968). $In(1)sc^{4L}sc^{V2R}$ is deficient for the distal half of *Xh* including half of the *rDNA* and is bb^+ (homozygous viable and normal). $In(1)sc^{V2}sc^8$ is missing the proximal half of *Xh* and half of the *rDNA* and is also bb^+.

In order to minimize genetic background differences, the three deficiencies along with $In(1)sc^{V2}$ controls were generated as brothers from a cross of $In(1)sc^{4L}sc^{8R}/In(1)sc^{V2}$ females by *y w*/B^SY males or *y w*/y^+Ymal^+ males. The male progeny were mated singly to *y w*/*y w* females to test for nondisjunction and drive (for the B^SY males) or for fertility (for the y^+Ymal^+ males).

The results are shown in Table 1. $In(1)sc^{V2}$ males are, as expected, wild type with respect to drive, nondisjunction, and fertility. $In(1)sc^{4L}sc^{8R}$ males are deficient for most of the heterochromatin and exhibit all of the mutant phenotypes. $In(1)sc^{4L}sc^{8R}$ has been shown to pair occasionally with the *Y* but always at the tip. Apparently no pairing sites are located proximal to the $In(1)sc^8$ break (COOPER 1964). Both smaller deficiencies, $In(1)sc^{4L}sc^{V2R}$ and $In(1)sc^{V2L}sc^{8R}$, behave as wild type even though together they are missing all the material deficient from $In(1)sc^{4L}sc^{8R}$. $In(1)y^{X2}$ is similar to $In(1)sc^{V2L}sc^{8R}$; it is broken in the *rDNA* and at the $In(1)sc^8$ break and thus is missing part of the *rDNA* and most of the proximal heterochromatin (SCHALET 1968, 1969). It, like $In(1)sc^{V2L}sc^{8R}$, exhibits regular disjunction and normal sperm recovery ratios. The same pattern was observed when these inversions were combined with a *Y*;autosome translocation—$In(1)sc^{4L}sc^{8R}$ males were sterile and the others were fertile (C. PEARSON and D. L. LINDSLEY, unpublished data). The simplest interpretation of these results is that all the phenotypes are controlled by repeated material of which at least one copy is distal to the $In(1)sc^{V2}$ break and at least one copy is proximal to it.

Since the $In(1)sc^{V2}$ break falls approximately in the middle of the *rDNA* (LINDSLEY, APPELS and HILLIKER 1982) the repeated material responsible for these phenotypes might be the *rDNA*. To test this possibility, males carrying the bb^- deficiency $In(1)w^{m4L}w^{m51bR}$ and either B^SY or Y^+Ymal^+ were crossed to *y w*/*y w* females. The heterochromatic breakpoints of both $In(1)w^{m4}$ and $In(l)w^{m51b}$ have been shown by in situ hybridization to fall just within the borders of the *rDNA* but at opposite ends (HILLIKER and APPELS 1982) so that no more than 3–4 *rDNA* cistrons lie outside either break. The deficiency $In(1)w^{m4L}w^{m51bR}$ retains 6–8 copies of the *rDNA*, not enough for survival. It proved to be wild type for all three spermatogenesis phenotypes (Table 1, line 5). If *rDNA* controls the spermatogenesis phenotypes, a few cistrons must suffice for wild type function. Alternatively, pairing and sperm viability might be controlled by other sequences distal and proximal to the *rDNA*.

The deficiencies in group B were selected as null

TABLE 1

Nondisjunction, meiotic drive, and *mal⁺Y* sterility in *Xh⁻Y* males

Group	X chromosome	*bb*	Sperm genotype				Parameters		Fertility over *mal⁺Y*[a]
			X	*Y*	*XY*	*O*	*P*	R_Y/R_X	
A	*In(1)sc^{V2}*	+	1865	1931	1	0	1.0	1.035	+
	In(1)sc^{4L}sc^{V2R}	+	2370	2330	1	2	1.0	0.983	+
	In(1)sc^{V2L}sc^{8R}	+	2525	2417	4	2	1.0	0.957	+
	In(1)y^{x2}	+	872	849	1	2	1.0	0.974	+
	In(1)w^{m4L}w^{m51bR}	−	1045	1039	3	1	1.0	0.994	+
	In(1)sc^{4L}sc^{8R}	−	809	401	113	1144	0.613	0.496	−
B	*Df(1)R-8*	+	4568	4368	3	0	1.0	0.956	+
	Df(1)R-6	+	2956	2811	2	0	1.0	0.951	+
	Df(1)R-17	+	2478	2388	0	0	1.0	0.964	+
	Df(1)R-8A	−	2313	2277	0	0	1.0	0.984	+
	Df(1)K-5	−	938	730	1	1	1.0	0.778	±
	Df(1)17-87	−	403	209	51	748	0.598	0.519	−
	Df(1)GA-90	−	979	301	88	2396	0.542	0.307	−
	Df(1)y^{X15}	−	360	90	13	721	0.650	0.250	−
	Df(1)X-1	−	605	138	32	1983	0.534	0.228	−
C	*Df(1)bb452*	−	3761	2905	57	243	0.966	0.772	−
	Df(1)bb3a	−	2445	1540	330	2067	0.789	0.630	−
	Df(1)bb74	−	2054	986	153	2656	0.691	0.480	−
	Df(1)bb158	−	1013	434	130	1735	0.583	0.428	−

[a] Fertility results for all deficiencies in groups B and C with the exception of *Df(1)bb3a* and *In(1)y^{X2}* are from RAHMAN and LINDSLEY (1981). In this column a "−" indicates an average of less than 1 offspring per male, "±" indicates an average of less than 20 offspring per male and "+" indicates greater than 20 offspring per male. At least 15 males were tested for each genotype.

mutants for *su(f)*, the most proximal locus known in the *X* euchromatin. All of them have one break distal to *su(f)* in the euchromatin and one break proximal to it in the heterochromatin. The euchromatic breakpoints of many of these deficiencies have been mapped cytologically but, for the most part, the heterochromatic breakpoint position can only be inferred from phenotypes. These deficiencies have all been examined previously for their phenotypes with respect to *mal⁺Y* sterility (RAHMAN and LINDSLEY 1981). It was found that males carrying any of the *bb⁺* deficiencies, *i.e.*, those with their proximal breaks in the distal half of the heterochromatin, were fertile. Males carrying some of the *bb⁻* deficiencies were fertile as well but most *bb⁻* deficiencies caused sterility in combination with *y⁺Ymal⁺*. The latter class could be divided into two groups, those associated with slight fertility in combination with *y⁺Ymal^{126}*, an X-ray induced derivative of *y⁺Ymal⁺* (SCHALET and FINNERTY 1968), and those that completely sterilized *y⁺Ymal^{126}* males. These results were taken to imply the existence of two sites proximal to the *rDNA* which contribute additively to fertility in the presence of duplication-bearing *Y* chromosomes.

The results in Table 1 with the group B deficiencies imply that nondisjunction and meiotic drive, like sterility, are controlled by more than one proximal heterochromatic site. All of the *bb⁺* deficiencies proved to be wild type with respect to nondisjunction and drive. *Df(1)K-5* and *Df(1)R-8A*, the two *bb⁻* deficiencies that were wild type with respect to *y⁺Ymal⁺* sterility, caused no nondisjunction. *Df(1)R-8A/B^{S}Y* males also showed no drive but *Df(1)K-5/B^{S}Y* males exhibited mild meiotic drive suggesting that *Df(1)K-5* is near the threshold of mutant expression, at least for the drive phenotype. The four other *bb⁻* deficiencies in group B caused substantial nondisjunction and drive in *Xh⁻/B^{S}Y* males. Although all four *su(f)* deficiencies are strong mutants, they vary considerably in terms of degree of both nondisjunction and drive. In the strongest mutant, *Df(1)X-1*, the level of nondisjunction is high enough ($P = 0.534$) to be consistent with random assortment of *X* and *Y*. Meiotic drive is correspondingly strong. To determine if the sex chromosomes were really assorting randomly, testes from *Df(1)X-1/B^{S}Y* males were squashed in lactic acetoorcein and examined with phase contrast microscopy. No first metaphase spermatocytes were found (out of 41 examined) in which the *X* and *Y* were paired. Secondary spermatocytes were distributed as: 32*X*, 33*Y*, 29*XY*, 34nullo-*X*; nullo-*Y*, which is consistent with random assortment. There is no evidence in these data for the preferential co-segregation of unpaired homologs described by PEACOCK (1965) working with similar deficiencies. W. J. PEACOCK and D. C. MORIZOT [unpublished thesis, cited in PEACOCK and MIKLOS (1973)] described one deficiency *Df(1)bb481*, now lost) that produced 61–68% nondisjunction measured cytologically. Fifty percent is the theoretical maximum nondisjunction observable, assuming random assortment of unpaired homologs. Since they observed only 60–65% univalents at first metaphase, only 30–33%

nondisjunction would have been expected. The reason for the discrepancy between these and the present results is unclear.

Df(1)X-1 defines the proximal limit of the sequences responsible for *X–Y* pairing. Whether it also defines the proximal limit of the sequences responsible for drive cannot be ascertained because of the possibility that a deficiency could be found that is more severe in its effects. The other three deficiencies are all somewhat less severe than *Df(1)X-1*, ranking, in order of severity, $Df(1)y^{X15}$, *Df(1)GA-90*, *Df(1)17-87*. The 95% confidence intervals for *Df(1)X-1*, $Df(1)y^{X15}$, and *Df(1)GA-90* overlap but *Df(1)17–87* is significantly less severe than the other three. Thus these data are consistent with the existence of at least two sites in the proximal heterochromatin proximal to the *R-8A* breakpoint responsible for controlling meiotic pairing, sperm viability and fertility in combination with $mal^{+}Y$.

RAHMAN and LINDSLEY (1981) argued for the existence of a third site in the *X* heterochromatin distal to the $In(1)sc^{4}$ breakpoint on the grounds that $In(1)sc^{4L}sc^{8R}$ lacks almost all the heterochromatin proximal to the *rDNA* yet has a comparatively mild phenotype—$In(1)sc^{4L}sc^{8R}/y^{+}Ymal^{+}$ males are slightly fertile. The difference between $In(1)sc^{4L}sc^{8R}$ and the deficiencies with more severe effects seems to reside in a small amount of heterochromatin derived from the distal end, distal to the $In(1)sc^{4}$ break, which is moved to the tip of the *X* in $In(1)sc^{4L}sc^{8R}$. The data in Table 1 imply that this material must contain a site involved in controlling nondisjunction and drive. The levels of these phenotypes, while substantial, are considerably less for $In(1)sc^{4L}sc^{8R}$ than for *Df(1)X-1* flies. Cytological studies have shown that $In(1)sc^{4L}sc^{8R}$ pairs some of the time with the *Y* and that pairing always occurs at the tip of the *X* (COOPER 1964; PEACOCK 1965). The data in Table 1 also indicate the existence of a second more proximal pairing site in the distal half of the heterochromatin. As mentioned above, $In(1)sc^{V2L}sc^{8R}$ lacks most of the proximal heterochromatin yet is wild type for all phenotypes. This second site in the distal half must lie between the $In(1)sc^{4}$ and $In(1)sc^{V2}$ breakpoints.

Thus the data in Table 1 imply the existence of at least four heterochromatic sites involved in control of sex chromosome pairing and sperm viability—two proximal to the $In(1)sc^{V2}$ break in the middle of the *rDNA* and two distal to it. The presence of either the two distal or the two proximal sites suffices to ensure wild-type function. If all four sites are absent (*Df(1)X-1*), *X–Y* disjunction is random, sperm recovery ratios are extremely aberrant, and both $y^{+}Ymal^{+}$ and $y^{+}Ymal^{126}$ males are sterile. If either the distalmost site ($In(1)sc^{4L}sc^{8R}$) or the proximal most site (*Df(1)17–87*) remains, the *X* and *Y* pair some of the time, sperm recovery ratios are moderately distorted and males carrying duplicated *Y*s exhibit weak fertility. It is equally valid to interpret at least the drive and nondisjunction data to mean that the phenotypes are controlled by two blocks of repeated material, one distal and one proximal to at least most of the *rDNA*. On this interpretation, one whole block suffices for wild type function; part of a block gives partial function. The $In(1)sc^{4}$ break splits the distal block while the *Df(1)17–87* break splits the proximal block. It is also possible that the two blocks are really the ends of one long block, *e.g.*, the *rDNA*. The possible role of *rDNA* in this system is discussed more fully below.

The group C deficiencies in Table 1 were selected as bb^{-} mutants in a normal-sequence *X* (LINDSLEY, EDINGTON and VON HALLE 1960). All are mutant for the spermatogenic phenotypes although they cover a very substantial range of mutant expression. *Df(1)bb452* is the mildest mutant in Table 1 with respect to nondisjunction and drive and *Df(1)bb158* is one of the most severe. Since nothing is known about the cytological breakpoints of these deficiencies—other than that they delete most of the *rDNA*—they do not contribute much to the analysis of heterochromatic structure. They do, however, supply more material to help answer the question of the separability of the various spermatogenic functions.

With the exception of *Df(1)K-5*, which exhibits some drive without nondisjunction or sterility, all of the deficiencies in Table 1 are either mutant for all phenotypes or wild type for all phenotypes. This argues strongly that the phenotypes are all controlled by the same heterochromatic sites. Further evidence for this interpretation comes from a comparison of the degree of nondisjunction with the severity of drive. For both phenotypes the mutant deficiencies in Table 1 exhibit a wide range of variation—from $P = 0.966$ in *Df(1)bb452* to $P = 0.534$ in *Df(1)X-1* and from $R_Y/R_X = 0.772$ in *Df(1)bb452* to $R_Y/R_X = 0.228$ in *Df(1)*X-1. Figure 2 is a plot of R_Y/R_X *vs.* *P* for the mutant deficiencies in Table 1. It shows a very strong correlation between the degree of nondisjunction and the severity of meiotic derive. A similar correlation has been noted before based on data from individual $In(1)sc^{4L}sc^{8R}$ males and from $In(1)sc^{4L}sc^{8R}$ males raised at various temperatures (ZIMMERING 1963; PEACOCK and MIKLOS 1973; PEACOCK, MIKLOS and GOODCHILD 1975). This argues strongly for a fundamental, probably causal relationship between sex-chromosome pairing and sperm viability.

The data in Table 1 provide the material for a test of an "all or none" model of the relationship between pairing and drive. That model can be described in the following way. In males carrying an *X* heterochromatic deficiency, there are two classes of primary spermatocyes: those in which *X–Y* pairing succeeds (A) and those in which it fails (B). The relative frequencies of the two classes depend upon the amount of pairing

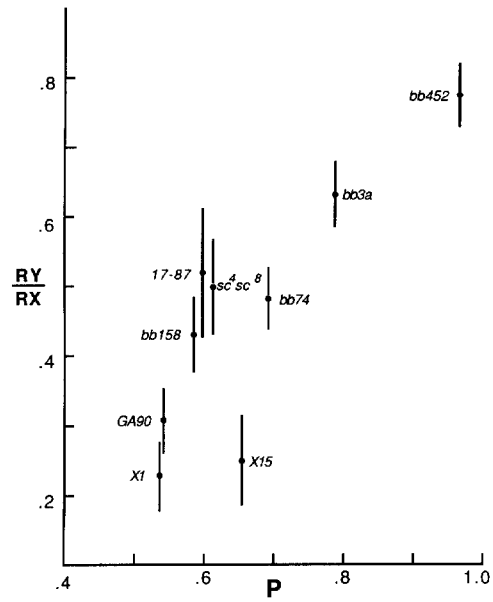

FIGURE 2.—Drive in Xh^-/Y genotypes as a function of X:Y pairing. R_Y/R_X (disjunctional drive) is plotted against P for mutant deficiencies. Data are from Table 1. Bars indicate 95% confidence intervals.

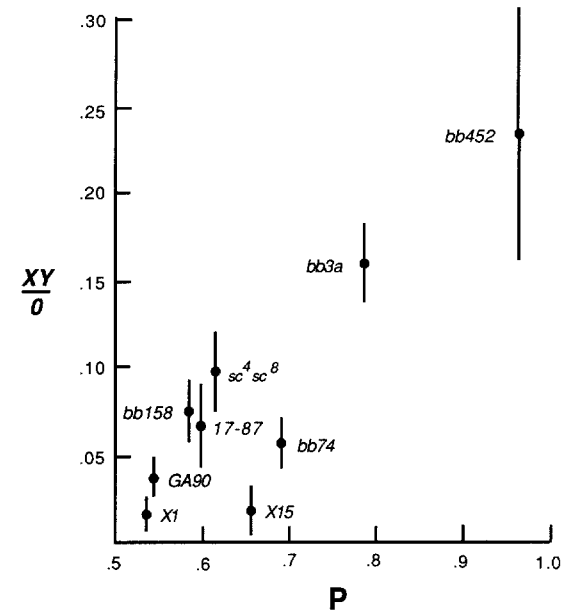

FIGURE 3.—Nondisjunctional drive in Xh^-/Y males as a function of probability of X:Y pairing. XY/O is plotted against P for the mutant deficiencies. Data are from Table 1. Bars indicate 95% confidence limits.

material present in the X chromosome. Class A spermatocytes produce only X-bearing sperm and Y-bearing sperm and their products experience no meiotic drive. Class B spermatocytes produce all four sperm classes in equal frequencies and their products do experience drive. The observed correlation between nondisjunction and drive arises because both parameters vary with the relative frequency of A and B. The central assumption is that overall drive levels vary among deficiencies only because the relative frequencies of class A and class B spermatocytes vary. The level of drive is the same in all class B spermatocytes whether those spermatocytes are found in low drive or high drive males. This assumption is easily tested using the data in Table 1 because all XY-bearing sperm and nullo-X; nullo-Y sperm come from class B spermatocytes. If the assumption is correct, the ratio of XY-bearing sperm to sperm that are nullo-X and nullo-Y should be constant across deficiencies. This ratio is plotted against P in Figure 3. It is clearly not constant. Instead, it correlates with P, a measure of pairing competence. Thus the level of drive is a function of the strength of the X–Y pairing bond but does not depend upon the disjunction-nondisjunction decision. The same conclusion follows from the fact that the probability of recovery of an autosome arm or of an autosomal free duplication from an Xh^-/Y male is the same in sex chromosome disjunctional and in nondisjunctional sperm (McKee 1984). Sperm recovery ratios are no more normal in class A than in class B spermatocytes. These observations imply that the pathways leading from pairing to disjunction and from pairing to sperm viability diverge before the nondisjunction-disjunction decision is made.

X:Y pairing and meiotic drive in $Xh^-/Y/Dp$ males

The simplest interpretation of the results in Table 1 is that nondisjunction, drive, and mal^+Y sterility all depend upon X–Y pairing and are controlled by collochores. Other possibilities remain, however. The phenotypes might be controlled by different but very closely linked repeated sequences. Or they might be controlled independently by a single multifunctional repeated sequence. A critical test of the functional relatedness of at least the nondisjunction and drive phenotypes can be carried out using an X fragment, $Dp(1;f)3$, that consists of all of the X heterochromatin but very little of the euchromatin. When this fragment is added to an $In(1)sc^{4L}sc^{8R}/Y$ genotype, it pairs regularly with the Y and causes univalent behavior of $In(1)sc^{4L}sc^{8R}$ McKee 1984). If the free duplication also outcompetes other X heterochromatic deficiencies for Y pairing sites, it will eliminate variability among deficiencies in the pairing parameter. If drive is a consequence of pairing failure, then the free duplication should also eliminate variability in the drive parameter. But if drive and nondisjunction are functionally unrelated, eliminating pairing variability should not affect drive variability. Low drive deficiencies like $Df(1)bb452$ should still exhibit low drive; high drive deficiencies like $Df(1)bb158$ should still exhibit high drive.

The results, presented in Table 2, of crosses between Xh^-/B^SY/Dp$(1;f)3$ males and $y\ w/y\ w$ females indicate that the free duplication has the same competitive effect (elimination of Xh^--Y pairing) on all of the "mutant" deficiencies (those that exhibit nondisjunction and drive over B^SY alone), except $Df(1)bb452$, the weakest mutant. This conclusion follows from the

TABLE 2

Disjunction and drive in $Xh^-/Y/Dp(1;f)3$ males

Group	X Chromosome	Sperm genotype						R_Y/R_{Dp}	R_X	Percent X and YDp
		X	XY	XDp	Y	Dp	YDp			
A	$In(1)sc^{V2}$	1797[a]	351	—[a]	702	360	565	—[b]	—[b]	—[b]
	$In(1)sc^{4L}sc^{V2R}$	492	209	400	385	235	420	0.925	0.961	43
	$In(1)sc^{V2L}sc^{8R}$	1686[a]	605	—[a]	1366	766	231	—[b]	—[b]	—[b]
	$In(1)w^{m4L}w^{m51bR}$	621	82	324	451	695	159	0.405	0.291	33
	$In(1)sc^{4L}sc^{8R}$	7	283	926	833	1859	0	0.370	0.411	0.2
B	*Df(1)R-8*	514	406	658	625	53	980	0.846	0.891	29
	Df(1)R-6	1285	1140	1916	1812	1220	1056	0.940	0.994	28
	Df(1)R-17	1331	962	1536	1532	1481	1002	0.805	0.807	30
	Df(1)R-8A	465	236	516	475	472	203	0.678	0.737	28
	Df(1)K-5	201	45	343	368	632	66	0.276	0.258	16
	Df(1)GA-90	80	136	615	570	2100	0	0.245	0.264	2.3
	$Df(1)y^{X15}$	0	4	14	9	40	0	0.254	0.394	0
	Df(1)X-1	5	50	270	281	1180	1	0.210	0.202	0.3
C	*Df(1)bb452*	100	71	386	508	1486	9	0.251	0.191	4.2
	Df(1)bb3a	2	56	222	264	764	0	0.295	0.248	0.2
	Df(1)bb74	15	45	217	186	746	0	0.227	0.265	1.2
	Df(1)bb158	8	80	296	292	899	1	0.296	0.300	0.6

[a] In the $In(1)sc^{V2}$ and $In(1)sc^{V2L}sc^{8R}$ crosses, offspring derived from X-bearing sperm could not be distinguished from offspring derived from *XDp*-bearing sperm.

[b] Cannot be calculated.

absence of significant numbers of *X* and *YDp* progeny in the crosses involving these deficiencies. The "wild-type" deficiencies (those that do not exhibit nondisjunction or drive over B^SY alone) retain the ability to compete in these three sex chromosome genotypes. *Df(1)bb452* and *Df(1)K-5* appear to be borderline for this phenotype, as for the phenotypes in Table 1, as the relative numbers of *X* and *YDp* progeny are significant but depressed compared to the wild-type deficiencies. Thus one effect of addition of a heterochromatic free *X* duplication to an Xh^- genotype is to eliminate Xh^-:*Y* pairing. Consequently the variability among mutant *Xh* deficiencies in terms of Xh^-:*Y* pairing so obvious in Table 1 is also eliminated. Table 2 also shows that addition of a free *Dp* has striking effects on meiotic drive. For each of the mutant deficiencies, sperm viability is inversely proportional to chromatin content, with *Dp*-bearing sperm outsurviving reciprocal *Y*-bearing classes and nullo-*X* sperm outsurviving reciprocal *X*-bearing classes. Thus all of the mutant deficiencies behave like $In(1)sc^{4L}sc^{8R}$ (McKee 1984) in that they show both multiplicative chromosome recoveries and sperm viabilities that are inversely proportional to chromatin content. But what about differences in levels of meiotic drive? These deficiencies are associated with radically different drive levels in Xh^-/Y males. Do they exhibit the same variability in the presence of a free *X* duplication? Figure 4 is a plot of R_Y/R_{Dp} in the $Xh^-/B^SY/Dp$ cross against *P* in the Xh^-/B^SY cross. The line is flat, indicating no correlation. Addition of the free duplication has eliminated the variability in both Xh^-:*Y* pairing and in meiotic drive. This argues strongly for a causal relationship between the two parameters.

DISCUSSION

The evidence presented above bears out in general the interpretation of sex chromosome meiotic drive advanced by Baker and Carpenter (1972) and Peacock and Miklos (1973), that breakdown of *X–Y* meiotic pairing leads to gametic lethality. Three lines of evidence have been presented in support of this interpretation.

1. The heterochromatic sites responsible for sex chromosome pairing, normal sperm development, and fertility in the presence of duplicated *Y*s are at least very closely linked and probably identical. Eighteen *X* heterochromatic deficiencies were tested for *X*:*Y* pairing, induction of sex chromosome meiotic drive, and fertility in conjunction with y^+Ymal^+. With one exception all proved either to be wild type for all three phenotypes or mutant for all three. The one exception, *Df(1)K-5*, disjoins regularly from B^SY and is fertile over y^+Ymal^+ yet causes mild distortion of *Y*-bearing sperm recovery. On closer inspection, however, it is clear that *Df(1)K-5* is not completely wild type with respect to pairing or fertility either. In the presence of a free duplication with a full complement of *X* pairing sites, *Df(1)K-5* exhibits weakened ability (relative to wild-type *X*s) to compete for pairing sites, as shown by the relatively low percent of *X*-bearing and *YDp*-bearing sperm (Table 2, line 11). *Df(1)K-5*/y^+Ymal^+ males are fertile but produce fewer progeny per male than males carrying any of the other wild

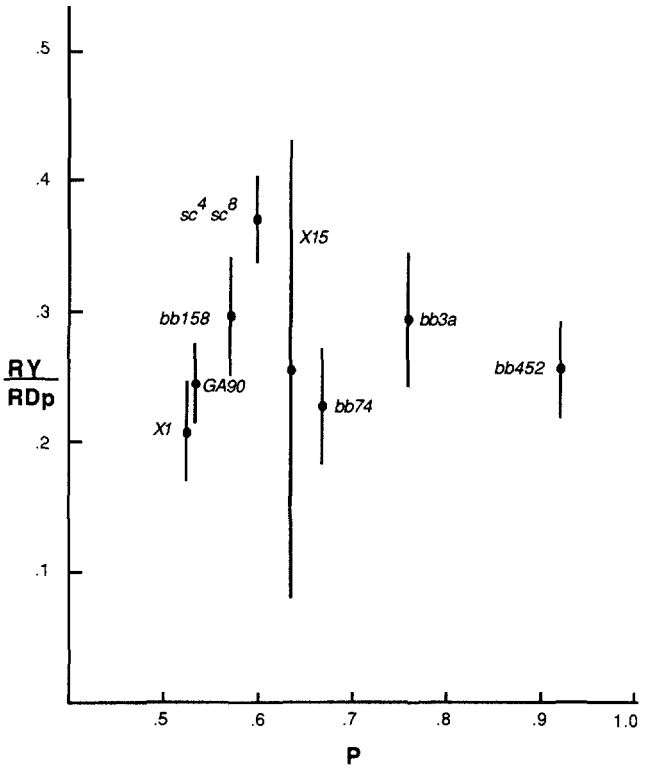

FIGURE 4.—Drive in $Xh^{-}/Y/Dp(1;f)3$ genotypes as a function of pairing competence of Xh^{-}. R_Y/R_{Dp} (from Table 2) is plotted against P (from Table 1) for mutant deficiencies. Bars indicate 95% confidence limits.

type deficiencies examined by RAHMAN and LINDSLEY (1981). Given these facts, *Df(1)K-5* is best viewed as a borderline case for all three phenotypes. The expression threshold for meiotic drive is evidently slightly lower than that for nondisjunction.

Df(1)C4 is evidently a similar borderline case. This deficiency is derived from $In(1)w^{m51b}$, an inversion in which all but a few of the *rDNA* cistrons and all of the heterochromatin distal to the *rDNA* is moved to the tip of the *X*. *Df(1)C4* is an X-ray-induced $su(f)^{-}$ derivative of $In(1)w^{m51b}$ from which virtually all of the distally located heterochromatin has been deleted (APPELS and HILLIKER 1982). APPELS and HILLIKER (1982) report a low but significant level of nondisjunction in males carrying this chromosome. L. SANDLER personal communication) compared sperm recovery ratios in $Df(1)C4/Ymal^{+}$ and control $X/Ymal^{+}$ males and found evidence for mild meiotic drive in *Df(1)C4*. $R_{Y/X}$ relative to the control *X* crosses ranged from 0.67 to 0.95 depending on the control *X* and was significantly different from 1 for all but one comparison out of six. Controls included Canton S and two $rDNA^{+}$ $su(f)^{-}$ deficiencies derived from $In(1)w^{m51b}$. These figures are comparable to $R_{Y/X}$ reported for *Df(1)K-5* in Table 1 but are considerably "milder" than those for any of the mutant deficiencies.

2. Among mutant deficiencies, there is a very strong correlation between the level of nondisjunction and the severity of meiotic drive. Also, it is clear that high nondisjunction, high drive deficiencies are more likely to cause sterility in conjunction with both $y^{+}Ymal^{+}$ and $y^{+}Ymal^{126}$ than are deficiencies more moderate in their disjunctional and recovery ratio phenotypes. The level of all three phenotypes is correlated with the probability of pairing with the *Y* which in turn depends upon the amount of pairing material present in the *X*.

3. The dependence of drive level on amount of remaining pairing material is abolished by addition of an *X*-heterochromatic free duplication. Free duplications completely outcompete partial *X*-heterochromatic deficiencies for *Y* pairing sites. Thus in the presence of a free duplication the probability of pairing with the *Y* is the same for all of the mutant deficiencies—0. The observed leveling of drive coefficients is exactly what the pairing hypothesis would predict and is difficult to explain any other way.

The experiments described above have implications for the structure as well as the function of pairing sites. The wild-type phenotype of both $In(1)sc^{4L}sc^{V2R}$ and $In(1)sc^{V2L}sc^{8R}$ implies that material either distal or proximal to the $In(1)sc^{V2}$ break in the middle of the *rDNA* is sufficient for wild-type function. Partial function is obtained with material distal to the $In(1)sc^{4}$ break or with material proximal to the *Df(1)17–87* break. These observations imply that *X–Y* pairing is controlled by more than one discrete site or by a repeated sequence. The strength of the *X–Y* pairing bond depends upon the number of sites present or the amount of the repeated sequence remaining.

The sequence(s) responsible for *X–Y* pairing has yet to be identified. The satellite sequences are unlikely candidates because none of them is distributed appropriately. Except for the 1.688 satellite which is unique to the *X* (HILLIKER and APPELS 1982), all the satellite sequences are found on all the chromosomes (PEACOCK *et al.* 1978). The *rDNA* is an obvious candidate. It is a repeated sequence and it is found only at the base of *YS* and in the *X* heterochromatin, where the pairing sites are found. The difficulty with this interpretation is that several $bb^{-}X$ heterochromatic deficiencies have been identified that exhibit normal disjunction, normal sperm recovery ratios, and fertility over $mal^{+}Y$ (*e.g.*, *Df(1)K-5*, *Df(1)R-8A*, and $In(1)$-$w^{m4L}w^{m51bR}$, Table 1). If the *rDNA* is the pairing material, it is clear that the threshold for mutant expression is much higher than for the bobbed phenotype or for viability. HILLIKER and APPELS (1982) estimate that $In(1)w^{m4L}w^{m51bR}$ retains no more than 6–8 ribosomal cistrons; it is certainly bobbed lethal but pairs regularly with a *Y*.

APPELS and HILLIKER (1982) obtained evidence that deletion of ribosomal cistrons can weaken the pairing capacity of an *X* chromosome. They tested two $su(f)^{-}$ derivatives of $In(1)w^{m51b}$, one broken in the middle of the *rDNA* (*Df(1)C1*) and one (*Df(1)C4*) that appeared to delete all the *rDNA* that is translocated to the tip

in w^{m51b}. *Df(1)C1* proved to be wild type but *Df(1)C4* exhibited a low but significant level of nondisjunction. As discussed above, L. SANDLER has found *Df(1)C4* to cause mild meiotic drive. The only difference between the two chromosomes is in the amount of *rDNA* remaining. This suggests that *rDNA* can contribute to *X–Y* pairing. However, this is a minor effect and does not demonstrate that the major pairing sites are *rDNA*. Whether the major role in *X–Y* pairing is played by the *rDNA* or by some unidentified sequence remains to be determined.

Whatever the composition of the pairing sites, it is clear that they play a role in sperm development in addition to their role in chromosome disjunction and that this role depends on interaction with euchromatic sequences. The nature of this interaction and the role of the euchromatic sequences remain mysterious.

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