Are Unpaired Chromosomes Spermicidal?: A Maximum-Likelihood Analysis of Segregation and Meiotic Drive in *Drosophila melanogaster* Males Deficient for the Ribosomal-DNA

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ABSTRACT

Meiosis in *Drosophila melanogaster* males is achiasmate and requires special systems to ensure normal segregation. Several situations that yield frequent nondisjunction also produce high levels of chromatin-dependent sperm lethality, suggesting the possibility of a simple and direct connection between defective disjunction and defective sperm development. One hypothesis that has been offered is that pairing not only ensures disjunction, but also changes the physical state of chromosomes so that they can be packaged in sperm. Here, I present an analysis of extensive data on disjunction and sperm survival in rDNA-deficient males collected by B. McKee and D. Lindsley. This analysis demonstrates that, although nondisjunction and sperm lethality are indeed correlated, the basis of this is not the presence of unpaired chromosomes in the sperm. Chromosomes that have failed to disjoin are not themselves spermicidal.

USING comfortably familiar language, Baker and Carpenter (1972) proposed that the element responsible for pairing in the achiasmate meiosis of *Drosophila melanogaster* males is a "bomb" capable of disrupting sperm maturation and that pairing "defuses" the bomb. Peacock and Miklos (1973) later proposed much the same idea. In a more general form, the hypothesis is that chromosomes that have failed to pair are themselves spermicidal. This hypothesis was proposed because of the apparent correlation between nondisjunction and distorted sperm ratios (meiotic drive), and, to test it, I have used maximum-likelihood methods to examine data from McKee and Lindsley (1987) on nondisjunction and meiotic drive in males deficient for the ribosomal RNA genes (rDNA).

The occurrence of both nondisjunction and distorted sperm recovery was first noted in males that bear the *In(1)sc^{4L}sc^{8R} X* chromosome (*sc^{4}sc^{8}*; Gershenson 1933; Sandler and Braver 1954; Peacock and Erickson 1965; Peacock *et al.* 1975). This chromosome is a crossover product of two inversions and is a synthetic deficiency of the basal heterochromatin of the *X*. In addition to *X*-bearing and *Y*-bearing sperm, *sc^{4}sc^{8}/Y* males produce large numbers of sperm that carry both sex chromosomes (*XY* sperm) and sperm that carry neither (*nullo* or *0* sperm). Although the frequencies of *X*- and *Y*-bearing sperm should be equal, as should the frequencies of *XY* and *nullo* sperm, their recoveries are grossly distorted. More *X*-bearing that *Y*-bearing sperm, and manifold more *0* than *XY* sperm, are recovered. The result is meiotic drive—a change in genotype frequency in the offspring without zygote mortality (Sandler and Novitski 1957). McKee and Lindsley (1987) tested a number of deficiencies of the basal heterochromatin of the *X* chromosome and showed that the reponsible segment is the rDNA cluster. Involvement of the rDNA was directly confirmed by demonstrating that a single transformed rDNA copy ameliorates both problems and that two copies give function even closer to normal (McKee and Karpen 1990; McKee *et al.* 1992). A third phenotype of rDNA-deficient *X* chromosomes, sterility in combination with certain duplication-bearing *Y* chromosomes, also responds to addition of rDNA transgenes (McKee 1991). A particular segment of the rDNA repeat sufficient to promote disjunction has also been identified (Merrill *et al.* 1992). It has recently been shown that this intergenic spacer fragment also reduces meiotic drive and corrects the sterility of many examples of *rDNA-deficiency/T(Y;A)* males (McKee *et al.* 1998).

Deficiencies of the *crystal* locus of the *Y* (*cry*, also known as *Su(Ste)*) also cause nondisjunction and meiotic drive. This system involves an interaction between *cry* and the multi-copy *Stellate* (*Ste*) locus of the *X* chromosome. Males that are deleted for *cry* and that have a high *Ste* copy number are completely sterile (Gatti and Pimpinelli 1983; Hardy *et al.* 1984). Both nondisjunction and meiotic drive, however, occur in males that are either deleted for *cry* but have a low-copy-number

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Ste locus (Palumbo *et al.* 1994) or that bear partial *cry* deficiencies and have a high-copy-number *Ste* locus (Hardy *et al.* 1984). As for rDNA deficiencies, these males produce *X*, *Y*, *XY*, and *0* sperm, and their recoveries are $X > Y$ and $0 \gg XY$. In contrast to rDNA deficiencies, however, the *cry-Ste* interaction also yields nondisjunction and meiotic drive of the autosomes. These males produce large numbers of *diplo-2* and *nullo-2* sperm, and the *nullo-2* sperm are recovered far more frequently.

A third case of nondisjunction and meiotic drive is the compound second chromosome *C(2)EN*. *C(2)EN* contains the complete euchromatic content of two second chromosomes, and *C(2)EN*/*0* males should produce equal numbers of *C(2)EN*-bearing and *nullo-2* sperm. Instead, with some variants of *C(2)EN*, virtually all of the progeny come from fertilization by *nullo-2* sperm (Novitski *et al.* 1981; Strommen 1982). (Note that *C(2)EN*/*0* females do produce eggs of both genotypes.) Most recently, making quite elegant use of direct karyotyping of sperm, Dernburg, Daily, and co-workers showed that the poor recovery of the *C(2)EN* compound second chromosome from males is also a case of sperm dysfunction (Dernberg *et al.* 1996). Most importantly in the present context, Dernburg *et al.* also found that there is nondisjunction and drive of other chromosomes of the complement in *C(2)EN* males.

With at least three quite diverse situations yielding both nondisjunction and sperm lethality, the bomb hypothesis has substantial heuristic appeal. Although each system might cause pairing defects via different routes, they would share a common developmental pathway in which the unpaired chromosomes, the still-fused bombs, are spermicidal. In its simplest form, however, this hypothesis is certainly not correct—the armed bomb cannot itself be the pairing element since complete deletion of the rDNA, along with most of the rest of the heterochromatin of the *X* chromosome, entirely prevents pairing (or, to be more precise, yields random disjunction) but there is still sperm lethality. Neverthless, even if the pairing site and the bomb are not one and the same, if unpaired chromosomes are spermicidal, we would need to understand only one, hopefully simple, mechanism. But is this hypothesis correct?

One way to ask whether a correlation results from direct causation is to ask whether there are exceptions. In this case, we would ask whether there are instances in which unpaired chromosomes do not cause drive, or instances in which drive occurs despite proper pairing. As considered in more depth in the discussion, such exceptions to the correlation of nondisjunction and drive do exist, but they are complex situations that might not tell us much about the archetypal case of rDNA deficiencies. An alternative approach is to ask whether the posited mechanism can actually produce the type of correlation observed. To that end, the data of McKee and Lindsley (1987) have been reexamined using maximum-likelihood methods.

McKee and Lindsley (1987) did two series of crosses. In the first, they examined males carrying a series of *X*-heterochromatin deficiencies, some that deleted part or all of the rDNA and some that did not. Three defects were found in all of the rDNA-deficient males and were not found in males bearing any of the other deficiencies: (1) frequent nondisjunction, (2) meiotic drive, and (3) sterility of *rDNA-deficiency*/$mal^{+}Y$ and *rDNA-deficiency*/$mal^{126}Y$ males. Moreover, the frequency of nondisjunction and the level of sperm mortality appeared to be correlated. In the second series of crosses, the males carried different *rDNA-deficient X* chromosomes, a *Y* chromosome, and a third chromosome—a small free duplication that contained an rDNA array. In these males, the *Y* chromosome and the rDNA-containing duplication disjoined most of the time, and there was little variation in sperm survival from one deficiency to another. Because adding a functional pairing partner for the *Y* reduced deficiency-to-deficiency variation in the level of drive (although its level remained substantial) they concluded that there is a causal relationship between pairing failure and sperm dysfunction.

The following examines the nature of the correlation between disjunction and sperm survival in *rDNA-deficiency*/*Y* males, asks whether a spermicidal effect of unpaired chromosomes can produce that correlation, and takes another look at the relationship between pairing propensity and the level of sperm dysfunction in *rDNA-deficiency*/*Y*/*rDNA-duplication* males.

BASIC METHODOLOGY

Maximum likelihood estimates and hypothesis testing: All of the analyses reported here made use of the MLIKELY.PAS computer program, a general-purpose program for numerical approximation of maximum-likelihood solutions for data generated by crosses. Details of the program and its applications will be presented separately. In the interim, the program and some examples are available at http://www.unisi.it/ateneo/dipart/bio_evol/mlikely.htm/. In addition to providing an estimate of the logarithm of the maximum likelihood of the data given a hypothesis ($\ln \hat{L}$; Fisher 1922; Edwards 1992), MLIKELY.PAS calculates the maximum-likelihood estimates of the parameters of the hypothesis and the expected frequencies and expected numbers for each progeny class. Among all unbiased estimates, the maximum-likelihood estimates (if they exist) have the lowest variance and hence provide the most statistical power.

In general, a series of hypotheses (H_i) is evaluated by first estimating $\ln \hat{L}_{Hi}$ and then making pairwise comparisons using the statistic: $G = 2(\ln \hat{L}_{H_1} - \ln \hat{L}_{H_2})$. G is distributed approximately as χ^2 with degrees of freedom equal to the difference between the number of parameters of the two hypotheses (Bishop *et al.* 1975, Chap.

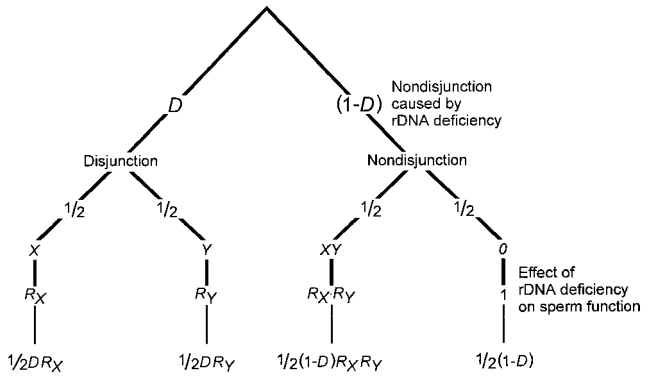

Figure 1.—The descriptive model of McKee (1984) in which the effects of rDNA deficiencies are separately measured by the frequency of sex-chromosome disjunction (D) and the survival of X-bearing sperm (R_X) and Y-bearing sperm (R_Y). *Nullo* sperm are unaffected and have a recovery of 1. These parameters describe the outcomes of the disjunctional process and sperm maturation, but do not imply a mechanism. Differences among deficiencies in D alone will alter the proportion of nondisjunctional progeny, but not the fraction of each sperm type that fail to function. All variation in sperm survival must be accounted for by differences in R_X and R_Y.

4). The approximation to χ^2 is asymptotic and becomes more exact as sample size increases. The particular hypotheses and comparisons needed are described in the following sections.

A note on wording: Except in a cytological context, pairing is something inferred rather than observed, and it is rather easy to ascribe diverse meanings to the words "pairing" or "paired." In this article I have attempted to consistently use paired to refer to "chromosomes that have conjoined well enough to disjoin in a directed fashion to opposite poles, whether they have had a normal or a difficult pairing history," and to use unpaired to refer to "chromosomes that have not disjoined from each other in a directed fasion even if they happen to end up at opposite poles."

ANALYSES

The correlation of disjunction and sperm survival in *rDNA-deficiency/Y* males: Males carrying an X-heterochromatin deficiency and a marked Y chromosome (*e.g.*, a B^sY) produce sperm of four karyotypes that yield distinguishable offspring when crossed to chromosomally normal females. As shown in Figure 1, McKee (1984) described sperm production in terms of the probability of disjunction of the X and Y chromosomes [denoted P in McKee (1984), but here denoted D as a more mnemonic name for "disjunction," and to avoid confusion with pairing itself] and the recoveries (gametic survivals) of sperm bearing the X and Y chromosomes (R_X and R_Y). Note that these parameters are meiotic end points rather than meiotic processes; they describe the results rather than define a mechanism. The probabilities of the four sex-chromosome gamete types are then: $X = \frac{1}{2}DR_X$, $Y = \frac{1}{2}DR_Y$, $XY = \frac{1}{2}(1 - D)R_XR_Y$, and $0 = \frac{1}{2}(1 - D)$. This formulation assumes that the parameters are independent (multiplicative). This assumption is not only a convenience, but it is also consistent with the cytological demonstration of independence made by McKee (1984) in $X/Y/Dp$ males.

The probabilities of the four gamete classes do not add to one because there is another class of sperm—the nonfunctional sperm. The proportions of each genotype among the progeny are therefore these probabilities divided by the total survivors. In other words, the observed frequency of each progeny type, including the *nullo* class, confounds the frequency of sperm lethality with the frequency of nondisjunction. To disentangle disjunction and drive we must calculate D, R_X, and R_Y [for a more extended discussion of the confusion provoked by failing to separately estimate disjunction and sperm survival, see Robbins *et al.* (1996)].

For any individual cross, the four classes contain three independent observations, and there are three parameters. Hence, for any one cross, these equations have unique solutions. Those solutions (McKee and Lindsley 1987) are $D = 1/(1 + \sqrt{XY{\cdot}0/X{\cdot}Y})$, $R_X = \sqrt{X{\cdot}XY/Y{\cdot}0}$, and $R_Y = \sqrt{Y{\cdot}XY/X{\cdot}0}$. The relevant subset of data from McKee and Lindsley (1987) and the calculated values of these descriptive parameters are shown in Table 1.

To examine the relationship of disjunction and sperm survival we need to consider three hypotheses for each of the sperm-survival parameters (R_X and R_Y). For R_X these are:

H1: all three parameters differ among the crosses in the set.

$H2_X$: R_X is the same in all crosses, and the other two parameters differ among the crosses.

$H3_X$: D and R_Y vary among the crosses, but R_X is correlated to D (*e.g.*, by a simple linear correlation $R_X = m \times D + b$).

To give some idea of how these hypotheses are entered into MLIKELY.PAS, Figure 2 shows them in Pascal syntax. Because the nine crosses produce the same offspring classes, a loop is used to calculate expected fractions and expected numbers, with the appropriate parameters referred to for each cross. Only a few lines have to be changed to accommodate the different hypotheses, and the simple linear correlation is truncated at 0 and 1 because sperm survival must fall in that range.

Three G-test comparisons then allow evaluation of variation and correlation. For R_X, the comparisons and their interpretations are:

1. $H2_X$ *vs.* H1: Is there significant variation from cross to cross in the survival of X-bearing sperm? A large G value would indicate that R_X differs among the crosses.

TABLE 1

Disjunction and meiotic drive data for *X/Y* males from McKee and Lindsley (1987) and calculated values of the descriptive parameters *D*, R_X, and R_Y

	Sperm genotype				Meiotic parameters		
	X	*Y*	*XY*	*0*	*D*	R_X	R_Y
Probability	$\frac{1}{2}DR_X$	$\frac{1}{2}DR_Y$	$\frac{1}{2}(1-D)R_XR_Y$	$\frac{1}{2}(1-D)$			
Df(1)X-1	605	138	32	1983	0.534	0.266	0.061
Df(1)GA-90	979	301	88	2396	0.542	0.346	0.106
Df(1)bb158	1013	434	130	1735	0.583	0.418	0.179
Df(1)17-87	403	209	51	748	0.598	0.363	0.188
In(1)sc^{4L}sc^{8R}	809	401	113	1144	0.613	0.446	0.221
Df(1)y^{X15}	360	90	13	721	0.650	0.269	0.067
Df(1)bb74	2054	986	153	2656	0.691	0.346	0.166
Df(1)bb3a	2445	1540	330	2067	0.701	0.503	0.317
Df(1)bb452	3761	2905	57	243	0.966	0.551	0.426

The probabilities of the four classes of sperm are described in terms of the frequency of disjunction (*D*) and the probabilities of recovery of sperm that bear an *X* chromosome (R_X) or a *Y* chromosome (R_Y). The derivation of these probabilities is illusrated in Figure 1, and the solutions for *D*, R_X, and R_Y are described in the text.

2. $H3_X$ *vs.* $H2_X$: Is there a statistically significant correlation between survival of *X*-bearing sperm and disjunction? The higher the value of *G*, the tighter the coupling of the two parameters.
3. $H3_X$ *vs.* H1: How well does the correlation explain the variation in survival of *X*-bearing sperm? If all of the variation of R_X were explained by differences in *D*, and there were no sampling variation, this comparison would yield $G = 0$. If the correlation with *D* explains everything except sampling variation, we would get a low, statistically nonsignificant *G* value. A significant *G* value for this comparison would indicate that there are sources of variation in survival of *X*-bearing sperm beyond that produced by a linear correlation with disjunction. The relative sizes of *G* for this comparison and the preceding then give us an idea of how much of the variation is explained by the correlation. In other words, the larger the value of *G* for the H3 *vs.* H2 comparison, the more statistically significant the correlation, and the smaller the value of *G* for the H3 *vs.* H1 comparison, the more biologically important it is.

Note that under H1 the maximum-likelihood estimates of the parameters are the same as the algebraic solutions for the individual crosses. In contrast, under H2 and H3 the number of parameters is less than the number of independent observations and the maxi-

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VAR
  D, Rx, Ry, m, b, X, Y, XY, NULLO, SurvivingSperm      :REAL;
  Cross, ObsGroup, ParGroup, I                          :BYTE;
  CrossSum                                              :INTEGER;

BEGIN
 FOR Cross := 1 TO 9 DO BEGIN
  ObsGroup := 4*(Cross-1);
 {H1: D, Rx & Ry differ in each cross}   {H2x: Rx same for all crosses}   {H3x: Rx=m*D+b}
  ParGroup := 3*(Cross-1);               ParGroup := 2*(Cross-1);         ParGroup := 2*(Cross -1);
  D := Par[ParGroup+1];                  D := Par[1];                     m := Par[1];
  Rx := Par[ParGroup+2];                 Rx := Par[ParGroup+2];           b := Par[2];
  Ry := Par[ParGroup+3];                 Ry := Par[ParGroup+3];           D := Par[ParGroup+3];
                                                                          Rx := m*D+b;
                                                                          IF Rx > 1 THEN Rx := 1;
                                                                          IF Rx < 0 THEN Rx := 0;
  X := 0.5*D*Rx;                                                          Ry := Par[ParGroup+4];
  Y := 0.5*D*Ry;
  XY := 0.5*(1.0-D)*Rx*Ry;
  NULLO := 0.5*(1.0-D);
  ExpFr[ObsGroup+1] := X;
  ExpFr[ObsGroup+2] := Y;
  ExpFr[ObsGroup+3] := XY;
  ExpFr[ObsGroup+4] := NULLO;
  SurvivingSperm := X + Y + XY + NULLO;
  CrossSum := Obs[ObsGroup+1]+Obs[ObsGroup+2]+Obs[ObsGroup+3]+Obs[ObsGroup+4];
  FOR I := 1 TO 4 DO BEGIN
   ExpFr[ObsGroup+I] := ExpFr[ObsGroup+I]/SurvivingSperm;
   ExpNo[ObsGroup+I] := ExpFr[ObsGroup+I]*CrossSum;
  END;   END;   END;
```

Figure 2.—Pascal coding of three hypotheses about the relationship of disjunction and the effect of the *X* chromosome on sperm function: (H1) all parameters differ in each cross, ($H2_X$) R_X is the same in all crosses, and ($H3_X$) R_X is correlated with disjunction. Similar coding was used to evaluate the relationship of R_Y and *D*.

TABLE 2

Results of maximum-likelihood analysis of the descriptive model

	H1 D, R_X, and R_Y vary	H2$_X$: R_X same; D and R_Y vary	H3$_X$: $R_X = mD + b$; D and R_Y vary	H2$_Y$: R_Y same; D and R_X vary	H3$_Y$: $R_Y = mD + b$; D and R_X vary
No. parameters	27	19	20	19	20
ln $\hat{L}$	−35077.7	−35130.6	−35098.9	−35412.9	−35113.2
G *vs.* H1		106	42	670	71
		(8 d.f., $P < 10^{-10}$)	(7 d.f., $P = 5.2 \times 10^{-7}$)	(8 d.f., $P < 10^{-10}$)	(7 d.f., $P < 10^{-10}$)
G *vs.* H2			63		599
			(1 d.f., $P < 10^{-10}$)		(1 d.f., $P < 10^{-10}$)

Values of ln $\hat{L}$ and G-test comparisons are shown (along with the corresponding probabilities of getting this G value by chance alone). There is highly significant variation of each of the parameters (H2$_X$ or H2$_Y$ *vs.* H1) and a highly significant correlation between chromosome-specific sperm recoveries and disjunction (H2$_X$ *vs.* H3$_X$ and H2$_Y$ *vs.* H3$_Y$). Although significant variation in sperm survival remains, the correlations with D account for most of the differences in sperm function (H3$_X$ or H3$_Y$ *vs.* H1). These results are shown graphically in Figure 3.

mum-likelihood estimates are the minimum variance unbiased averages derived from all of the data.

The results of these comparisons for R_X and R_Y are given in Table 2 and shown graphically in Figure 3. It it clear that McKee and Lindsley's impression of a correlation between drive and disjunction was correct. First, there is substantial, highly significant variation of survival of both *X*-bearing and *Y*-bearing sperm. Second, sperm survival is highly significantly correlated with disjunction. Third, these correlations explain most, although not all, of the variation in sperm survival. For the effect of the *X* chromosome on sperm survival, the single degree of freedom of the correlation explains the major part of the variation, while a minor part is divided among the remaining seven degrees of freedom. In the case of the effect of the *Y* chromosome on sperm recovery, the correlation with disjunction explains nearly 90% of the variation.

Are disjunction and sperm survival correlated because unpaired chromosomes kill sperm? Since McKee subsequently identified a single heterochromatic element responsible for both the disjunctional and sperm-development defects, it would have been indeed surprising if the two had not been correlated in the deficiency series. The deficiency data, however, also allow us to ask about the mechanistic basis of the correlation. One possibility, as embodied in the bomb hypothesis, is that the correlation is causal; sperm carrying unpaired chromosomes are dysfunctional, while sperm carrying chromosomes that had paired survive.

One way to examine this hypothesis is to consider the ratio of *XY* sperm to *nullo* sperm as was done in McKee and Lindsley (1987). Because these two sperm classes come only from cells in which the *X* and *Y* were unpaired, this proportion should be constant under the hypothesis that unpaired chromosomes are themselves spermicidal. The ratio *XY*/*nullo* reduces, in terms of the descriptive parameters, to R_XR_Y and for that product to remain constant one term must decrease if the other increases. In the deficiency series, however, R_X and R_Y are both positively correlated with D. Is this departure from constancy of R_XR_Y (or, equivalently, *XY*/*nullo*) significant? McKee and Lindsley (1987, their Figure 3) considered this and concluded that there was enough spread in the (overlapping) 95% confidence bars for the *XY*/*nullo* ratio that they rejected the hypothesis. A more concrete statistical approach is a simple 2 × 9 contingency test of the numbers of progeny derived from *XY* and *nullo* sperm. The result is $\chi^2 = 441$ with 8 d.f. Quite clearly, the *XY*/*nullo* ratio is not constant as the spermicide model would require.

This test, despite its intuitive appeal, uses only part of the data and does not consider the possibility that only one or the other of the chromosomes conforms to the hypothesis that unpaired chromosomes are themselves spermicidal. Moreover, the fit of this mechanism for one of the chromosomes might be so good, even though the predicted response of sperm bearing the other chromosome is opposite to that observed, that we would still want to consider it. To examine these possibilities, we again call upon likelihood methods.

The "unpaired chromosomes are spermicidal" hypothesis is diagramed in Figure 4. If the *X* and *Y* chromosomes pair (with probability P), they disjoin giving us *X* and *Y* karyotypes, and, because the chromosomes have paired, all of these sperm survive. If the *X* and *Y* chromosomes do not pair, random movement will yield all four gamete types, but because the chromosomes have not paired they will kill some of the sperm that get them. The less often the chromosomes pair, the more sperm will bear a lethal cargo. In other words, the hypothesis that it is the unpaired state of the chromosomes that causes the correlation of drive and disjunction does not require that chromosome-specific sperm survival differ from cross to cross. The only thing that needs to change is the frequency of unpaired chromosomes. The recoveries of sperm that carry unpaired chromosomes in this hypothesis are named R'_X and R'_Y

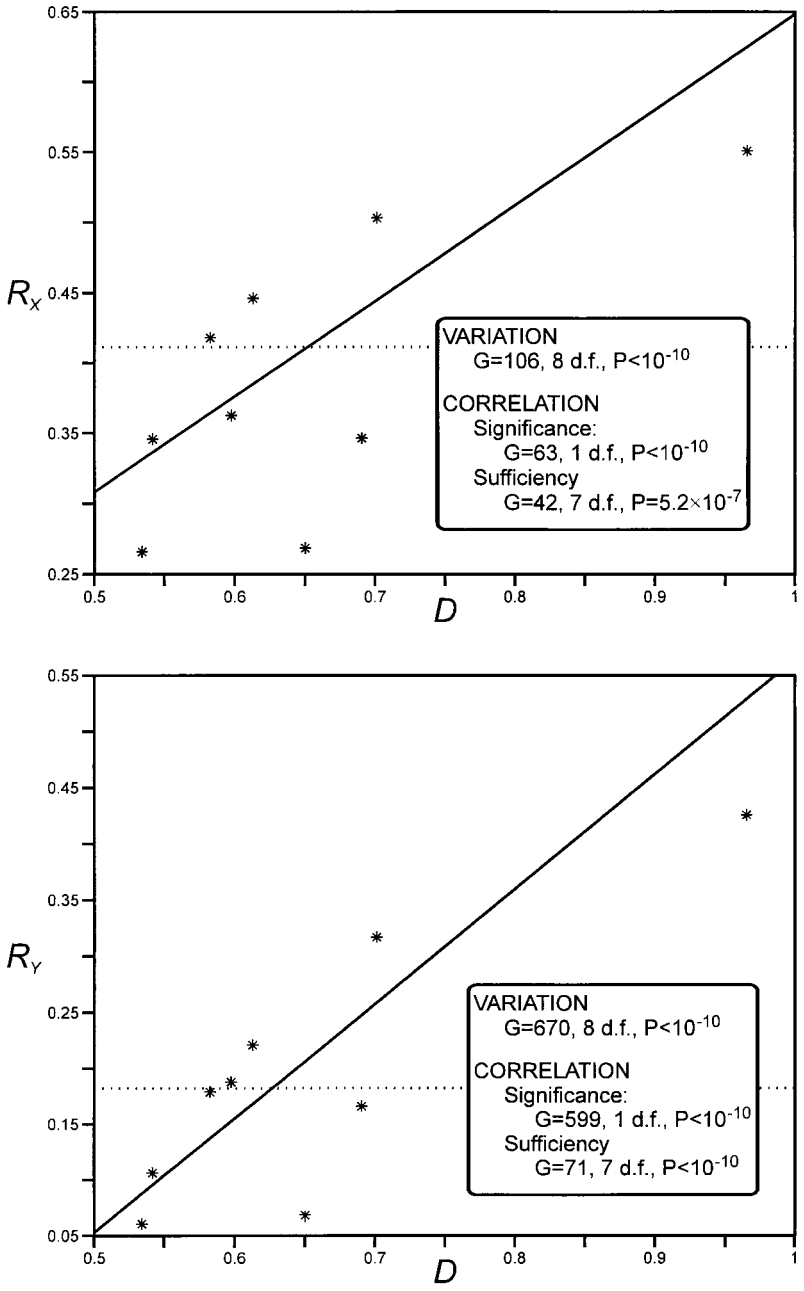

Figure 3.—The observed relationship of disjunction and sperm survival. The plotted points are the parameters calculated H1. The dotted lines are the maximum-likelihood estimates of invariant sperm survival ($H2_X$ and $H2_Y$). The solid lines are the maximum-likelihood estimates of sperm recoveries if correlated to disjunction ($H3_X$ and $H3_Y$).

to emphasize that they do not mean the same thing as the survivals among all sperm (R_X and R_Y) in the descriptive algebra.

The probabilities of the four gamete types are then: $X = \frac{1}{2}P + \frac{1}{4}(1 - P)R'_X$, $Y = \frac{1}{2}P + \frac{1}{4}(1 - P)R'_Y$, $XY = \frac{1}{4}(1 - P)R'_Y$, and $0 = \frac{1}{4}(1 - P)$, and the solutions, for individual crosses, are

$$R'_X = \frac{X - Y + \sqrt{Y^2 - 2X{\cdot}Y + X^2 + 40{\cdot}XY}}{20},$$

$$R'_Y = \frac{Y - X + \sqrt{Y^2 - 2X{\cdot}Y + X^2 + 40{\cdot}XY}}{20},$$

$$P = \frac{X - R'_X 0}{20 - R'_X 0 + X}.$$

P, R'_X, and R'_Y are probabilities and, as such, must have values between 0 and 1, but the data for *Df(1)bb452/Y* yield $R'_X = 3.5881$. Although tempted to reject the model forthwith, we note that disjunction in *Df(1)bb452/Y* males is much closer to normal than in any of the others. Because so few of the progeny of *Df(1)bb452/Y* males are nondisjunctional, a small zygotic inviability of the regular sons, *e.g.*, a marker effect, could lead to an apparent $R'_X > 1$. Because *Df(1)bb452* may be an outlier, the likelihood analysis was done twice: first with R'_X for *bb452* allowed to exceed one, and second using only the data for the other eight deficiencies.

The design of this analysis is outlined in Figure 5. First, we consider the two hypotheses: (H1) all three parameters vary from cross to cross (the solutions given

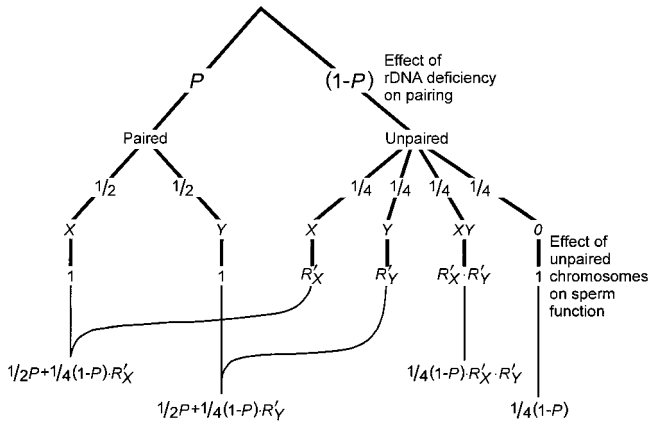

Figure 4.—A model in which unpaired chromosomes cause sperm lethality. Here, P is the frequency of pairing of the X and Y chromosomes, and if they fail to pair they disjoin at random. Only unpaired chromosomes cause sperm dysfunction, and the recoveries of sperm bearing the X and Y chromosomes are R'_X and R'_Y. In this view, differences in P alone will change sperm survivals—the more often chromosomes remain unpaired, the more often sperm die.

above apply to each individually) and (H2) P differs from cross to cross, but there is a single value of R'_X and a single value of R'_Y for all of the crosses. If the spermicide hypothesis is correct for both chromosomes, there should be no significant difference between the two. The results of the maximum-likelihood analysis, shown in the first line of Table 3, shows this is extremely improbable, just as the comparison of *XY* and *nullo* sperm already indicated.

HYPOTHESES FOR TESTING THE SPERMICIDAL-CHROMOSOME MODEL

P = pairing, R'_X = recovery of sperm bearing an unpaired X, R'_Y = recovery of sperm bearing an unpaired Y

H1	P, R'_X and R'_Y are different in each cross
H2	P differs among the crosses, but R'_X and R'_Y are the same in all crosses
$H2_X$	P and R'_Y differ among the crosses, but R'_X is the same in all crosses
$H2_Y$	P and R'_X differ among the crosses, but R'_Y is the same in all crosses

COMPARISONS $G = 2\times(\ln\hat{L}_{Hi} - \ln\hat{L}_{Hj})$

H2 *vs.* H1	Are only unpaired X and Y chromosomes spermicidal?
$H2_X$ *vs.* H1	Are only unpaired X chromosomes, but both paired and unpaired Y chromosomes, spermicidal?
$H2_Y$ *vs.* H1	Are only unpaired Y chromosomes, but both paired and unpaired X chromosomes, spermicidal?

Figure 5.—Hypotheses and comparisons for a maximum-likelihood analysis of the causal model. If only unpaired chromosomes can cause sperm dysfunction, differences in survival will occur if the frequency of pairing changes even if the chromosome-specific recoveries remain unchanged. A hypothesis was evaluated in which both unpaired sex chromosomes are spermicidal (H2), as were two hypotheses that asked whether this might be true for only one or the other ($H2_X$ and $H2_Y$).

Is the variation in recovery of sperm bearing just one or the other of the sex chromosomes accounted for by changes in the frequency of pairing? As indicated in Figure 5, we now compare two other hypotheses to H1: ($H2_X$) a single value of R'_X applies to all of the crosses, while both P and R'_Y vary from cross to cross and ($H2_Y$) a single value of R'_Y applies to all of the crosses, while both P and R'_X vary from cross to cross. As shown in the remainder of Table 3, neither situation provides a fit to the data. Hence, a spermicidal effect of unpaired X chromosomes and a more indirect effect on Y-bearing sperm, or of unpaired Y chromosomes and a more indirect effect on X-bearing sperm, can also be excluded.

Are there two populations of sperm? It is clear from the preceding that sperm derived from nondisjunctional cells are not uniformly unhealthy. At this point, however, there are two further things to ask about the behavior of sperm derived from disjunctional *vs.* nondisjunctional cells. The answers will not be as clear-cut as they were for the preceding, but they will at least tell us something about the range of possible mechanisms.

First, do the data actually provide evidence for two different populations of sperm? If sperm derived from nondisjunctional cells are, on average, less healthy than sperm derived from cells in which disjunction was successful, the spermicide model, even though it leaves a significant part of the experimental variation unexplained, should explain some of it. That is, the expectations for $H2_X$ and $H2_Y$ in the causal model should be closer to the observations than the expectations for $H2_X$ and $H2_Y$ in the descriptive formulation. In other words, the values of G should be smaller for the causal model. Because there are equal numbers of parameters in both models, there are no degrees of freedom left for a statistical test, and there is only a prediction of the direction of change. The fit of the causal model is somewhat better in the case of lethality of Y-bearing sperm ($G_{descriptive} = 670$ *vs.* $G_{causal} = 283$ with *bb452* included), but for lethality of X-bearing sperm it is not ($G_{descriptive} = 106$ *vs.* $G_{causal} = 329$). Hence, as far as these data tell us, sperm derived from both populations of cells could be equally unhealthy. To decide whether there are two populations of sperm requires either an assumption or data beyond those provided by studying segregation.

Second, if we wish to think about mechanisms in which only sperm derived from nondisjunctional cells are unhealthy (albeit nonuniformly unhealthy), we can ask how they would have to behave in order to conform to the data. That is, we can ask how the survivals of sperm bearing nondisjunctional chromosomes (R'_X and R'_Y) are related to pairing. When we do, we find that there is a significant positive correlation of R'_X with P ($G = 288$, 1 d.f., $P < 10^{-10}$) and of R'_Y with P ($G = 96$, 1 d.f. $P < 10^{-10}$). Hence, any proposed mechanism must provide for increased chromosome-specific sperm lethality with declining meiotic health within the affected

TABLE 3

Results of maximum-likelihood analysis of the spermicidal-chromosome model

	With *Df(1)bb452*	Without *Df(1)bb452*
H2 *vs.* H1	$G = 668$, 16 d.f., $P < 10^{-10}$	$G = 518$, 14 d.f., $P < 10^{-10}$
$H2_X$ *vs.* H1	$G = 329$, 8 d.f., $P < 10^{-10}$	$G = 219$, 7 d.f., $P < 10^{-10}$
$H2_Y$ *vs.* H1	$G = 282$, 8 d.f., $P < 10^{-10}$	$G = 266$, 7 d.f., $P < 10^{-10}$

Maximum-likelihood analyses were done for the hypotheses outlined in Figure 5. Initial analysis (see text) indicated that the data for *bb452* are inconsistent with this model, but its disjunction is so nearly normal that it may be an outlier. The analyses were therefore done in two fashions. In one case, survival of *X*-bearing sperm was allowed to exceed 1 (an exception that affects only *bb452*). In the other, the *bb452* data were excluded. Whether one evaluates a hypothesis in which both unpaired sex chromosomes are spermicidal (H2) or the two hypotheses that asked whether this might be true for only one or the other chromosome ($H2_X$ and $H2_Y$), there is a large difference between the predictions of the spermicidal-chromosome model and the observations, and the probabilities of getting differences this big by chance are exceedingly low.

population of sperm, even if that population comes only from nondisjunctional cells.

Nondisjunction and meiotic drive in *X/Y/Dp* males: McKee and Lindsley also asked whether meiotic drive varies with different deficiencies in *rDNA-deficiency/Y/rDNA-duplication* males despite the fact that the *Y* and *Dp* elements disjoin most of the time. Their data are shown in Table 4. They simplified their analysis by assuming that the *Y* and *Dp* always disjoin, and they excluded *X* and *YDp* sperm from their calculations. Plotting measures of sperm survival against the frequencies of *X-Y* disjunction observed in the crosses of *X/Y* males (*D* in the foregoing), they saw little variation and were unable to perceive any correlation. Maximum-likelihood analysis, however, proves more sensitive than this eyeball test.

As shown in the top portion of Table 5, we start this analysis, as did McKee and Lindsley, by ignoring *X* and *YDp* sperm and assuming that the *Y* and *Dp* always disjoin. If the *Y* and *Dp* always disjoin, only four gamete types will be observed: *XY*, *XDp*, *Y*, and *Dp*. Because all of these gametes contain either the *Y* or the *Dp*, recovery of those two elements cannot be separately evaluated. Therefore, following McKee (1984) and McKee and Lindsley (1987), recovery of the *Y* chromosome relative to the *Dp*, rather than absolute recovery, is calculated. Among meioses in which the *Y* and *Dp* disjoin, the *X* chromosome might go to either the *Dp* or *Y* pole. With D'' = the proportion of $XDp{\leftrightarrow}Y$ disjunction, R''_X = the recovery of *X*-bearing sperm, and $R_{Y/Dp}$ = the recovery of *Y*-bearing sperm relative to *Dp*-bearing sperm, we have $XDp = \frac{1}{2}D''R''_X$, $Y = \frac{1}{2}D''R''_{Y/Dp}$, $XY = \frac{1}{2}(1 - D'')R''_XR''_{Y/Dp}$, and $Dp = \frac{1}{2}(1 - D'')$. The solutions are

$$D'' = \frac{1}{1 + \sqrt{XY{\cdot}Dp/XDp{\cdot}Y}},$$

$$R''_X = \sqrt{XDp{\cdot}XY/Y{\cdot}Dp},$$

$$R''_{Y/Dp} = \sqrt{Y{\cdot}XY/XDp{\cdot}Dp}.$$

To compare these parameters with *D*, the analysis must account for the sampling variation in the data from both the *X/Y/Dp* and *X/Y* crosses. That is, we must simultaneously estimate *D*, R_X, and R_{YDp} (using the *X/Y* data) and D'', R''_X and R''_{Y/D_p} (using the *X/Y/Dp* data). For each parameter to be examined, we then evaluate three hypotheses: (H1) all six parameters vary among the crosses; (H2) one parameter of the *X/Y/Dp* crosses is the same for all crosses, and the other parameters vary; and (H3) one parameter of the *X/Y/Dp* crosses is correlated to *D*, but the other parameters vary.

The results of this analysis are summarized in the bottom part of Table 5. First, the proportions of $XDp{\leftrightarrow}Y$ and $Dp{\leftrightarrow}XY$ disjunctions do not vary significantly, and none of the values of D'' (not shown) are far from $\frac{1}{2}$. Second, recovery of *X*-bearing sperm does vary and shows a highly significant *negative* correlation with disjunction of the *X* and *Y* in *X/Y* males. Although the correlation leaves a majority of the variation of R''_X unexplained, suggesting that this is a secondary phenomenon, the slope is substantial. Last, although the recovery of the *Y* relative to that of the *Dp* also varies significantly, there is no correlation of that compound parameter with the disjunctional propensity of the *X* and *Y* chromosomes.

The foregoing analysis included, as did McKee and

TABLE 4

Sperm produced by *X/Y/Dp* males

	Sperm genotype					
	XDp	*Y*	*XY*	*Dp*	*X*	*YDp*
Df(1)X-1	270	281	50	1180	5	1
Df(1)GA-90	615	570	136	2100	80	0
Df(1)bb158	296	292	80	899	8	1
$In(1)sc^{4L}sc^{8R}$	926	833	283	1859	7	0
$Df(1)y^{X15}$	14	9	4	40	0	0
Df(1)bb74	217	186	45	746	15	0
Df(1)bb3a	222	264	56	764	2	0
Df(1)bb452	386	508	71	1486	100	9

Data from McKee and Lindsley (1987) for crosses of *X/Y/Dp* males bearing eight of the nine deficiencies that they had tested in *X/Y* males.

TABLE 5

Maximum-likelihood analysis of chromosome behavior in *X/Y/Dp* males using a model that excludes *X* and *YDp* sperm

Parameters for *X/Y/Dp* crosses		Probabilities of sperm genotypes
XDp↔*Y* and *Dp*↔*XY* disjunctions only; *X* and *YDp* sperm classes ignored		
$D'' = XDp \leftrightarrow Y$ disjunction		$XDp = \frac{1}{2}D''R''_X$
$1 - D'' = Dp \leftrightarrow XY$ disjunction		$Y = \frac{1}{2}D''R''_{Y/Dp}$
R''_X = recovery of *X*-bearing sperm		$XY = \frac{1}{2}(1 - D'')R''_X R''_{Y/Dp}$
$R''_{Y/Dp}$ = relative recovery of *Y*-bearing sperm		$Dp = \frac{1}{2}(1 - D'')$
	D''	R''_X	$R''_{Y/Dp}$
Variation (7 d.f.)	8.9 (0.26)	143 ($<10^{-10}$)	74.3 ($<10^{-10}$)
Correlation with *D*			
Slope	0.11	−0.31	−0.05
Significance (1 d.f.)	5.6 (0.02)	41 (2×10^{-10})	0.7 (0.40)
Sufficiency (6 d.f.)	3.4 (0.76)	102 ($<10^{-10}$)	73.7 ($<10^{-10}$)

Maximum-likelihood analysis was done simultaneously using the *X/Y/Dp* and corresponding *X/Y* data. The parameters and sperm probabilities for the *X/Y/Dp* data are shown here and described in the text, while the parameters and sperm probabilities for the *X/Y* males were the same as those shown in Figure 1. Values for $\ln \hat{L}$ and parameter estimates were obtained for three classes of hypotheses: (H1) all parameters vary among crosses, ($H2_i$) one parameter for the *X/Y/Dp* males is constant in all crosses, and ($H3_i$) one parameter for the *X/Y/Dp* males is correlated to the disjunctional behavior of *X/Y* males (*D*). Values for *G*-test comparisons of these hypotheses are shown (along with the corresponding probabilities of getting this *G* value by chance alone).

Lindsley's, the assumption that the *Y* and *Dp* always disjoin from one another and ignored *X* and *YDp* sperm. For some deficiencies, however, a substantial number of *X*-bearing sperm and a small number of *YDp*-bearing sperm were actually recovered. Because those sperm must come from meioses in which the *Y* and *Dp* went to the same pole, the assumption fails. It is possible that meiotic drive is constant in sperm derived from *Y*↔*Dp* disjunctions, as McKee and Lindsley thought, and that the apparent variation seen in the preceding analysis depends on the size of the subpopulation of cells in which the *Dp* did not disjoin from the *Y*—a subpopulation where *X*-*Y* interactions were strong. To evaluate this possibility, we must revise the algebraic description to include all of the progeny classes.

An alternative description of events in *X/Y/Dp* males is outlined in Table 6. In this model, corresponding to the descriptive view of *X/Y* males, all three disjunctions (*XY*↔*Dp*, *Y*↔*XDp*, and *X*↔*YDp*) occur, and sperm survival depends on chromosome content and not on disjunctional origin. With the additional offspring classes, effects of the *Y* and the *Dp* on sperm survival (R''_Y and R''_{Dp}) can be separately evaluated and the compound parameter $R''_{Y/Dp}$ is not needed. The results are summarized in the bottom portion of Table 6. There is a highly significant correlation of the frequency of *X*↔*YDp* disjunction in *X/Y/Dp* males with the frequency of *X*↔*Y* disjunction in *X/Y* males (*D*), but, with little *X*↔*YDp* disjunction in any of the crosses, the slope is quite shallow. Although this disjunctional class is now explicitly included, there nevertheless remains a highly significant negative correlation, with slope = −0.31, of recovery of *X*-bearing sperm produced by *X/Y/Dp* males with *X*↔*Y* disjunction in *X/Y* males. As with the model considered in Table 5, although significant and strong, the correlation explains only about a third of the variation of *X*-bearing sperm survival in *X/Y/Dp* males. Nevertheless, it is clearly not an artifact of having ignored *X*↔*YDp* disjunction and *X* and *YDp* offspring.

DISCUSSION

Obviously, the central conclusion reached in McKee and Lindsley (1987), that the nondisjunction and meiotic drive (sperm lethality) caused by *X*-heterochromatin deficiencies in *XY* males are strongly correlated with each other, is vigorously supported by the analysis presented here. The more formal analysis does, however, permit asking whether unpaired chromosomes *per se* poison sperm development. A model in which only unpaired chromosomes affect sperm development does not predict the positive correlation of disjunction with survival of both *X*-bearing and *Y*-bearing sperm among total surviving sperm seen in the data, and it does not account for any significant portion of the variation in sperm lethality. Failure of disjunction of a chromosome does not convert it into a spermicide; the two phenomena, disjunctional failure and sperm lethality, share a common underlying cause.

For *X/Y/Dp* males, the eyeball test by which McKee and Lindsley discerned no pattern proves to be somewhat misleading. In most cells of *X/Y/Dp* males, the *Y* disjoins from the duplication. Nevertheless, (1) the more likely the *X* and *Y* are to disjoin in *X/Y* males, the

TABLE 6

Maximum-likelihood analysis of chromosome behavior in *X/Y/Dp* males using a model that includes all sperm classes

Parameters for *X/Y/Dp* crosses	Probabilities of sperm genotypes
XDp↔*Y*, *Dp*↔*XY*, and *X*↔*YDp* disjunctions	
D_1 = *X*↔*YDp* disjunction	$XDp = \frac{1}{2}D_2R''_XR''_{Dp}$
D_2 = *Y*↔*XDp* disjunction	$Y = \frac{1}{2}D_2R''_Y$
$1 - D_1 - D_2$ = *XY*↔*Dp* disjunction	$XY = \frac{1}{2}(1 - D_1 - D_2)R''_XR''_Y$
R''_X = recovery of *X*-bearing sperm	$Dp = \frac{1}{2}(1 - D_1 - D_2)R''_{Dp}$
R''_Y = recovery of *Y*-bearing sperm	$X = \frac{1}{2}D_1R''_X$
R''_Y = recovery of *Dp*-bearing sperm	$YDp = \frac{1}{2}D_1R''_YR''_{Dp}$

	D_1	D_2	R''_X	R''_Y	R''_{Dp}
Variation (7 d.f.)	73 ($<10^{-10}$)	5.1 (0.65)	143 ($<10^{-10}$)	11.5 (0.12)	11.5 (0.12)
Correlation with *D*					
Slope	0.05	0.06	−0.31	0.07	0.26
Significance (1 d.f.)	43 (1×10^{-10})	1.7 (0.19)	41 (2×10^{-10})	3.0 (0.08)	3.1 (0.08)
Sufficiency (6 d.f.)	30 (4×10^{-5})	3.4 (0.76)	102 ($<10^{-10}$)	8.5 (0.20)	8.4 (0.21)

Maximum-likelihood analysis was done simultaneously for the *X/Y/Dp* and corresponding *X/Y* data using a model for disjunction and sperm recovery in *X/Y/Dp* males that includes all sperm types. The design of the analysis and the format of the table are the same as for Table 5.

more likely they are to disjoin in *X/Y/Dp* males, and (2) the better the *X* and *Y* are able to interact, the lower the survival of *X*-bearing sperm in the *X/Y/Dp* males. In other words, in rDNA-deficient *X/Y/Dp* males, a healthier interaction between the *X* and *Y* does not lead to healthier sperm. Although strong and highly significant statistically, this interaction is only evident in *X*-bearing sperm and does not account for the majority of the variation in drive. In these males, where *Y-Dp* disjunction predominates, interaction of the *X* and *Y* is disruptive, but this is a second-order phenomenon superposed on the basic damage to sperm survival caused by rDNA deficiency.

There are several previously described situations in which sex-chromosome meiotic drive is not tightly correlated to pairing failure, but none of them definitely rules out the spermicidal-chromosome hypothesis. First, there is the case of *rDNA-deficiency/Y/rDNA-duplication* males (McKee 1984; McKee and Lindsley 1987), the minor effects of different deficiencies aside. In these males, disjunction of the *Y* chromosome is quite regular; it almost always goes to the pole opposite the *Dp*. Nevertheless, recovery of *Y*-bearing sperm is very severely depressed. By itself this would not necessarily rule out the bomb hypothesis. The bomb cannot be the rDNA itself, and defusing might require a pairing-mediated interaction between the *Y* chromosome and some other site in the *X* chromosome that does not exist in the duplication.

A second exception is the behavior of certain *X*-chromosome translocations such as *T(1;4)B*^{S} [the first situation in which meiotic drive was ever demonstrated (Novitski and Sandler 1957)]. McKee (1987) showed that translocation-related meiotic drive also involves the rDNA; sperm recovery is distorted only if the translocation breakpoint separates the rDNA from a euchromatic site near salivary segment 13. Although segregation is quite regular in these males, there is a substantial deficit of *Y*-bearing sperm. Nevertheless, this too would not by itself eliminate the hypothesis that unpaired chromosomes are spermicidal. "Regular" segregation in a translocation heterozygote does not have the same meaning as regular segregation of two simple homologs. Although the base of the *X* and the *Y* invariably go to opposite poles, the distal part of the *X* is not always included. Indeed, *X*^{basal}-only sperm are recovered more frequently than any other type. As for rDNA-deficient *X/Y/Dp* males, all one would have to posit to make these data fit with an "unpaired chromosomes are themselves spermicidal" model would be failure of an interaction of the *Y* and the distal part of the *X*.

A third exception is the behavior of *cry*$^{-}$ males (Palumbo *et al.* 1994; Robbins *et al.* 1996). When the *X* chromosome carries fewer than about 60 copies of *Ste*, *cry*$^{-}$ males are more or less fertile, but exhibit both disjunctional failures and meiotic drive. There is a very tight inverse correlation of fertility and disjunction with *Ste* copy number in these males, being close to normal with about 15 copies of *Ste*. Chromosome-specific sperm recovery, however, hardly varies with *Ste* copy number. Although here pairing and drive are seemingly uncoupled or only weakly coupled, this situation might have, *ab initio*, little to do with the rDNA-related cases. Not only does the *cry-Ste* interaction produce nondisjunction and sperm dysfunction when the rDNA arrays are intact, but it also causes nondisjunction and drive of the autosomes.

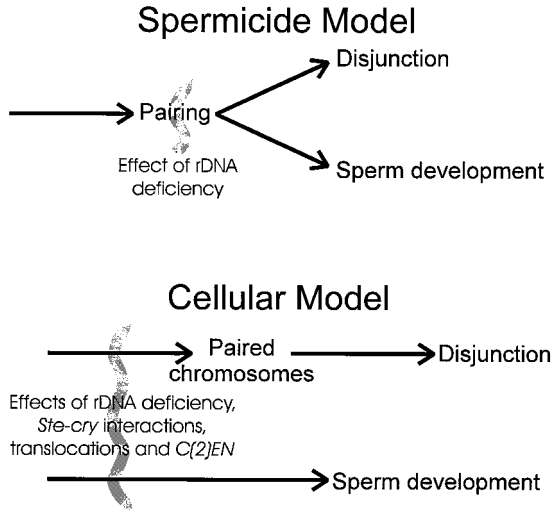

Figure 6.—Models that posit that only unpaired chromosomes cause sperm dysfunction are inconsistent with the data from McKee and Lindsley (1987) and are inconsistent with the behavior of *X/Y/Dp* males, the *Ste-cry* interaction, and *X-4* translocations. Thus, rDNA deficiencies, and these other systems, affect one or more processes that are common to both disjunction and sperm development. The cellular process or processes may be (or may include) pairing, if by pairing one means the physical behavior of homologs in prophase I, rather than just the conjunction of chromosomes requisite for disjunction.

Last, when McKee (1984) examined the behavior of a grossly asymmetrical autosomal translocation in rDNA-deficient males, he found that sperm survival was lower when the larger half-translocation was present, but that the level of autosomal meiotic drive was the same in *X-Y* disjunctional and *X-Y* nondisjunctional sperm. In other words, autosomal drive was independent of the disjunctional state of the sex chromosomes. However, we do not know the pairing state of the autosomal elements themselves in this situation, and that is what is really pertinent to the model.

Our current state of knowledge is illustrated in Figure 6. Having now ruled out the spermicidal chromosome model in even the relatively simple situation of rDNA-deficient *X/Y* males, we will perforce have to consider more complex mechanisms. But having ruled out that mechanism in the simplest situation, we also have no reason now to think of the *X/Y/Dp*, translocation, *cry-Ste*, or *C(2)EN* situations as involving distinctly different processes.

From segregation experiments alone, it is not possible to decide whether only sperm derived from nondisjunctional cells are unhealthy, so the range of possible cellular mechanisms is very broad. Nevertheless, we do know that, whether all sperm or only a subpopulation of sperm are affected in rDNA-deficient *X/Y* males, within the affected population *per se* the worse the meiotic anomaly, the lower the chromosome-specific sperm survival.

McKee *et al.* (1998) have recently suggested a mechanism by which pairing difficulties could lead to sperm elimination downstream, without depending on the disjunctional state of the chromosomes contained in the particular sperm. They suggest that misalignment of chromosomes at metaphase could trigger a checkpoint system that tags the meiotic products for elimination instead of causing anaphase delay. Rather than correcting or preventing aneuploidy, the potentially aneuploid products would be eliminated. A mechanism like this would, quite neatly, allow a variety of aberrant situations to produce similar sperm-dysfunction phenotypes. While this is a concrete example of a cellular hypothesis, it is, however, not entirely devoid of complexity. First, as McKee *et al.* note, the response to triggering the checkpoint cannot be all-or-none. First, not all products of cells with bad metaphase alignment are eliminated since some *XY* and *nullo* sperm do survive. Second, the response must, in some way, depend on the degree of meiotic anomaly since the ratio of *XY* to *nullo* sperm varies with the frequency of disjunction, even though these products come only from nondisjunctional meioses. To provide a variable reponse while retaining a qualitative trigger, McKee *et al.* suggest the possibility of competition among sperm. However, that competition, if it exists, has a peculiar property—when disjunction is better, there are more "normal" competitors, but survival of the "abnormal" sperm increases rather than decreases. Alternatively, we could imagine that the signal itself could have some quantitative, or competitive, aspect. There is one inference from the present analysis that is consistent with competition, but it does not tell us whether it is at the level of the trigger or at the level of the response. In rDNA-deficient *X/Y/Dp* males, a healthier interaction between the *X* and *Y* reduces overall survival of *X*-bearing sperm.

Although statistical analysis is revealing, it is certainly no substitute for doing experiments. For meiotic behavior in *D. melanogaster* males, what it tells us is that there is not a simple, direct, mechanistic connection between sperm survival and whether a chromosome has or has not paired. This, in turn, suggests that we should be able to find additional regulatory elements. Moreover, the negative correlation of survival of *X*-bearing sperm with *X-Y* disjunctional propensity in *X/Y/Dp* males could arise, if not from competition, from the existence of both negative and positive controls. The several systems that disrupt meiosis and sperm development suggest some candidates to look at, and it will probably be worthwhile to examine interactions among these systems. A systematic search for loci that interact with or mimic rDNA, *Ste*, or *cry* deficiencies might reveal others, and two examples of autosomal, single-gene mutations that mimic *cry* deficiencies, *sting* (Schmidt *et al.* 1998) and *scratch* (G. Palumbo, personal communication), are already in hand.

This is not a simple system, however. Understanding

regulation of the achiasmate meiosis of *D. melanogaster* males will require experimental analysis of the interactions of these loci with each other, with the rDNA, and with different combinations of pairing partners. Parsing these interactions will require statistical analyses that are just as careful as the design of the experiments.

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