The Genetic Analysis of Distributive Segregation in *Drosophila melanogaster*. I. Isolation and Characterization of *Aberrant X segregation* (*Axs*), a Mutation Defective in Chromosome Partner Choice

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ABSTRACT

We describe the isolation and characterization of *Aberrant X segregation* (*Axs*), a dominant female-specific meiotic mutation. Although *Axs* has little or no effect on the frequency or distribution of exchange, or on the disjunction of exchange bivalents, nonexchange *X* chromosomes undergo nondisjunction at high frequencies in *Axs*/+ and *Axs*/*Axs* females. This increased *X* chromosome nondisjunction is shown to be a consequence of an *Axs*-induced defect in distributive segregation. In *Axs*-bearing females, fourth chromosome nondisjunction is observed only in the presence of nonexchange *X* chromosomes and is argued to be the result of improper *X* and fourth chromosome associations within the distributive system. In *XX* females bearing a compound fourth chromosome, the frequency of nonhomologous disjunction of the *X* chromosomes from the compound fourth chromosome is shown to account for at least 80% of the total *X* nondisjunction observed. In addition, *Axs* diminishes or ablates the capacity of nonexchange *X* chromosomes to form trivalents in females bearing either a *Y* chromosome or a small free duplication for the *X*. *Axs* also impairs compound *X* from *Y* segregation. The effect of *Axs* on these segregations parallels the defects observed for homologous nonexchange *X* chromosome disjunction in *Axs* females. In addition to its dramatic effects on the *X* chromosome, *Axs* exerts a similar effect on the segregation of a major autosome. We conclude that *Axs* defines a locus required for proper homolog disjunction within the distributive system.

THE general mechanism of disjunction in most eukaryotes consists of homologous pairing, exchange, and segregation of exchange partners. The basic features of the exchange-mediated segregation system and our current understanding of the mechanisms by which exchange chromosomes disjoin has recently been reviewed by HAWLEY (1988).

Although exchange itself is sufficient to ensure proper chromosome segregation in *Drosophila melanogaster* females, several lines of evidence suggest the existence of another exchange-independent system of segregation. In females with structurally normal *X* chromosomes, the frequency of nonexchange bivalents is approximately 6.0% while the frequency of spontaneous *X* nondisjunction is approximately 0.5% (BRIDGES 1916; WEINSTEIN 1936). In addition, inversion heterozygotes with a reduced frequency of exchange between *X* chromosomes exhibit spontaneous frequencies of *X* nondisjunction significantly lower than expected if the exchange-mediated system were the only disjunction mechanism present in Drosophila females (COOPER 1945). Finally, although the fourth chromosomes in Drosophila are always nonexchange (STURTEVANT 1951), they segregate from each other with great efficiency; the frequency of fourth chromosome nondisjunction is approximately 0.1% (*cf.* BAKER and CARPENTER 1972). These results demonstrate that an alternative mechanism of exchange-independent segregation must exist.

BRIDGES (1916) obtained evidence that such exchange-independent segregations followed a different set of rules than did segregations mediated by exchange. When examining sex chromosome disjunction in *XXY* females, he observed frequencies of *X* nondisjunction much higher than those observed in *XX* females. These *X* nondisjunctional events involved primarily nonexchange *X* chromosomes that appeared to segregate from the *Y* chromosome. When the *X* chromosomes underwent exchange, they segregated from each other faithfully with the *Y* going at random. Bridges argued that this nondisjunction resulted from pairing of an *X* and *Y* chromosome while the remaining *X* segregated at random; this process was termed secondary nondisjunction. COOPER (1948) later showed that secondary nondisjunction can be accounted for by the formation of an *XXY* trivalent in which the *Y* directs the two nonexchange *X* chromosomes (*XX* ↔ *Y*).

GRELL (1962a, b) first proposed the existence of a novel segregation system, termed distributive segregation, to explain both secondary nondisjunction and

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the disjunction of the always nonexchange fourth chromosomes. Grell also proposed that the segregation of other nonhomologs, of nonexchange *X* chromosomes (GRELL 1967), and of compound chromosomes (GRELL 1963) was the responsibility of the distributive system. She argued that this female-specific distributive system was independent of the exchange-mediated system and does not play a role in the achiasmate meiosis found in Drosophila males (GRELL 1970).

GRELL also presented evidence that the choice of disjunctional partners within the distributive system is based on factors such as availability, size, and shape of chromosomes, and not on homology (GRELL 1964a, b; MOORE and GRELL 1972). In other words, if two nonexchange elements are present in a given meiocyte, they will segregate from each other faithfully regardless of their identity. When more than two elements are present however, they will choose disjunctional partners solely on the basis of the size and shape of chromosomes.

An example of the dependence on size within the distributive system is observed in experiments performed by O'TOUSA (1982). In these studies, a number of free *X* chromosome duplications of varying sizes were tested for their ability to interfere with fourth chromosome segregation. When the free duplication equals the size of the fourth chromosome, four nondisjunction rises dramatically, presumably as a consequence of duplication ↔ fourth chromosome segregation while the remaining four segregates at random. The observation that such segregations were much less frequent when the duplication was larger or smaller than the fourth chromosome provides an example of the dependence on size within the distributive system.

In addition to secondary nondisjunction (described above), the nonrandom assortment of other nonhomologs (*i.e.*, *X* and *4*, compound autosomes, and various other nonhomologous combinations) has also been shown to result from nonhomologous disjunction, and has provided evidence that two acrocentric chromosomes will generally be oriented by a metacentric chromosome when the elements involved are nonexchange (COOPER, ZIMMERING and KRIVSCHENKO 1955; SANDLER and NOVITSKI 1956; GRELL 1959a, b, 1962a; OKSALA 1958; FORBES 1960; GRELL and GRELL 1960). These experiments demonstrate the dependence of distributive disjunction on shape.

Based on models proposed by CARPENTER (1973) and O'TOUSA (1982) we outline a three phase model for distributive segregation: (1) selection of nonexchange chromosomes that will enter the distributive system; (2) determination and orientation of proper (generally homologous) chromosome partners; and (3) disjunction of chromosomes to opposite poles at meiosis I. Although many mutations affecting the frequency and distribution of exchange have been isolated and characterized (reviewed in BAKER and HALL 1976), only three mutations affecting the distributive system have so far been isolated; *ald*, *mei-S51* and *nod*. The three proposed phases of distributive segregation have been genetically defined by these mutations.

The first phase of the model, the selection of nonexchange chromosomes, is defined by the *ald* (*altered disjunction*) mutation (O'TOUSA 1982), which allows exchange chromosomes to enter the system and participate in nonhomologous disjunctions (such as *XX* ↔ *Y* and *XX* ↔ *44*). The locus may define a component required for proper chiasma maintenance (HAWLEY 1988). The *ald* mutation also effects the proper choice of disjunctional partners and thus defines the second phase of the model. This defect in partner choice induces high frequencies of nonhomologous disjunctions involving the *X* and fourth chromosomes (*i.e.*, *XX* ↔ *44*).

The *mei-S51* mutation has also been shown to affect partner choice within the distributive system (ROBBINS 1971). It is a synthetic mutation composed of two recessive genes on the second and third chromosomes. Females homozygous for *mei-S51* exhibit reduced exchange and high frequencies of nonhomologous disjunction, particularly with respect to the *X* and fourth chromosomes (*i.e*, *XX* ↔ *44* segregations are frequent). In addition, *mei-S51* decreases the frequency of secondary nondisjunction in structurally normal *XXY* females and increases the frequency of nondisjunction in *X* inversion heterozygotes. ROBBINS proposed that *mei-S51* disrupts a number of aspects of chromosome pairing and alignment prior to metaphase and therefore both reduces exchange and prevents proper partner choice within the distributive system.

The third and final phase of the model is the proper segregation of oriented chromosomal partners to opposite poles and is defined by the *nod* (*no distributive disjunction*) mutation (CARPENTER 1973). The wild-type product of this locus is required for the actual disjunction of chromosomes within the distributive system. Females bearing the *nod* mutation and structurally normal *X* chromosomes produce approximately 90% fourth chromosome loss. Moreover, nonexchange *X* chromosomes disjoin at random in females homozygous for *nod*. The *nod* mutation also disrupts secondary nondisjunction, such that the *Y* chromosome is still capable of committing two nonexchange *X* chromosomes to segregate to the same pole, but the disjunction of the *Y* and *XX* pair is disrupted (*i.e.*, *XXY* ↔ O and *XX* ↔ *Y* segregations occur at equal frequencies).

In this report we describe a new dominant mutation *Axs* (*Aberrant X segregation*) whose effect is specific to the distributive system. *Axs* induces high frequencies of *X* nondisjunction in *X* chromosome inversion het-

erozygotes, increases frequencies of fourth chromosome nondisjunction in response to the presence of *X* chromosome structural heterozygosity, and strongly decreases the efficiency of *XX* ↔ *Y* trivalent formation in *XXY* females. *Axs* also impairs the segregation of nonexchange major autosomes. The primary phenotype of *Axs* may then be summarized as follows: *Axs* decreases the frequency of homologous segregations within the distributive system while increasing the frequency of nonhomologous disjunctions. The data suggest that *Axs* defines a locus required primarily for homologous disjunctions of distributively segregating chromosomes, and reveals the existence of a homology dependent component of the distributive system.

MATERIALS AND METHODS

All crosses were performed at 23.5° on standard medium (LEWIS 1960). Both bottles and vials were set up on day 0, transferred on day 5 and the parents discarded on day 10. Scoring was performed on days 14 and 18.

Mutations and chromosomes used: With the exception of *Axs*, all mutations and chromosomes referred to in this report are described in LINDSLEY and GRELL (1968) except for *FM7* which is described in LINDSLEY and ZIMM (1987).

Throughout this report, the balancer *X* chromosome *Inversion(1) Basc* (*Muller-5*) will be abbreviated as *Mu-5* and the fourth chromosome mutation spa^{pol} will be referred to as *pol*. In addition, *Inversion(1) delta-49* will be referred to as *dl-49*, and *Inversion(1) scute-8 Left scute-4 Right* will be referred to as sc^4sc^8.

Calculations: An example of the basic experiment for monitoring *X* and fourth chromosome nondisjunction is a cross between *y/y*; *pol/pol* females and $Y^yX{\cdot}Y^L$, *In(1)EN, v f B*/O; *C(4)RM, ci* ey^R/O males. This cross allows one to recognize *X* chromosome nondisjunctional offspring from the mother either as yellow females (diplo-*X* exceptions) or as vermillion, forked, Bar males (nullo-*X* exceptions). Similarly one can recognize fourth chromosome nondisjunctional offspring from the mother either as sparkling poliert flies (diplo-*4* exceptions) or as cubitus interuptus eyeless-Russian flies (nullo-*4* exceptions). Nondisjunction frequencies are calculated as the sum of exceptional progeny classes divided by the sum of all progeny classes. Except where noted, for crosses in which the female test parent contained free *X* chromosomes, the number of exceptional-*X* progeny are doubled before computations are made to correct for the inviability of triplo-*X* and nullo-*X* progeny.

Because they cause male inviability, the following two *X* chromosomes required slightly different methods of calculating nondisjunction frequencies. The *dl-49 Axs* chromosome used in all experiments carries an unmapped distal lethal that requires the presence of y^+Y in males; *X*/O males bearing this *dl-49* chromosome are therefore inviable. This chromosome originally carried both the *f* and *Rex* mutations and was graciously provided by L. ROBBINS. The proximity of *Axs* to *f* allowed us to generate a *dl-49, Axs car* chromosome via recombination between *dl-49, f Rex* and a *y cv v Axs car* chromosome. Finally, sc^4sc^8 carries a complete deficiency for the rDNA genes (bb^o), and thus, *X*/O males bearing this chromosome are also inviable. Thus, in crosses involving *dl-49/y* females (*i.e.*, those in which the *dl-49 Axs*-bearing *X*/O males would be inviable), the reported number of males represents only the *y*/O progeny. This number is doubled to estimate the total number of regular male zygotes produced. This adjusted total is used to calculate nondisjunction as described above.

In those crosses that involved sc^4sc^8/*dl-49* females (in which all sc^4sc^8/O and *dl-49*/O males are inviable) no adjustment was attempted. The listed value for total progeny reflects only regular and fourth chromosome nondisjunctional female progeny and *X* exceptional offspring. In these crosses the percent *X* nondisjunction is calculated by dividing the total number of *X* exceptional progeny by the total number of progeny and multiplying by 100.

Females bearing a free *Y* chromosome also pose a computational difficulty due to the lowered viability of *XXYY* progeny (LINDSLEY and GRELL 1968). In these crosses, the number of *XXYY* zygotes is estimated using the number of regular *XY* male progeny. These males are the reciprocal product of segregations generating these *XXYY* females.

All other corrections or adjustments are listed in the footnotes and adjusted totals are recorded in each table.

Exchange rank distributions are calculated by the method of WEINSTEIN (1936) for regular-*X* progeny and by the method of MERRIAM and FROST (1964) for exceptional-*X* progeny.

Nonhomologous disjunction frequencies are calculated in the following manner as described by O'TOUSA (1982). For two pairs of chromosomes (AA;BB) being assayed, the nonhomologous disjunction frequency is calculated as: [(AA; O + O;BB) − (AA;BB + O;O)] divided by the total progeny. The subtraction is carried out in order to account for those cases in which AA and BB disjoined independently and segregated to opposite poles. When a pair of homologous chromosomes and a heterologue (AA;E) is being assayed, the nonhomologous disjunction frequency is calculated as: [(AA + O;E) − (AA;E + O;O)] divided by the total progeny. For a detailed discussion of the equations see O'TOUSA (1982). When two pairs of chromosomes and a third chromosome (AA;E;BB) are being assayed, the frequency of AAE ↔ BB nonhomologous disjunctions is calculated as [(AA;E;O + O;O;BB) − (AA;E;BB + O;O;O)] divided by the total progeny.

The data shown in Figures 2 and 4 were fitted to regression lines using a commercial software plotting program (Cricket Graph).

Isolation of the *Axs* mutation: *Axs* (*Aberrant X segregation*) is an ethyl methanesulfonate-induced *X* chromosomal mutation. It was fortuitously recovered in this laboratory in the course of a screen for mutations on a bb^2 *X* chromosome which block rDNA magnification, a process which occurs primarily in the male germline and results in reversion of bb^2 (*i.e.*, stable and heritable increases in rDNA redundancy) (RITOSSA 1968; TARTOF 1973). During the stock construction in this screen it was frequently necessary to make mutagenized *X* chromosomes (*X**) homozygous by crossing *X*/Mu-5* females to X^*/B^SY brothers. Females heterozygous for the *Mu-5* balancer chromosome and the *Axs*-bearing *X* chromosome generated high levels of *X* nondisjunction. On this basis, the *Axs* chromosome was selected for further testing.

Construction and use of *Axs*-bearing and $Axs^+C(1)RM$ chromosomes: A compound *X* chromosome heterozygous for the *Axs* mutation was constructed by irradiating *y pn cv m f*·y^+/*y cv v Axs car* females and crossing to y^+bb^2/B^SY males. Yellow daughters carrying B^SY (*i.e.*, females arising from diplo-*X* exceptions in which the y^+ marker had been lost) were selected and backcrossed to y^+bb^2/B^SY males to generate a number of stocks. We will present evidence below that the *Axs* mutation is recombinationally inseparable from the *forked* locus. Thus, for the purposes of constructions, the *Axs* mutation was followed by the presence or absence of *f*. To maintain heterozygosity of *Axs* in each stock, phenotypically heterozygous females were selected each generation and their heterozygous daughters were used for the next

generation only if both *f* (*Axs*$^+$) and *v car* (presumed *Axs*/*Axs*, see below) females were present.

A single heterozygous *Axs* stock was chosen and used for all subsequent steps in both constructions and experiments. In order to ensure that all *C(1)*RM chromosomes used in the experiment were derived from the same irradiation-induced attachment event, a single *f* (*Axs*$^+$) female from this heterozygous stock was used to generate a stock of control females while the putative *Axs* homozygotes (*v car*) were selected directly from the heterozygous *Axs* stock. Cytological analysis of larval neuroblasts revealed that the compound *X* chromosome constructed in this manner is a *C(1)*RM chromosome (data not shown).

Control and heterozygous *Axs* crosses were performed in batch matings. For the heterozygous *Axs* crosses, we assumed that (1) if the females tested were wild type with respect to *v* and *car*, and (2) each bottle gave *f* progeny, then the females used were probably heterozygous for the *Axs* mutation. All homozygous *Axs* crosses were performed in vials in the following manner. F_1 progeny from a single *v car* mother were scored, and five of the F_1 daughters were used to generate F_2 progeny that were then scored. Ten of the F_2 daughters were used to generate F_3 progeny. These were scored and the sum of all generations was used to calculate frequencies of nondisjunction. Each generation, and two generations subsequent to F_3 were scored for the presence of *f*. The lines yielding *f* flies were discarded. Approximately 50 single *v car* females initiated the experiment, and five of these lines were finally used to generate the data reported.

RESULTS

Axs (*Aberrant X segregation*) maps to position 56 on the standard map and was not separable from *f* (56.7). Among the progeny of +*Axs* + +/*v* + *f car* females, no crossovers between *Axs* and *f* were recovered among 40 *v-f* recombinants or 40 *f-car* recombinants (all *f* recombinants were *Axs*$^+$ and all *f*$^+$ recombinants carried the *Axs* mutation). Cytological analysis performed by D. WRIGHT in the laboratory revealed that chromosomes bearing the *Axs* mutation also carry a small aberration at band 15D1 on the polytene map (Figure 1). This aberration is not observed in the parent strain (*bb*^{2}) and is absent in a single spontaneous revertant of *Axs* (data not shown). This is in agreement with the genetic mapping described above. Experiments are underway to determine the relationship between this aberration and the *Axs* mutation.

Axs does not increase either sex or fourth chromosome nondisjunction in *Axs*/*Y* males. Control males exhibit frequencies of sex and fourth chromosome nondisjunction of 0.079% and 0.119% respectively (N = 1269). These frequencies are similar to those reported previously (*cf.* BAKER and CARPENTER 1972). In *Axs* males, only a single *X,4* simultaneous nondisjunctional offspring was recovered out of 2131 progeny scored. Thus *Axs* has little or no effect on meiotic disjunction in males.

Sex chromosome disjunction in *Axs* females: Table 1 presents the results of measuring *X* and fourth chromosome disjunction in females which are either homozygous for two structurally normal *X* chromosomes or heterozygous for an inverted, or multiply-inverted, *X* chromosome. It has been shown previously that frequencies of *X* nondisjunction are not greatly increased by inversion heterozygosity in otherwise genetically normal females (STURTEVANT and BEADLE 1936; COOPER 1945). Indeed *Axs*$^+$/*Axs*$^+$ control females in Table 1 exhibit frequencies of *X* nondisjunction that are low, regardless of heterozygosity for inverted balancer chromosomes. In females with structurally normal *X* chromosomes, *X* nondisjunction is elevated only slightly in *Axs*/*Axs*$^+$ or *Axs*/*Axs* females. In the presence of *Axs* and a balancer, however, *X* chromosome nondisjunction is increased some 10- to 50-fold.

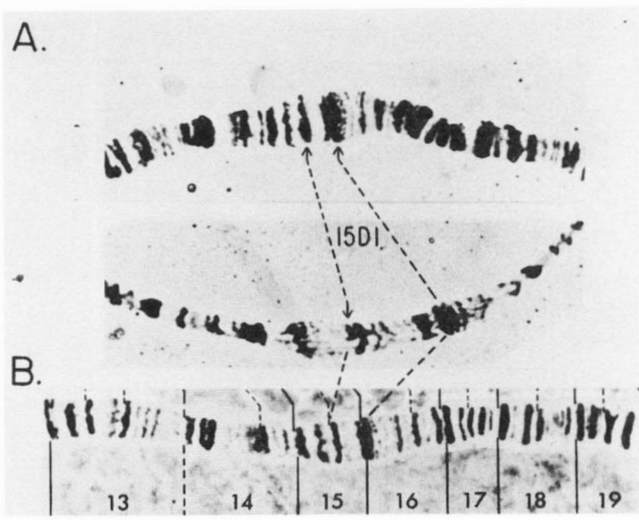

FIGURE 1.—Cytological analysis of the *Axs* mutant chromosome. (A) The top spread is +/*Axs*. The bottom spread is *Axs*/+ which is stretched to reveal the aberration at 15D1. (B) The wild-type polytene map, reprinted from LEFEVRE (1976).

The data in Table 1 also show that *Axs* increases the frequency of *X* nondisjunction in a semidominant fashion. Both the *y/dl-49* and the *sc*^{4}*sc*^{8}*/dl-49* females exhibit an approximately two-fold increase in *X* nondisjunction when comparing one versus two doses of the *Axs* mutation. For normal sequence *X* chromosomes bearing the *Axs* mutation, the effect is in the same direction although much less dramatic. These experiments demonstrate a clear zero, one, two dosage effect of the *Axs* mutation on *X* chromosome disjunction in females.

X nondisjunction in *Axs*/Balancer females appears to increase with the degree of structural heterozygosity. These data suggest that the frequency of *X* nondisjunction is correlated with the frequency of *X* chromosome nonexchange bivalents (E_0 tetrads) in *Axs*-bearing females. To test this hypothesis it is necessary to estimate the frequency of E_0 tetrads for each inversion combination. Because exchange cannot be directly measured in balancer heterozygotes, an indirect parameter must be used in tetrad analysis. As noted above, in *XXY* females, nonexchange *X* chromosomes

TABLE 1

Results of crossing $Y^sX \cdot Y^L$, *ln(1)EN, v f B*/O; *C(4)RM, ci ey^R*/O males to *X/X; pol/pol* females

Gamete types		Maternal genotype												
Mother	Father	*y/y*	*yAxs/y*	*yAxs/yAxs*	*y/dl-49*	*yAxs/dl-49*	*yAxs/dl-49Axs*	*dl-49/ sc^{4}sc^8*	*dl-49Axs/ sc^{4}sc^8*	*dl-49Axs/ sc^{4}sc^{8}Axs*	*w^a/Mu-5*	*w^{a}Axs/Mu-5*	*y/FM7*	*yAxs/FM7*
Regular														
X4	*XY44*	968	2301	793	3757	2795	2687	3494	1681	2503	1275	2452	8115	888
X4	O44	1702	2577	465	(2110)[a]	(1414)	(1521)				1898	1628	6063	715
X Nondisjunctional														
O4	*XY44*	1	14	10	13	174	375	25	149	638	0	84	10	83
XX4	O44	2	34	6	11	183	367	20	167	658	0	101	10	71
4 Nondisjunctional														
X44	*XYO*	1	51	25	1	20	65	4	11	104	1	59	2	45
XO	O44	2	19	4	(1)	(13)	(32)				0	2	2	5
X44	OO	3	33	13	(0)	(16)	(51)				0	29	10	53
XO	*XY44*	1	33	11	3	16	67	2	12	93	0	27	12	20
X,4 Nondisjunctional														
XX44	OO	0	4	0	0	2	3	2	2	11	3	18	0	3
OO	*XY44*	0	2	1	0	2	8	0	3	23	0	12	1	1
XXO	O44	0	1	0	1	17	41	1	15	126	1	9	0	26
O44	*XYO*	0	13	3	3	34	88	5	13	235	0	26	0	17
Total progeny		2680	5082	1331	5900	4686	5305	3553	2073	4391	3178	4447	14225	1929
Adjusted total		2683	5150	1351	8039	6541	7791	3553	2073	4391	3182	4697	14246	2131
% Nondisjunction														
X		0.22	2.64	2.96	0.70	12.60	22.64	1.49[b]	17.80[b]	38.51[b]	0.25	10.65	0.29	19.04
4		0.26	3.42	4.52	0.17	3.12	7.42	0.42	3.67	13.48	0.26	5.26	0.20	10.37
% Simultaneous *X,4* nondisjunction		0	0.78	0.59	0.10	1.68	3.59	0.23	2.56	9.00	0.25	2.77	0.01	4.41
% Nonhomologous disjunction		NA	0.31	0.30	0.10	0.96	3.03	0.11	2.07	7.45	NA	0.21	0	3.66

Chromosomes denoted as *y Axs* are in fact *y cv v Axs car*, and those denoted as *w^{a}Axs* are *y w^{a}v Axs car*. The *y* chromosome used in the *FM7* control is *y cv v f car* and chromosomes referred to as *w^a* also carry *y*, *ct^6*, *f*, and *car*.

[a] Because the *dl-49 Axs* X/O males in these crosses are inviable (see MATERIALS AND METHODS) and the *Axs$^+$ dl-49* chromosome is not isogenic to the *dl-49 Axs* chromosome, for the purpose of consistency, no *dl-49*/O males are recorded. Thus, the number (in parentheses) includes only *y* X/O males which was doubled for purposes of calculation.

[b] Due to lethality of X/O males in these crosses (see MATERIALS AND METHODS), % *X* nondisjunction calculated as follows: (total *X* exceptional progeny/total progeny)100.

NA, not applicable due to the formation of a negative number.

TABLE 2

Results of crossing $Y^sX \cdot Y^L$, *In(1)EN, y B*/O males to *X/X/Y* females

Gamete types		Maternal genotype			
Mother[a]	Father	*dl-49/ y w^a ct^6 m f car/ Y*	*Mu-5/ y w^a ct^6 mf/ Y*	*FM7/ y cv f/ Y*	*dl-49/ sc^{4}sc^{8}B$^+$/ Y*
Regular ($X \leftrightarrow XY$)					
$X(Y)$	$\hat{XY}$	241	492	651	991
$X(Y)$	O	311	406	944	663[b]
X Nondisjunctional ($XX \leftrightarrow Y$ and $XXY \leftrightarrow$ O)					
$XX(Y)$	O	224	478	1156	1274
$O(Y)$	$\hat{XY}$	179	331	757	858
Total progeny		955	1707	3508	3786
% *X* Nondisjunction[c]		56.44	66.58	67.01	70.69

[a] Due to the use of an unmarked *Y* chromosome, the presence or absence of the *Y* in a given offspring cannot be assessed. This is denoted by placing the *Y* in parentheses.

[b] The sc^4sc^8/O males in this class are inviable. These males represent ¼ of the total number of males in this class and thus, this number is multiplied by ⁴/₃ for use in the calculation of nondisjunction frequency.

[c] Due to low viability of *XXYY* females, the percent *X* nondisjunction is calculated as follows: [total *X* nondisjunctional progeny/regular males + total *X* nondisjunctional progeny]100.

segregate from the *Y* chromosome at high frequencies. Moreover, the frequency of secondary nondisjunction has been shown to increase with the extent of structural heterozygosity (COOPER 1948), as a consequence of an increased frequency of E_0 tetrads. Since the *Y* has little or no effect on the frequency of exchange (STURTEVANT and BEADLE 1936), the frequency of secondary nondisjunction may be used as a valid estimate of E_0 tetrad frequencies.

The use of secondary nondisjunction to estimate the frequency of nonexchange tetrads has been recently reviewed by RUTHERFORD and CARPENTER (1988), however, two points require further mention here. First, $XX \leftrightarrow Y$ segregations have been shown by a number of investigators to account for most, if not all, of the total *X* nondisjunction observed (BRIDGES 1916; COOPER 1948; CARPENTER 1973). Second, although secondary nondisjunction represents the predominant class of segregational events when *XXY* females are nonexchange (for a review, see ZIMMERING 1976), $X \leftrightarrow XY$ segregational events involving nonexchange *X* chromosomes are also observed. For example, STURTEVANT and BEADLE (1936) concluded that only 90% of the E_0 tetrads in *In(1)*dl-*49*/+/Y females underwent $XX \leftrightarrow Y$ segregations. Thus, the frequency of secondary nondisjunction is in fact an underestimate of the true E_0 tetrads.

We have measured the frequency of *X* nondisjunction in *X/X/Y* females (secondary nondisjunction) and thus, have derived an estimated frequency of E_0 tetrads for each type of inversion-bearing female used in these experiments. The data are presented in Table 2.

As shown in Figure 2, *X* chromosome nondisjunction increases linearly with the estimated frequency of E_0 tetrads for both *Axs* heterozygotes (closed diamonds) and *Axs* homozygotes (closed squares). No such effect is observed in Axs^+ control females (open squares). These data argue that (1) in the presence of *Axs*, nondisjunction increases linearly with the frequency of E_0 tetrads and (2) the *Axs* mutation acts primarily on nonexchange chromosomes (see below).

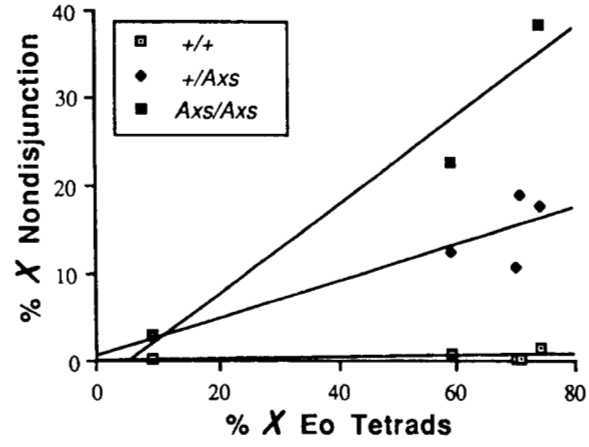

FIGURE 2.—Percent *X* E_0 tetrads (data taken from Tables 2 and 3) *vs.* percent *X* nondisjunction (data taken from Table 1).

Four additional features of the *X* nondisjunction associated with *Axs* should be noted. First, unlike most previously described meiotic mutants in which the frequency of nullo-*X* bearing eggs is substantially higher than diplo-*X* bearing eggs (DAVIS 1969; BAKER and CARPENTER 1972; HALL 1972; CARPENTER 1973; PARRY 1973; O'TOUSA 1982), the nondisjunction observed in *Axs* females results in nearly equal frequencies of nullo- and diplo-*X* bearing ova. This observation indicates that *Axs* primarily induces nondisjunction; chromosome loss, if it occurs at all, is at best a minor component of the *Axs* defect.

Second, since in *Axs* homozygotes the frequency of *X* nondisjunction is approximately one half the fre-

quency of X E_0 tetrads, it appears that the disjunction of nonexchange X chromosomes is random or nearly random. For example, *sc*^{4}*sc*^{8}*Axs/dl-49 Axs* females, which yield an apparent X chromosome E_0 tetrad frequency of approximately 71% (Table 2), exhibit 38.5% X nondisjunction.

Third, most, if not all, *Axs*-induced X nondisjunction occurs at meiosis I. If a given chromosome has not undergone exchange, nondisjunction at meiosis II can be observed through the homozygosis of all markers contained on that chromosome. There was no significant increase in meiosis II nondisjunction of chromosomes in either hetero- or homozygous *Axs* females. In fact, among all the experiments described, the only possible example of meiosis II nondisjunction was a single yellow crossveinless vermillion carnation female among 202 diplo-X female progeny recovered from *y cv v Axs car/dl-49* mothers.

Fourth, *Axs* does not appear to affect mitotic chromosome stability or the stability of chromosomes in early zygotic cleavages that have been transmitted by *Axs*-bearing mothers. In the experiments in which females were heterozygous for the cell-autonomous markers *f* and/or *y*, no patches of mutant tissue were observed in either hetero- or homozygous *Axs* mothers, or their progeny. In addition, only one gynandromorph from *Axs* females was observed among the progeny from all the experiments performed. Although these experiments do not rigorously test for the presence of an effect of the *Axs* mutation on somatic chromosome disjunction, the results suggest that *Axs* does not induce mitotic chromosome loss at high frequencies. This result is in contrast to those observed for mutations like *nod* and *ca*nd which produce high frequencies of zygotic chromosome loss (CARPENTER 1973; BAKER and HALL 1976).

All of these data strongly suggest that the effect of *Axs* is limited to the first meiotic division.

Exchange chromosomes and *Axs*: The effect of *Axs* is much more pronounced in balancer heterozygotes. This suggests that the *Axs* mutation only influences the segregation of nonexchange X chromosomes (*i.e.*, X chromosomes in the distributive system). To further examine the effects of the *Axs* mutation on the meiotic exchange system, we monitored the frequency and distribution of exchange on the X chromosome in +/+, +/*Axs* and *Axs/Axs* females (Table 3). To test the ability of *Axs*-bearing females to respond to standard modulators of exchange frequency, the effect of *Axs* on the interchromosomal effect was also examined. The interchromosomal effect has been reviewed by LUCHESSI (1976); briefly, females that are heterozygous for an autosomal balancer have an increased frequency of exchange on other bivalents. To examine the effect of *Axs* in this process, X chromosomal exchange was monitored in females heterozygous for the second chromosome balancer *SM1* in the presence of one or two doses of the *Axs* mutation (Table 3).

The effect of the *Axs* mutation on exchange frequency and on the interchromosomal effect are presented in Table 3 and are represented graphically in Figure 3. The data show that recombination levels in +/*Axs* and *Axs/Axs* females are similar to those observed in +/+ control females either in the presence or absence of the *SM1* chromosome. For both sets of crosses, however, a 4×3 χ^2 test for homogeneity revealed statistically significant differences between the proportions of noncrossover (NCO), single crossover (SCO), double crossover (DCO) and triple crossover (TCO) progeny produced by +/+, +/*Axs* or *Axs/Axs* females. (For females bearing normal second chromosomes $\chi^2 = 27.34$, d.f. $= 6$, $P < 0.001$ and for *SM1*-bearing females $\chi^2 = 14.46$, d.f. $= 6$, $P < 0.01$.) Despite the finding of statistically significant differences in the frequency of crossover recovery, we note that the observed differences are small (less than 10%) and that there is no consistent dosage effect of the *Axs* mutation. Indeed, in females bearing normal second chromosomes, +/*Axs* females display levels of recombination which are virtually identical to those displayed in +/+ females; while in the presence of *SM1*, the total frequency of X chromosome recombination observed in +/*Axs* exceeds that observed in +/+ controls. Thus, we conclude that *Axs* has little or no consistent effect on the frequency or distribution of X chromosome exchange, and that the differences observed most likely reflect other genetic differences between the three tested genotypes. The observation that *Axs* exerts little or no effect on the interchromosomal effect further suggests that, for most intervals, exchange responds to normal controls, even in the presence of the *Axs* mutation.

In the crosses reported in Table 3, control females exhibited a remarkably high frequency of X nondisjunction (Table 4). Indeed, Table 4 also shows that *Axs*-bearing mothers did not exhibit increased frequencies of X nondisjunction when compared to these controls (approximately 2% in all cases). These observations are not understood. We have repeated the experiment utilizing a different set of tester chromosomes and, in addition to corroborating the tetrad analysis, we have observed substantial increases in X nondisjunction in *Axs* females relative to frequencies observed in controls (data not shown).

In the experiments described in Tables 3 and 4, it was observed that a significant fraction of diplo-X offspring recovered from all six crosses were homozygous for one or more X chromosomal markers (data not shown). Because such exceptional progeny arise by nondisjunction of bivalents that have undergone exchange, this observation raised the possibility that there was a weak effect of *Axs* on the disjunction of exchange bivalents. To test this hypothesis, diplo-X exceptions from crosses involving normal sequence

TABLE 3

Results of crossing $Y^SX \cdot Y^L$, *In(1)EN, v f B*/O; *C(4)RM, ci ey*^{R}/O males to *y cv*/*y w*^{a} *ct*^{6} *m car*; *pol*/*pol* or *y cv*/*y w*^{a} *ct*^{6} *m car*; *SM1*/+; *pol*/*pol* females

Maternal genotype	X Chromosome recombination and tetrad analysis									
2nd chromosome:	+/+					*SM1*/+				
X Chromosome:	+/+	+/*Axs*		*Axs*/*Axs*		+/+	+/*Axs*		*Axs*/*Axs*	
	4	4	O	4	O	4	4	O	4	O
NCO	688	1580	19	1248	33	799	732	3	510	5
SCO										
1 (*w-cv*)	138	412	0	239	7	216	264	0	137	1
2 (*cv-ct*)	47	124	4	85	5	93	89	2	37	1
3 (*ct-m*)	185	432	3	253	7	229	230	0	148	2
4 (*m-car*)	291	561	1	434	8	333	303	2	167	2
DCO										
1, 2	0	1	0	2	0	0	1	0	0	0
1, 3	10	21	0	6	0	28	34	1	13	0
1, 4	43	111	1	53	1	79	107	0	43	0
2, 3	2	5	0	4	0	5	6	0	4	0
2, 4	25	36	0	17	0	37	28	0	21	0
3, 4	29	55	0	37	0	68	58	0	31	0
TCO										
1, 2, 3	0	0	0	1	0	0	1	0	2	0
1, 2, 4	0	1	0	0	0	0	0	0	0	0
1, 3, 4	2	5	0	5	0	3	3	0	2	0
2, 3, 4	2	0	0	1	0	1	0	0	1	0
Total males	1462	3372		2446		1891	1864		1127	
Map distances for region										
1	13.2	16.4		12.8		17.2	22.1		17.6	
2	5.2	5.1		4.7		7.2	6.8		5.9	
3	15.7	15.5		12.8		17.6	17.9		18.0	
4	26.8	22.9		22.7		27.6	26.9		23.7	
Total	60.9	59.9		53.0		69.6	73.7		65.2	
Exchange rank										
E_0	0.09	0.09		0.15		0.07	0.04		0.11	
E_1	0.62	0.64		0.66		0.45	0.47		0.51	
E_2	0.27	0.26		0.17		0.46	0.47		0.34	
E_3	0.02	0.01		0.02		0.02	0.02		0.04	

second chromosomes were progeny tested and tetrad frequencies were calculated according to the method of MERRIAM and FROST (1964). The resulting data are presented in Table 4. The tetrad frequencies for diplo-*X* exceptional progeny from heterozygous *Axs* females are E_0, 0.54; E_1, 0.38; and E_2, 0.08. The frequencies for homozygous *Axs* females are E_0, 0.54; E_1, 0.29; and E_2, 0.17. Tetrad frequencies for diplo-*X* exceptional progeny from control females are E_0, 0.26; E_1, 0.25; and E_2, 0.48 (taken from MERRIAM and FROST 1964). Thus, the data reveal a two-fold excess of E_0 tetrads among the ova producing diplo-*X* exceptions in *Axs*-bearing females. This observed excess of nondisjunctional progeny generated from E_0 tetrads in *Axs*-bearing females suggests that *Axs* does not increase the nondisjunction of exchange tetrads, but rather produces diplo-*X* exceptional ova primarily as the result of failed disjunction of E_0 bivalents.

Both the low frequency of *X* chromosome nondisjunction in *Axs* females bearing normal sequence *X* chromosomes and the observed excess of E_0 tetrads among the bivalents producing diplo-*X* exceptions strongly suggest that the *Axs* mutation has little or no effect on the disjunction of exchange chromosomes.

Fourth chromosome disjunction in *Axs* females: Fourth chromosome nondisjunction remains relatively constant (0.12–0.39%) among *Axs*$^+$ control females and is not increased by structural heterozygosity on the *X* (Table 1). Although the fourth chromosomes are always nonexchange, the *Axs* mutation does not induce high levels of fourth chromosome nondisjunction in females bearing two structurally normal *X* chromosomes. Thus, where the frequency of *X* E_0 tetrads is low, *Axs* seems to have no direct effect on the disjunction of chromosome four.

In *Axs* females heterozygous for a number of struc-

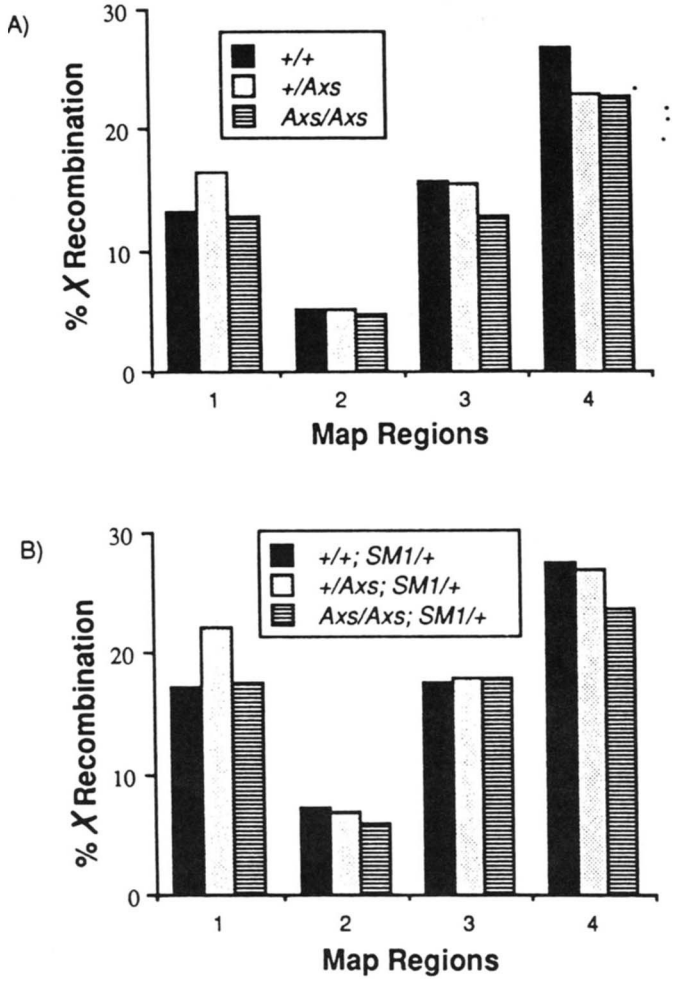

FIGURE 3.—Exchange in *Axs* females. (A) The effect of *Axs* on the frequency of exchange. (B) The interchromosomal effect and *Axs*.

turally abnormal balancer *X* chromosomes, fourth chromosome nondisjunction increases with the frequency of *X* chromosome nondisjunction. For example, in *y Axs/y Axs*, *y Axs/dl-49 Axs* and sc^4sc^8*Axs/dl-49 Axs*, frequencies of fourth chromosome nondisjunction are 4.52%, 7.42%, and 13.48%, respectively (Table 1). As was shown in the analysis of *X* misbehavior, the effect on the disjunction of chromosome four is also responsive to the dosage of the *Axs* mutation.

The correlation between *X* and fourth chromosome nondisjunction is expressed graphically in Figure 4. The data show that in *Axs* females, fourth chromosome nondisjunction is tightly coupled to the presence of nonexchange *X* chromosomes and thus, to *X* chromosome misbehavior. Indeed, Table 1 shows that approximately 50% of the fourth chromosome nondisjunction can be accounted for by simultaneous *X* and fourth chromosome nondisjunction. The vast majority of these simultaneous nondisjunctional events result from nonhomologous segregation of the *X* chromosomes from the fourth chromosomes (*i.e.*, *XX*;OO and OO;*44* ova are produced in vast excess compared to *XX*;*44* and OO;OO ova). The exception to this

TABLE 4

Results of crossing $Y^SX{\cdot}Y^L$, *ln(1)EN, v f B*/O; *C(4)RM, ci* ey^R/O males to *y cv*/*y* w^a ct^6 *m car*; *pol*/*pol* females

Gamete types		Maternal genotype		
Mother	Father	+/+	+/*Axs*	*Axs*/*Axs*
Regular				
X4	*XY44*	1624	6099	4231
X4	O*44*	1163	3509	2246
X Nondisjunctional				
O*4*	*XY44*	11	32	24
XX4	O*44*	14	50	23
4 Nondisjunctional				
X44	*XY*O	0	32	32
*X*O	O*44*	0	5	23
X44	OO	0	23	40
*X*O	*XY*O	4	26	37
X,4 Nondisjunctional				
XX44	OO	2	3	3
OO	*XY44*	0	1	3
*XX*O	O*44*	0	3	3
O*44*	*XY*O	1	4	3
Total progeny		2820	9787	6668
Adjusted total		2848	9880	6727
% Nondisjunction				
X		1.97	1.88	1.75
4		0.39	1.09	2.32
X,4		0.21	0.22	0.36
Number of exceptional females tested:		9	39	38[a]
Exchange rank				
E_0		(0.26)[b]	0.54	0.54
E_1		(0.25)	0.38	0.29
E_2		(0.48)	0.08	0.17

[a] Due to the low numbers of diplo-*X* exceptional females generated in this cross, we collected additional exceptional females from crosses that were not scored.

[b] The small numbers obtained in these crosses did not permit the calculation of tetrad frequencies using data from these females. The tetrad frequencies presented here are from MERRIAM and FROST (1964).

behavior is the w^a*Axs/Mu-5* heterozygote, where nonhomologous disjunction is low.

The anomalous behavior of the *Mu-5* chromosome: The *Mu-5* chromosome is eccentric in at least two aspects of its response to the *Axs* mutation. First, the overall frequency of *X* nondisjunction in *Axs/Mu-5* females is much lower than would be expected based on the estimated frequency of E_0 tetrads (Table 2). For example, the frequency of *X* nondisjunction in *Axs/Mu-5* females is half that observed in *Axs/FM7* females despite the fact that *X/Mu-5* females display an approximately equal frequency of *X* E_0 tetrads (66.58%) when compared to *X/FM7* females (67.01%) as derived from Table 2. Secondly, in *Axs/Mu-5* females, simultaneous *X,4* nondisjunctional events were divided equally between *XX* ↔ *44* and *XX*;*44* ↔ O; O disjunctions. The absence of a preference for *XX* ↔ *44* nonhomologous disjunctional events is in stark contrast to the observations made for several other *X* chromosomes tested (Table 1).

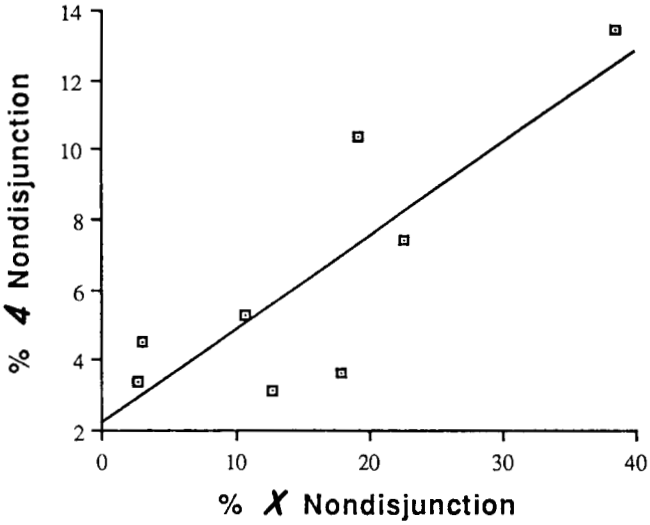

FIGURE 4.—Percent *X* nondisjunction *vs.* percent *4* nondisjunction (data taken from Table 1).

Although these observations are not understood, we propose two possible explanations. First, the *Mu-5* chromosome may carry an undefined structural aberration, such as a heterochromatic deficiency, which impairs *X* and fourth chromosome associations. This possibility is supported by the fact that *X*/O males bearing our *Mu-5* chromosome display good viability, despite the statement by LINDSLEY and GRELL (1968) that such males should be poorly viable due to position-effect variegation of an essential locus, *l(1)J1*. We have confirmed by polytene chromosome analysis that our *Mu-5* does possess the euchromatic inversions characteristic of a *Mu-5* chromosome (data not shown). Second, it is possible that *Mu-5* carries a cryptic meiotic mutation that interacts with *Axs*. Regardless of which hypothesis is correct, the basic observation points out the necessity of utilizing a number of different tester chromosomes when characterizing a novel meiotic mutation.

Distributive disjunctions of a major autosome and *Axs*: We tested the effect of *Axs* on the behavior of the second chromosomes in both the presence and absence of balancers for the *X* and second chromosomes. Because aneuploidy for the major autosomes is lethal, these experiments require that the males carry compound autosomes (for review see HOLM 1976). By providing a diploid complement from the father, nullo-bearing eggs can be recovered as euploid, and conversely, by providing a nullo-bearing sperm, one recovers the diplo-bearing egg. In these crosses all eggs produced from normal segregations are lost, and thus, these experiments only allow for the recovery of nondisjunctional progeny.

The data in Table 5a show that *Axs* induces a dosage dependent increase in the frequency of second chromosome nondisjunction. Heterozygous or homozygous *Axs* females carrying two structurally normal second chromosomes increase second chromosome nondisjunction by 3- and 12-fold respectively over control levels. In females heterozygous for the *SM1* balancer chromosome and a structurally normal second chromosome, *Axs* heterozygotes increase second chromosome nondisjunction over control levels by 1.5-fold and *Axs* homozygotes exhibit a 7-fold effect.

The *Axs* mutation also reduces the fraction of events that were simultaneously nondisjunctional for the *X* and second chromosomes in *Axs/Axs*; *+/SM1* females. Moreover, those simultaneous events which did occur were randomized such that among the diplo-*2* ova simultaneously nondisjunctional for the *X* chromosomes, nullo-*X* and diplo-*X* exceptions were recovered at nearly equal frequencies. This is in contrast to the vast excess of nullo-*X* ova observed among diplo-*2* ova in either *+/+* or *+/Axs* females. The effect was consistently observed in each bottle and has been subsequently repeated. The significance of this result is considered in the DISCUSSION.

The 7- to 12-fold effects on second chromosome nondisjunction observed in *Axs* homozygotes are similar to those observed for the *nod* mutation which is defective in distributive disjunction (CARPENTER 1973; and see Introduction). Two lines of evidence, however, argue that basing our conclusions solely on the fold-effects exerted by the *Axs* mutation may be misleading.

First, although *SM1* effectively suppresses the recovery of exchange products (MACINTYRE and WRIGHT 1966), it is not clear to what extent exchange is reduced in these heterozygotes (E_0 data are not available for these aberrations). Thus, it may not be reasonable to compare the effects of this autosomal balancer to those exerted by a given *X* chromosome balancer in *Axs*-bearing females. Second, as was observed for the *X* chromosomes, second chromosome nondisjunction may frequently involve nonhomologous *44* ↔ *22* disjunctions. This class of segregational events would be recovered less frequently in these crosses because the tester males did not carry a compound fourth chromosome. Thus, our calculations may tend to underestimate the frequency of second chromosome nondisjunction in *Axs* females.

In order to observe the effect of increased numbers of chromosomes in the distributive system, we tested the behavior of the *X* and second chromosomes in *Axs* heterozygotes in the presence of both *Mu-5* and *SM1* (Table 5b). Although the basal levels of second chromosome nondisjunction are elevated in *Mu-5/+; SM1/+* females, the effect of *Axs* on the frequency of second chromosome exceptions in these females is similar to that observed when comparing non-*Mu-5*-bearing females. Thus, although there is a slightly higher frequency of second chromosome misbehavior in response to the addition of the *Mu-5* chromosome in both control and *Axs* females, the effects of *Axs* parallel the observations made for females bearing structurally normal *X* chromosomes.

TABLE 5

Results of crossing X/y^+YB^S; *C(2)EN, bw sp*/O males to *X/X*; +/+ or *X/X*; *SM1*/+ females

Maternal genotype			Female gametic genotype							
			Diplo 2			Nullo 2[a]				
X	*2*	No. of mothers	*X*	*XX*	O	*X*	*XX*	O	2nd Chromosome exceptions/female	Fraction of simultaneous X-2 exceptions
a)										
+/+	+/+	888	19	0	0	3	0	1	0.026	0.044
+/*yAxs*[b]	+/+	744	40	1	0	6	0	4	0.069	0.098
yAxs/*yAxs*[b]	+/+	888	227	11	26	1	0	0	0.298	0.140
+/+	*SM1*/+	480	112	1	24	0	0	0	0.285	0.183
+/*Axs car*	*SM1*/+	480	141	5	46	2	0	0	0.404	0.273
Axs car/*Axs car*	*SM1*/+	504	949	45	43	0	0	0	2.058	0.085
b)										
+/*Mu-5*	*SM1*/+	456	655	18	722	30	2	3	3.136	0.521
Axs car/*Mu-5*	*SM1*/+	480	1071	53	963	21	3	3	4.404	0.483

[a] The tester males used in these crosses transmit the *C(2)EN* chromosome at low efficiency. Thus, the number of nullo *2* progeny is low. The numbers used for calculations are not adjusted to account for this behavior.

[b] The term *y Axs* denotes *y cv v Axs car*.

Based on the data presented in Table 5, a and b, we conclude that *Axs* exerts a significant effect on the disjunction of the nonexchange second chromosomes.

The effect of *Axs* in *C(1)RM/Y* females: In order to further test the effects of the *Axs* mutation on large metacentric chromosomes, disjunction of a metacentric compound *X* chromosome, *C(1)RM*, from a *Y* chromosome was monitored in the presence of either zero, one, or two doses of the *Axs* mutation. The construction of this *C(1)RM* chromosome is described in MATERIALS AND METHODS.

Although both arms of the *C(1)RM* chromosome are normal sequence and freely recombine, the *C(1)RM* segregates from the *Y* chromosome (*C(1)RM* ↔ *Y*) in the distributive system almost 100% of the time (for a review see GRELL 1976). In other words, exchange events do not preclude a compound *X* from entering the distributive system. One might expect, therefore, that in the presence of the *Axs* mutation the frequency of *C(1)RM* ↔ *Y* disjunctions observed would be strongly decreased.

As can be observed from the data reported in Table 6, the frequency of nondisjunction involving the *C(1)RM* and *Y* chromosomes (*i.e.*, *C(1)RM/Y* ↔ O) in Axs^+/Axs^+ control females is quite low (0.16%). However, nondisjunction of the *C(1)RM* and *Y* chromosomes is observed in *Axs* heterozygotes at a frequency of 3.1% (approximately 20-fold higher than control levels) and in *Axs* homozygotes at a frequency of 20.8% (approximately 130-fold higher than control levels).

These data show that *Axs* exerts a strong effect on disjunctions involving a compound *X* and a *Y* chromosome, however, there is no longer a clear zero, one, two dosage effect as was observed for free *X* chromosomes. In addition, the nondisjunction observed is not 50% in *Axs* homozygotes but rather 20% (*i.e.*, nondisjunction is not equal to one-half the frequency of *C(1)RM* ↔ *Y* segregations which, in this case, is 100%). Thus, these data demonstrate that there may be some effect of a change in *X* chromosome size or shape on the dominant nature of the *Axs* mutation.

Disjunction in *XX*; *C(4)RM* females: The experiments described above demonstrate that *Axs*-induced increases in *X* and fourth chromosome nondisjunction may be explained by a decrease in homologous disjunctions with a concomitant increase in nonhomologous *XX* ↔ *44* disjunctions. Unfortunately, the analysis of the crosses presented in Table 1 is complicated by the presence of four elements in the distributive system. It was possible that if the system could be simplified such that only two *X* chromosomes and one heterolog (E) were available, most, if not all, *X* nondisjunction could be accounted for by *XX* ↔ *E* segregational events. To test this prediction, the disjunction of the *X* chromosomes and a metacentric *C(4)RM* chromosome was tested in the presence and absence of the *Axs* mutation.

The results of examining *X* and *C(4)RM* disjunction in sc^4sc^8/*dl-49*; *C(4)RM*/O females bearing zero, one or two doses of the *Axs* mutation are reported in Table 7. As has been observed previously, *X* chromosome nondisjunction rises in a dosage dependent manner when comparing *Axs* heterozygotes to *Axs* homozygotes. Indeed, the frequencies of *Axs*-induced *X* nondisjunction observed in these females are virtually identical to those observed in females bearing two free fourth chromosomes. Yet, in all three classes of *C(4)RM*-bearing females (+/+, +/*Axs*, and *Axs*/*Axs*), 75–85% of the total *X* nondisjunctional events occur as the consequence of nonhomologous segregation from the *C(4)RM* chromosome. Thus, the proportion of *X* nondisjunction associated with *XX* ↔ *C(4)RM*

TABLE 6

Results of crossing *y cv v f car/y⁺Y* males to *C(1)RM/B^SY* females

Gamete types		Maternal genotype											
				Axs/Axs[a] vial numbers									
				1		2		3		4		5	
Mother	Father	+/+	+/*Axs*	$F_1 + F_2$	F_3	$F_1 + F_2$	F_3	$F_1 + F_2$	F_3	$F_1 + F_2$	F_3	$F_1 + F_2$	F_3
Regular													
C(1)RM	*Y*	4161	2667	20	226	11	340	12	197	14	108	32	251
Y	*X*	4524	2294	28	210	10	279	10	159	32	94	26	205
Nondisjunctional													
C(1)RM;Y	*Y*	0	6	2	10	1	4	0	15	1	6	1	14
O; O	*X*	7	73	9	67	0	75	5	50	13	35	4	40
Total progeny scored		8692	4640	572		720		448		303		573	
% Nondisjunction[b]		0.16	3.10	23.90		18.99		22.54		13.95		14.62	
				Average frequency of nondisjunction: 20.84%									

For complete genotypes of the *C(1)RM* chromosomes used see MATERIALS AND METHODS.

[a] Crosses were performed in vials. F_1 denotes progeny from a single mother. Five nonvirgin F_1 females were used to generate F_2 progeny and ten nonvirgin F_2 females were used to generate F_3 progeny (see MATERIALS AND METHODS for further discussion).

[b] For reasons detailed in text, the percent nondisjunction is calculated as follows: {2 (male nondisjunctional progeny)/[2 (male nondisjunctional progeny) + total regular progeny]} × 100.

TABLE 7

Results of crossing *XY^S·Y^L, In(1)EN,y B*/O; *C(4)RM, pol*/O males to *sc⁴sc⁸B⁺/dl-49;C(4)RM, ci ey^R*/O females

Gamete types		Maternal genotype		
Mother	Father	*sc⁴sc⁸/dl-49*	*sc⁴sc⁸Axs/dl-49*	*sc⁴sc⁸Axs/dl-49 Axs*
Regular[a]				
X C(4)	$\widehat{XY}$ O	1780	1609	773
X O	$\widehat{XY}$ *C(4)*	1434	1288	640
X Nondisjunctional				
XX C(4)	O O	12	21	39
O O	$\widehat{XY}$ *C(4)*	5	18	17
XX O	O *C(4)*	53	203	314
O *C(4)*	$\widehat{XY}$ O	69	233	425
Total progeny		3353	3372	2208
% *X* nondisjunction[b]		4.15 (1.49)[c]	14.09 (17.80)	36.01 (38.51)
% *XX* ↔ *C(4)* nonhomologous disjunction		3.13	11.77	30.93

[a] Because the *dl49 Axs X*/O males in these crosses are inviable (see MATERIALS AND METHODS) and the *Axs⁺ dl-49* chromosome is not isogenic to the *dl49 Axs* chromosome, for the purpose of consistency, no *dl-49*/O males are recorded.

[b] Due to lethality of *X*/O males in these crosses (see MATERIALS AND METHODS), the % *X* nondisjunction is calculated as follows: (total *X* exceptional progeny/total progeny)100.

[c] The numbers in parentheses represent frequencies of *X* nondisjunction for *sc⁴sc⁸/dl-49; pol/pol* females (see Table 1).

segregations remains the same in either control or *Axs* females, such that the vast majority of *X* nondisjunction is associated with *XX* ↔ *C(4)RM* segregation. The effect of *Axs* then, is to enhance the frequency of *XX* ↔ *C(4)RM* nonhomologous disjunctional events, rather than to allow the *X* chromosomes simply to disjoin at random.

We conclude from this experiment that most, if not all, of the *X* and fourth chromosome nondisjunction observed in *Axs* females is the consequence of an increase in nonhomologous associations. This conclusion is further supported by the following analysis of segregation in females bearing either a *Y* or small free duplication.

Disjunction in *XXY* females: To determine the effect of the *Axs* mutation on disjunction in *XXY* females, *y/y/y⁺Y; pol/pol* and *sc⁴sc⁸/dl-49/y⁺Y; pol/pol* females were tested in the presence and absence of the *Axs* mutation (Table 8). Because the frequency of secondary nondisjunction in *sc⁴sc⁸/dl-49/Y* females is lower here than in Table 2, we conclude that a *y⁺Y* chromosome induces secondary nondisjunction with reduced efficiency compared to a structurally normal *Y*. Although this difference is unexplained, it may reflect the altered structure of the *y⁺Y* derivative when compared to a normal *Y* (LINDSLEY and GRELL 1968). In order to observe all possible segregation events

TABLE 8

Results of crossing $Y^sX \cdot Y^L$, *In(1)EN, y B*/O; *C(4)RM, ci ey*^{R}/*O* males to *y/y/y*$^+$*Y; pol/pol*[a] or *sc*^{4}*sc*^{8}*B*$^+$*/dl-49/y*$^+$*Y; pol/pol* females

Gamete types		Maternal genotype											
		y/y/Y		*y/yAxs/Y*		*yAxs/yAxs/Y*		*sc*^{4}*sc*^{8}*/dl-49/Y*		*sc*^{4}*sc*^{8}*Axs/dl-49/Y*		*sc*^{4}*sc*^{8}*Axs/dl-49 Axs/Y*	
Mother	Father	O ova	*Y* ova	O ova	*Y* ova	O ova	*Y* ova	O ova	*Y* ova	O ova	*Y* ova	O ova	*Y* ova
Regular													
X4	*XY 44*[b]	461	169	1313	504	408	144	573	222	670	273	820	521
X4	O *44*	588	425	1486	1295	394	396		600		620		670
X Nondisjunctional													
O4	*XY 44*	3	30	11	71	11	25	5	378	13	355	67	267
XX4	O *44*	57	1	158	11	42	6	806	0	641	5	506	89
4 Nondisjunctional													
X44	*XY* O	3	1	13	2	16	3	3	1	14	3	57	11
XO	O *44*	0	3	7	7	9	12		(2)[c]		(7)		(26)
X44	O O	6	2	8	4	12	6		(2)		(2)		(3)
XO	*XY 44*	0	0	7	2	6	3	1	3	12	6	12	33
X,4 Nondisjunctional													
XX44	OO	2	0	4	0	0	1	0	1	3	3	14	4
O O	*XY 44*	3	1	3	3	3	0	0	0	1	0	3	8
XX O	O *44*	1	2	3	2	1	2	0	0	7	4	28	17
O *44*	*XY* O	0	1	5	8	5	0	0	3	8	6	37	26
Total progeny:		1759		4927		1505		2600		2653		3219	
Adjusted total:		2116		5997		1853		2374		2371		2669	
% Nondisjunction													
X		9.55		9.31		10.36		50.25[d]		44.12[d]		39.94[d]	
4		1.65		1.77		4.91		0.51		2.83		9.37	
% Nonhomologous disjunction													
XX ↔ *Y*		7.85		7.17		4.32		49.75		41.25		23.68	
XX ↔ *44*		NA		0.27		0.43		0.08		0.76		2.96	
Y ↔ *44*		0.52		0.28		1.24		0		0.13		3.07	
XXY ↔ *44*		NA		0.13		0.32		NA		0.34		1.76	

[a] Chromosomes denoted as *y* are in fact *y cv f* and those denoted as *y Axs* are *y w*^{a} *v Axs car*. All *sc*^{4}*sc*^{8} chromosomes used are B^+ derivatives.

[b] Due to inviability of *XXYY* females, the number of regular *y*$^+$*Y*-bearing males in this class replaces the number of *XXYY* females in all calculations.

[c] The *X/y*$^+$*Y,4* nondisjunctional males (in parentheses), are not used to calculate frequencies of nondisjunction due to the inviability of the corresponding classes of *X*/O *4* nondisjunctional males (see *d* below).

[d] Due to lethality of *X*/O males in these crosses (see MATERIALS AND METHODS and Table 7 footnote *a*), % *X* nondisjunction is calculated as follows: (total *X* exceptional progeny/total progeny)100.

NA, not applicable due to the formation of a negative number.

(including *XXY* ↔ O), it was necessary to use a marked *y*$^+$ *Y* chromosome in these experiments.

In *y/y/y*$^+$*Y; pol/pol* females, there is little or no effect of the *Axs* mutation on total frequencies of *X* chromosome nondisjunction (approximately 10% in control, heterozygous and homozygous *Axs* females). The frequency of secondary nondisjunction (*XX* ↔ *Y*), however, decreases from 82% of the total *X* nondisjunction in *Axs*$^+$*/Axs*$^+$ females to 41% of the total *X* nondisjunction in *Axs/Axs* females. Moreover, in *Axs* homozygotes, the frequency with which both *X* chromosomes and the *Y* segregate to the same pole (*XXY* ↔ O) is increased three-fold over control levels. (Including *XXY* ↔ *44* segregational events, the frequencies for *XXY* ↔ O segregations are as follows: 0.85% for control females; 1.07% for heterozygous *Axs* females; and; 3.07% for homozygous *Axs* females.) These results suggest that *Axs/Axs/y*$^+$*Y* females cannot facilitate *XX* ↔ *Y* segregational events as readily as *Axs*$^+$*/Axs*$^+$*/y*$^+$ *Y* control females.

In *sc*^{4}*sc*^{8}*/dl-49/y*$^+$ *Y; pol/pol* females there is a significant decrease in the frequency of *X* nondisjunction in *Axs* females when compared to *Axs*$^+$ females (from 50% to 40%). Based on a contingency test, this difference is highly significant ($\chi^2 = 81.6$, d.f. = 1, $P < 0.001$). In fact, in *Axs* homozygotes, the presence of the *Y* chromosome does not increase the frequency of *X* nondisjunction above that observed in *sc*^{4}*sc*^{8}*Axs/dl-49 Axs; pol/pol* non-*Y*-bearing females (the total frequency of *X* nondisjunction in these *Axs* homozygotes, 39.9%, is virtually the same as that observed in *sc*^{4}*sc*^{8}*Axs/dl-49 Axs; pol/pol* females, 38.5%, Table 1).

In *Axs* homozygotes and to a lesser extent in *Axs* heterozygotes, the *Y* chromosome segregates from two nondisjoining *X* chromosomes with reduced efficiency. *XX* ↔ *Y* segregation in *sc*^{4}*sc*^{8}*Axs/dl-49 Axs* females accounts for 59% of the total *X* chromosome nondisjunction, whereas in *sc*^{4}*sc*^{8}*Axs*$^+$*/dl-49 Axs*$^+$ control females, 99.0% of the *X* chromosome nondisjunc-

tion can be accounted for by *XX* ↔ *Y* segregations. The frequency of both the *X* chromosomes and the *Y* segregating to the same pole is increased 29-fold over controls in *Axs* homozygotes (6.74%) with a five-fold increase over controls in *Axs* heterozygotes (1.28%). Similarly, *FM7/Axs/y⁺Y* females also exhibit a similar reduction in secondary nondisjunction when compared to *FM7/+/y⁺Y* control females (35.8% compared to 57.5%) (see Table 10). These data, like those obtained for females with structurally normal *X* chromosomes, suggest that the ability of a *Y* chromosome to direct the segregation of both nonexchange *X* chromosomes is strongly reduced in *Axs* bearing females.

The effect of *Axs* on fourth chromosomes in *XXY* females is more complex. In females with structurally normal *X* chromosomes, fourth chromosome nondisjunction rises only threefold in *Axs* homozygotes. There is little or no effect in *Axs* heterozygotes. In contrast, *sc⁴sc⁸Axs/dl-49 Axs/y⁺Y; pol/pol* females exhibit an 18-fold increase in fourth chromosome nondisjunction. This increase is at least partially the result of dramatic increases in the frequency of *XX* ↔ *44* and *XXY* ↔ *44* nonhomologous disjunctional events when compared to controls. Indeed, in *Axs* homozygotes, *XXY* ↔ *44* disjunctional events account for 25% of the *XXY* or OO ova (of the 217 cases of *XXY* ↔ O segregations observed, 156 are the result of *XXY4* ↔ *4* segregations, 54 are the result of *XXY* ↔ *44* segregations, and only 7 cases are the result of all five chromosomes proceeding to the same pole). These data suggest that, in the presence of *Axs*, *XX* ↔ *44* nonhomologous disjunctions may prevent or interfere with proper *XX* ↔ *Y* segregational events in *XXY44* females.

Finally, the frequency of *Y* ↔ *44* disjunctional events is greatly enhanced in *sc⁴sc⁸Axs/dl-49 Axs/y⁺Y* females when compared with *sc⁴sc⁸/dl-49/y⁺Y* control females. A much smaller enhancement is observed in females bearing structurally normal *X* chromosomes. This curious dependence on the frequency of *X* chromosome E_0 tetrads is shown below to be a general property of *Axs*-induced mutant behavior. These data suggest that the *Axs* mutation also increases the ability of a *Y* chromosome to direct fourth chromosome disjunction (*Y* ↔ *44*).

Disjunction in *XX; Dp(1;f)1346* females: *X* and fourth chromosome disjunction was also examined in the presence of *Dp(1;f)1346, y⁺*, a free *X* chromosome duplication which is approximately two times the size of the fourth chromosome (Table 9).

In *sc⁴sc⁸/dl-49; Dp(1;f)1346, y⁺* control females, *XX* ↔ *Dp* segregations are observed twice as frequently as *44* ↔ *Dp* segregations despite the small size of this duplication. (Although females bearing structurally normal *X* chromosomes exhibit frequencies of *XX* ↔ *Dp* segregations lower than *44* ↔ *Dp* segregations, it must be kept in mind that the frequency of *X* E_0 tetrads is much lower in the absence of structural heterozygosity.) Thus, at least some component of the preferential *XX* ↔ *Dp* segregation is likely to reflect factors other than size or shape such as, homology (see DISCUSSION).

There is little or no effect of *Axs* on either *X* or fourth chromosome nondisjunction (twofold over control levels in each case) in *y/y; Dp(1;f)1346, y⁺* females. However, the frequencies of both *X* and fourth chromosome nondisjunction increase in *sc⁴sc⁸Axs/dl-49 Axs; Dp(1;f)1346, y⁺* mutant females when compared to *sc⁴sc⁸/dl-49; Dp(1;f)1346, y⁺* control females. As is observed for *sc⁴sc⁸Axs/dl-49 Axs/y⁺* Y females (Table 8), there is no increase in total *X* nondisjunction over that observed in *sc⁴sc⁸Axs/dl-49 Axs* females (Table 1). Rather, these duplication-bearing *Axs* females redistribute *X* nondisjunctional events among *XX* ↔ *Dp*, *XXDp* ↔ *44*, and *XX* ↔ *44* disjunctions. This result again indicates that the presence of an additional element in the distributive system does not significantly effect the frequency of *X* misbehavior, just the types of events that contribute to the nondisjunctions themselves.

The effect of the *Axs* mutation on the ability of *Dp(1;f)1346* to induce *X* chromosome nondisjunction can be assessed by comparing the proportion of *X* nondisjunctional events due to *XX* ↔ *Dp* segregations. Only 33% of the *X* nondisjunction can be accounted for by *XX* ↔ *Dp* segregational events in *sc⁴sc⁸Axs/dl-49 Axs* homozygotes as opposed to 73% in controls. Thus, in a manner similar to that observed for the *Y* chromosome, *Axs* impairs the ability of the duplication to facilitate *XX* ↔ *Dp* disjunctions.

A comparison of the disjunctional effects of the *Y* and *Dp(1;f)1346, y⁺* chromosomes allows two further inferences to be made.

First, in *sc⁴sc⁸Axs/dl-49 Axs* females the frequency of *XX* ↔ *Y* segregations (23.7%) is only twofold greater than that of *XX* ↔ *Dp* segregations (10.8%). These data are in stark contrast to the observations in *sc⁴sc⁸/dl-49* control females, in which the *Y* chromosome and the duplication differ by almost 15-fold in their capacity to direct the segregation of nonexchange *X* chromosomes. Thus, in *Axs* females, the segregational behaviors of a large metacentric *Y* and a small acrocentric *X* duplication are more similar in terms of their effects on *X* segregation than in *Axs⁺* control females.

Second, in *sc⁴sc⁸Axs/dl-49 Axs* females that carry either the duplication or the *Y* chromosome, the proportion of the *X* nondisjunctional events due to secondary nondisjunction (either *XX* ↔ *Dp* or *XX* ↔ *Y*) is reduced significantly. These data suggest that the *Axs* mutation interferes with those associations involving the *X* chromosome and either the *Y* or *Dp(1;f)1346* chromosomes that normally result in secondary nondisjunctional events.

The effect of *Dp(1; f)1346* on fourth chromosome segregation in *Axs*-bearing females is more complex. There is a fivefold increase in the total frequency of fourth chromosome nondisjunction in sc^4sc^8*Axs/dl-49 Axs* over control levels, but most of this increase can be accounted for by *XX* ↔ *44*, *XXDp* ↔ *44*, or *XX* ↔ *Dp44* segregations. Approximately 80% of all fourth chromosome nondisjunction in control females is due to nonhomologous *Dp* ↔ *44* segregation and this proportion remains relatively unchanged in *Axs* females, regardless of the frequency of *X* chromosome E_0 tetrads. These experiments again indicate that the effect of *Axs* on the fourth chromosomes results from an increase in the frequency of nonhomologous disjunctional events involving the fours and other chromosomes present in the distributive system.

Although there is no change in the overall proportion of fourth chromosome nondisjunction due to nonhomologous *Dp* ↔ *44* segregational events, there is an increase in the absolute frequency of *Dp* ↔ *44* disjunctions when comparing *y Axs/y Axs; Dp* to sc^4sc^8*Axs/dl-49 Axs; Dp* females. This observation parallels that observed for the *Y* ↔ *44* segregational events described above. Thus, as was observed for fourth chromosome disjunction (Table 1), the total frequency of nonhomologous *Dp* ↔ *44* or *Y* ↔ *44* disjunctions responds to an increase in *X* misbehavior due to the presence of high frequencies of *X* E_0 tetrads.

***Axs* is not a *cis*-acting site on the *X* chromosome:** Since *Axs* maps to the *X* chromosome and is associated with a small aberration, it might be suggested that the mutation defines a *cis*-acting chromosomal site on the *X* required for distributive disjunctions. To test this possibility, we examined disjunction in *Axs/FM7/*y^+Y females (Table 10). If the *Axs* mutation did in fact delete a *cis*-acting site and thus, render the *Axs*-bearing chromosome incapable of nonhomologously associating with the *Y* chromosome the following prediction could have been made: *FM7* ↔ *Y* segregations, with the *Axs* chromosome going at random, would be much more frequent than *Axs* ↔ *Y* segregations with the *FM7* chromosome going at random (*Axs*;*Y* ova would be recovered at much higher frequencies than *FM7*; *Y* ova). As shown in Table 10, in *FM7*-bearing females, progeny were derived from both classes of ova and were recovered at approximately equal frequencies in both Axs^+ and *Axs* heterozygous females. We conclude from these data that *Axs* does not define a *cis*-acting *X* chromosome site necessary for proper chromosome disjunction.

The nature of the *Axs* mutation: The ability of a duplication to rescue the *Axs* mutation has been tested in the presence of *Dp(1;4)*r^+f^+ which carries the normal *X* euchromatic DNA from *rudimentary* to *forked* (bands 14A1–16A1 on the polytene map) appended to the right arm of the fourth chromosome. Thus, this duplication is presumed to include the Axs^+ region which has been cytologically mapped to 15D (Figure 1). The effect of this duplication on both *X* and fourth chromosome segregation has been monitored in females bearing this duplication chromosome and the *Axs* mutation. It should be noted that males bearing this duplication chromosome have extremely low viability (Table 11, a and b) and thus, only female progeny will be presented.

The data in Table 12 indicate that, in *Dp(1;4)*r^+f^+-bearing *Axs* females, the effects of the *Axs* mutation are substantially rescued by the duplication. In the absence of the duplication, the number of diplo-*X*, haplo-*4* ova produced by *Axs* females is 58-fold greater than that observed for Axs^+ controls. In duplication-bearing females, however, the effects of the *Axs* mutation are less than two-fold over control levels for *FM7* females and less than three-fold over control levels for *Mu-5* females. The effects on the production of nullo *4* ova parallel those for haplo *4* ova production described above. These data suggest that *Axs* is a hypo- or nullomorphic mutation which can be substantially rescued by a duplication of the wild-type locus.

It is evident that the *Dp(1;4)*r^+f^+ chromosome alone exhibits dramatic effects on the segregation of chromosomes in control females, the strongest of which is on the *X* chromosome. The *Dp(1;4)*r^+f^+ chromosome is a fourth chromosome that contains two numbered units of *X* euchromatin and these results may indicate that homologous interactions between this *X* DNA and the multiply inverted *X* chromosomes in the meiocyte may disrupt homolog segregation in the distributive system. In addition, when comparing the segregational behavior of the *X* chromosomes in females bearing either *Mu-5* or *FM7*, the *FM7* chromosome seems to be affected to a greater degree. This could be due either to the higher frequency of E_0 tetrads generated by the *FM7* chromosome and/or to some structural differences between the two chromosomes.

Regardless of the basal levels of nondisjunction observed in control females, it is clear that the duplication substantially rescues the chromosomes from the *Axs* mutation when compared to these controls. Thus, the analysis of these data, coupled with the semidominant behavior of the *Axs* phenotypes described previously, suggests that *Axs* is a loss-of-function mutation at a dosage sensitive locus. Unfortunately, the regions both proximal and distal to the *Axs* mutation carry haplo-insufficient *Minute* mutations and thus, there are no available deficiencies for the region. Efforts are now underway in the laboratory to generate small deficiencies containing the *Axs* region.

DISCUSSION

The preceding analysis of the *Axs* mutation leads to six basic conclusions, namely: (1) *Axs* induces nondisjunction at meiosis I in Drosophila females, and its

TABLE 9

Results of crossing $Y^{S}X \cdot Y^{L}$, *In(1)EN, y B*/O; *C(4)*RM, *cl* ey^{R}/O males to *y/y:pol/pol*: *Dp(1:f)1346*, y^{+a} or $sc^{4}sc^{8}B^{+}$/*dl-49*; *pol/pol*; *Dp(1:f)1346*, y^{+} females

Gamete types		Maternal genotype											
		y/y/Dp		*y/yAxs/Dp*		*yAxs/yAxs/Dp*		$sc^{4}sc^{8}$/*dl-49/Dp*		$sc^{4}sc^{8}$*Axs/dl-49/Dp*		$sc^{4}sc^{8}$*Axs/dl-49Axs/Dp*	
Mother	Father	O ova	*Dp* ova	O ova	*Dp* ova	O ova	*Dp* ova	O ova	*Dp* ova	O ova	*Dp* ova	O ova	*Dp* ova
Regular													
X4	*XY 44*	1511	1399	1476	1515	751	548	1997	1764	1865	1795	1212	1096
X4	O *44*	1984	1705	1670	1491	700	435						
X Nondisjunctional													
O*4*	*XY 44*	2	10	5	8	3	6	5	35	72	166	107	251
XX4	O *44*	17	4	23	3	13	3	123	4	349	57	521	188
4 Nondisjunctional													
X44	*X Y* O	55	1	94	6	47	2	49	2	67	2	170	13
XO	O *44*	3	37	3	43	1	17						
X44	O O	63	0	79	5	37	0						
XO	*XY 44*	4	42	3	50	6	36	1	29	4	82	20	136
X,4 Nondisjunctional													
XX44	O O	2	1	2	3	1	1	4	6	4	2	26	5
O O	*XY 44*	3	0	1	2	1	0	4	0	3	3	6	11
XXO	O *44*	1	1	0	2	0	2	0	1	2	13	22	55
O *44*	*X Y* O	0	0	0	1	0	1	6	2	13	2	66	22
Total progeny		6845		6480		2611		4032		4501		3927	
Adjusted total		6886		6530		2642		4032		4501		3927	
% Nondisjunction													
X		1.19		1.53		2.35		4.71[b]		15.24[b]		32.60[b]	
4		3.21		4.67		5.98		2.58		4.38		14.06	
% Nonhomologous disjunction													
XX ↔ *Dp*		0.55		0.70		0.83		3.42		8.13		10.85	
XX ↔ *44*		NA		NA		0		NA		0.40		2.98	
Dp ↔ *44*		2.69		3.84		4.84		1.84		3.71		9.58	
XXDp ↔ *44*		NA		NA		0		NA		0.47		2.80	

[a] In *Axs* heterozygotes, *y Axs* denotes *y cv v Axs car* and *y Axs/y Axs* homozygotes are *y* w^{a} *v Axs car/y cv v Axs car*.

[b] Due to the lethality of *X*/O males (see MATERIALS AND METHODS and Table 7 footnote *a*) and the lowered viability of *X*/O; *Dp(1:f)1346* (which are not reported here), the % *X* nondisjunction is calculated as follows: (total *X* exceptional progeny/total progeny)100.

NA, not applicable due to the formation of a negative number.

TABLE 10

Results of crossing *X/Y* males to *FM7/X/Y* females

Segregation in mother	A) *y cv v f car/Y* × *FM7/y* w^{a} *ct*[6] *f car/*$y^{+}Y^{a}$		B) *y* w^{a} ct^{6} *f car/Y* × *FM7/y cv v Axs car/*$y^{+}Y^{a}$	
	Female progeny	Male progeny	Female progeny	Male progeny
X/Y ↔ *Y*	479	470	224	178
X/X/Y ↔ *Y*	4	2	13[b]	30
X ↔ *X/Y*				
FM7 ↔ *X/Y*	480	327	378	329
X ↔ *FM7/Y*	324	237	324	292
Total progeny	2339		1768	
Adjusted total	3290		2247	
% *X* nondisjunction	57.9		41.1	
% Nonhomologous *X/X* ↔ *Y* disjunction	57.5		35.8	

[a] The *FM7* chromosome carries the y^{31d}, w^{a}, *v*, and *B* markers and thus, segregation of all chromosomes can be followed in these crosses.

[b] Due to lowered viability of *XXYY* females, the number of males in this class replaces this number in all calculations.

TABLE 11

Results of crosses involving *Dp (1;4)r⁺f⁺*

Gamete types		
Mother	Father	No.
a) Results of crossing $Y^{S}X{\cdot}Y^{L}$, *In(1)EN, v f B*/O; *C(4)RM, ci ey*^{R}/O males to *C(1)Dx, y f*/$B^{s}Y$; *Dp(1;4) r⁺ f⁺/pol* females		
Y 4	*X Y C(4)*	59
Y Dp(1;4)	*X Y C(4)*	7
No. of mothers		20
b) Results of crossing *y* w^{a} ct^{6} *m f*/$y^{+}Y$; *pol/pol* males to *C(1)Dx, y f*/$B^{s}Y$; *Dp(1;4) r⁺ f⁺/pol* females		
XX 4	*Y 4*	98
XX Dp(1;4)	*Y 4*	152
Y 4	*X 4*	49
Y Dp(1;4)	*X 4*	8
No. of mothers		40

effect is limited to chromosomes in the distributive segregation system; (2) there is a simple and apparently linear relationship between the frequency of *X* nondisjunction and the frequency of *X* chromosome nonexchange tetrads, such that in *Axs* homozygotes *X* nondisjunction is apparently equal to one-half the frequency of E_0 tetrads; (3) nondisjunction of the fourth chromosomes results primarily from nonhomologous *XX* ↔ *44* disjunctions and occurs at high frequency only in the presence of nonexchange *X* chromosomes; (4) nonhomologous disjunctions involving the *X* and other chromosomes account for a large fraction of the total *X* chromosome nondisjunction observed; (5) *Axs* exerts a qualitatively similar effect on the disjunction of a nonexchange major autosome (second chromosome); (6) *Axs* is not a *cis*-acting site on the *X* chromosome, but rather is apparently a loss-of-function mutation at a dosage sensitive locus. This characterization of the *Axs* phenotype is based solely on the analysis of the one existing allele. Before we can conclude that these phenotypes are a general consequence of the loss of *Axs*$^{+}$ function, it will be necessary to characterize a number of alleles.

The data obtained so far, however, do allow us to present below a simple model in which the *Axs*$^{+}$ gene product is required for homolog recognition within the distributive system. We propose that the *Axs* defect may be understood by the failure of homologous chromosomes to properly identify their partners followed by the concomitant occurrence of nonhomologous segregations.

***Axs* specifically affects the distributive segregation system:** The data in Tables 3 and 4 show that *Axs* has little or no affect on exchange events along the length of the *X* chromosome. Moreover, the disjunction of *X* chromosomes which have undergone exchange seems to be unaffected by the presence of either one or two doses of the *Axs* mutation. *Axs* does, however, affect the disjunction of nonexchange *X* chromosomes, such that, as the frequency of *X* chromosome E_0 tetrads rises, so does the frequency of *X* nondisjunction (see below). Similar results have been obtained for the second chromosome, when comparing the effect of *Axs* in females bearing two structurally normal second chromosomes to those heterozygous for the autosomal balancer *SM1*. These data argue strongly that the province of the *Axs* defect is limited to the distributive system.

The relationship between the frequency of nonexchange *X* chromosomal bivalents and *X* chromosome nondisjunction: As shown in Figure 2, *X* chromosome nondisjunction increases in an apparently linear fashion with the frequency of *X* chromosome E_0 tetrads in both hetero- and homozygous *Axs* females. Indeed, in the presence of two doses of *Axs*, *X* chromosome disjunction within the distributive system is apparently random.

There are more than twenty mutations that result in increased frequencies of E_0 tetrads for all chromosomes in the genome by decreasing the frequency of exchange (BAKER and HALL 1976; see also HAWLEY 1988). Unlike the direct relationship between *X* nondisjunction and *X* E_0 tetrads observed in *Axs* females, in these exchange deficient mutations *X* nondisjunction rises in a nonlinear fashion (*X* nondisjunction is however, linear with respect to E_0 cubed, BAKER and HALL 1976).

BAKER and HALL (1976) have argued that the nonlinear relationship between *X* nondisjunction and *X* E_0 tetrads observed in exchange-defective mutations may reflect a requirement for the *X* chromosome and both arms of a major autosome (A) to be nonexchange in order for *X* nondisjunction to occur. The resulting *X* nondisjunctional events would presumably be the product of *XX* ↔ AA nonhomologous segregations (for reviews, see BAKER and HALL 1976; HAWLEY 1988). However, as shown in Table 5, *Axs* has little or no effect on simultaneous *X* and second chromosome nondisjunction except in *Axs/Axs; SM1/+* females where it acts to reduce the frequency of these simultaneous events. Thus, in females homozygous for *Axs*, *X* nondisjunction does not appear to require the presence of nonexchange autosomes, but rather, the absence of an *X* chromosome exchange event is itself sufficient to randomize *X* disjunction.

In addition, the relationship of *X* chromosome nondisjunction to the frequency of E_0 tetrads in the presence of *Axs* is unchanged by the addition of a *Y* chromosome, a free *X* duplication, or a compound fourth chromosome to the distributive system. The ability of *Axs* to induce *X* nondisjunction is therefore unaffected by the number, structure, or identity of other chromosomes undergoing distributive segregations. This observation, in addition to the apparently random disjunction of distributively segregating *X* chromosomes in *Axs* homozygotes, might suggest that the nonexchange *X* chromosomes simply segregate at random in the absence of *Axs*$^{+}$. Yet, paradoxically,

TABLE 12

Results of crossing $Y^SX \cdot Y^L$, *In(1)EN, v f B*/O; *C(4)RM, ci ey*^{R}/O males to *X/X; pol/pol* or *X/X; Dp(1;4)r*$^+$ *f*$^+$*/pol* females

Gamete types		Maternal genotype							
Mother	Father	*FM7/y*	*FM7/yAxs*	*FM7/y/ Dp(1;4)*	*FM7/yAxs/ Dp(1;4)*	*Mu-5/w*^{a}	*Mu-5/w*^{a} *Axs*	*Mu-5/w*^{a}*/Dp(1;4)*	*Mu-5/w*^{a}*Axs/Dp(1;4)*
Regular[a]									
X 4	$\hat{XY}$ $\hat{44}$	8115	888	1566	1442	1275	2452	581	704
X Nondisjunctional									
XX 4	O $\hat{44}$	10	71	50	83	0	101	2	7
4 Nondisjunctional									
X O	$\hat{XY}$ $\hat{44}$[b]	12	20	4	6	0	27	0	6
X,4 Nondisjunctional									
XX O	O $\hat{44}$	0	26	3	12	1	9	0	0
Total progeny		8137	1005	1623	1543	1276	2589	583	717
% Progeny from									
XX;4 ova		0.12	7.07	3.08	5.38	0	3.90	0.34	0.98
X;O ova		0.15	1.99	0.25	0.39	0	1.04	0	0.84
XX;O ova		0	2.59	0.19	0.78	0.08	0.35	0	0

Data are taken from Table 1 and treated in a manner similar to those obtained for duplication-bearing females.

Chromosomes denoted as *yAxs* are in fact *y cv v Axs car*, and those denoted as *w*^{a}*Axs* are *y w*^{a} *v Axs car*. The *y* chromosome used in the *FM7* control is *y cv v f car* and chromosomes referred to as *w*^{a} also carry *y*, *ct*^{6}*f*, and *car*.

[a] Due to low viability of male progeny from duplication bearing females (see Table 11), only female progeny are reported here.

[b] Because the *Dp(1;4)r*$^+$*f*$^+$ chromosome is heterozygous with a normal *pol* fourth chromosome, the only fourth chromosome nondisjunctional products able to be scored are from nullo-*4*-bearing ova.

nonhomologous disjunctional events involving the *X* chromosome, specifically *XX* ↔ *44* and *XX* ↔ *C(4)RM*, are frequently observed in *Axs* females.

The effect of *Axs* on fourth chromosome disjunction: In females with structurally normal *X* chromosomes, *Axs* exhibits a weak effect on the disjunction of the nonexchange fourth chromosomes, while fourth chromosome nondisjunction dramatically increases in *Axs* females heterozygous for *X* chromosome balancers. Thus, fourth chromosome nondisjunctional events induced by the *Axs* mutation require the presence of nonexchange *X* chromosomes. Two lines of evidence suggest that the *Axs*-induced increase in fourth chromosome nondisjunction results from the failure of nonexchange *X* and fourth chromosomes to properly recognize their homologs, followed by the formation of improper *X-4* associations.

First, approximately one-half of the fourth chromosome nondisjunction observed in *Axs* females carrying an *X* chromosome balancer occurs concurrently with *X* nondisjunction, and these simultaneous *X,4* nondisjunctional events are almost always the consequence of nonhomologous *XX* ↔ *44* segregations. Second, as shown in Figure 4, fourth chromosome nondisjunction is linear with respect to *X* nondisjunction in both *Axs* hetero- and homozygotes. These observations suggest that the primary effect of *Axs* on fourth chromosome disjunction may be a result of improper associations with a nonexchange *X* chromosome. This would lead to frequent *X* ↔ *44* disjunctions, leaving the other *X* to segregate at random.

A relationship between increased *X* chromosome nondisjunction and increased fourth chromosome nondisjunction has also been demonstrated for a number of exchange defective meiotic mutants (BAKER and HALL 1976). As more chromosomes enter the distributive system in females bearing one of these mutations, fourth chromosome nondisjunction increases. Although this is similar to the trend seen in *Axs*-bearing females, the high frequency of nonhomologous *X* and fourth chromosome segregation observed in *Axs*-bearing females is in stark contrast to observations made for exchange-defective meiotic mutants. In these mutants, simultaneous nondisjunction of the *X* and fourth chromosomes is apparently random, such that approximately equal frequencies of *XX*;OO, OO;*44*, *XX*;*44* and OO;OO ova are produced (for review see BAKER and HALL 1976).

Although the mechanism by which the *X* chromosome can interfere with proper fourth chromosome disjunction remains unclear, BAKER and HALL (1976) have speculated that "nonexchange *X* chromosomes could associate with fourth chromosomes, and thus disrupt fourth chromosome disjunction, but that these associations were not sufficiently stable to allow nonhomologous segregations of the *X* and fourth chromosomes." Both cytological and genetic evidence suggest that the *X* and fourth chromosomes are evolutionarily related (for review see HOCHMAN 1976). Moreover, MIKLOS *et al.* (1988), have recently shown that the fourth chromosome and the proximal euchromatin of the *X* are highly homologous with respect to the number, composition, and distribution of certain classes of repetitive sequences. Thus, it is possible that homologous sequences present on both the *X* and fourth chromosomes provide opportunities

for frequent associations that can lead to improper segregational events.

Finally, we note that the frequent *X-4* associations and improper disjunctions observed in females bearing structurally aberrant *X* chromosomes are much less common in either Axs^+ or *Axs* females bearing structurally normal *X* chromosomes (*i.e.*, a much smaller fraction of fourth chromosome nondisjunction is associated with *X* nondisjunction). This suggests the possibility that naturally occurring nonexchange *X* chromosomes are in some sense different from, or unique, compared to those nonexchange *X* chromosomes produced by inversion-bearing females.

Nonhomologous disjunctions involving the *X* chromosome in *Axs*-bearing females: The random disjunction of nonexchange *X* chromosomes in *Axs/Axs* females is not due to a failure of the *X* chromosomes to participate in distributive disjunctions *per se*, because nonhomologous disjunctions involving the *X* and, in particular, the fourth chromosomes, are observed at high frequencies in *Axs* females. Indeed, in *Axs* homozygous females bearing a compound fourth chromosome, $XX \leftrightarrow C(4)RM$ nonhomologous disjunctions account for more than 80% of the *X* nondisjunction observed.

The relationship of *Axs* to nonhomologous distributive disjunctions involving the *X* chromosome may be summarized as follows; the frequency of "improper" segregations (such as $XX \leftrightarrow 44$ and $XX \leftrightarrow C(4)RM$), are dramatically increased in females with high *X* E_0 tetrads, while the frequency of "proper" distributive disjunctions (such as $XX \leftrightarrow Y$) are strongly decreased. The effect of *Axs* on secondary nondisjunction in fact parallels the *Axs* defect in $X \leftrightarrow X$ distributive disjunctions, in that failed secondary nondisjunction (such as, $XXY \leftrightarrow O$) frequently occurs as the result of $XX \leftrightarrow 44$ segregational events (*i.e.*, $XXY \leftrightarrow 44$). Perhaps the effect of *Axs* on both proper and improper distributive segregations is a consequence of an *Axs*-induced defect preventing homolog recognition which allows for a more promiscuous choice of partners for the *X* and fourth chromosomes.

A simple model of Axs^+ function: The observations described above are most easily understood in light of a model in which the Axs^+ gene product is required for homolog recognition within the distributive system. Such an assertion contradicts the widely held belief that distributive disjunctions are entirely independent of homology (for reviews see GRELL 1976; HAWLEY 1988).

Several studies have demonstrated however, that the parameters of size and shape alone may be insufficient to explain partner choice and have suggested a possible role of homology in distributive disjunctions. For example, GERSHENSON (1940) and LINDSLEY and SANDLER (1958) observed that in females heterozygous for an *X* chromosome inversion which suppresses exchange, small *X* chromosomal free duplications were capable of inducing high levels of *X* nondisjunction. Indeed, as shown in Table 9, in Axs^+ females heterozygous for two *X* inversions, a free *X* duplication approximately two times the size of the fourth chromosome induces greater than twice as many $XX \leftrightarrow Dp$ disjunctions as it does $44 \leftrightarrow Dp$ disjunctions. This is so despite a greater similarity in size of the duplication to the fourth chromosome. Thus, in competitive situations, homology may well play some role in partner choice within the distributive system.

We propose that one function of the Axs^+ locus is to mediate homolog recognition within the distributive system by promoting tight pairings between homologs, thus preventing pairings (such as *X-4*) which are based on more limited homologies. In this sense, the *Axs* mutation might be likened to the *Ph* mutation on chromosome 5B in wheat which allows homeologous pairing at the expense of proper meiotic pairing of homologs (for review see SEARS 1976).

According to this model, the *Axs* mutation impairs the mechanism by which homologous chromosomes recognize each other. In this way, *Axs* allows for the formation of less homologous pairings and/or complex multivalents in which improper nonhomologous associations occur as a secondary effect. Our model also requires that not all secondary associations are equally likely to occur or to cause nondisjunction. This assumption is necessary to explain the decreased ability of the *Y* or *Dp(1;f)1346* chromosomes to impair $4 \leftrightarrow 4$ segregation in *Axs* females bearing structurally normal *X* chromosomes where *X* E_0 tetrads are low.

For example, we propose that in $sc^4sc^8Axs/dl\text{-}49Axs$; *C(4)RM* females, the nonexchange *X* chromosomes are incapable of pairing with each other and consequently one *X* pairs with the *C(4)RM* chromosome while the other segregates at random. Thus, these *X-C(4)RM* nonhomologous disjunctions would result in *X* nondisjunction in approximately one-half of the *X* E_0 tetrads. We would further propose that, as more elements are added to the distributive system, nonhomologous associations involving the *X* chromosomes and other heterologs continue to occur in the form of more complicated multivalents.

This model explains the following observations: (1) all of the $sc^4sc^8Axs/dl\text{-}49Axs$ females tested exhibit frequencies of *X* chromosome nondisjunction approximately equal to one-half the E_0 tetrads, regardless of the presence or absence of other potential disjunctional partners; (2) $XX \leftrightarrow Y$ distributive disjunctions are disrupted by the *Axs* mutation in a manner similar to $X \leftrightarrow X$ distributive disjunctions and thus, the frequency of secondary nondisjunction in *XXY* females homozygous for the *Axs* mutation is reduced; (3) as is strikingly apparent in the *C(4)RM* experiment (where only three elements are present in the distributive

system), *X* chromosomes in *Axs* females are free to disjoin from a nonhomolog with high efficiency; and (4) fourth chromosome nondisjunction rises in response to increases in *X* E_0 tetrad frequency regardless of the presence of other elements in the system.

It should be noted that in *X/X/y*$^+$*Y* or *X/X/Dp(1; f)1346* females bearing two structurally normal *X* chromosomes, *Y* ↔ *44* or *Dp* ↔ *44* disjunctions are not increased in *Axs* heterozygotes and are only moderately increased in *Axs* homozygotes. Dramatic increases, however, in these disjunctional events, as well as *XXY* ↔ *44* or *XXDp* ↔ *44* disjunctions, are observed in the presence of heterozygosity for structural aberrations that suppress *X* chromosome exchange. To explain these results, we propose that all four types of nonhomologous segregation result from the formation of complex multivalents involving both nonexchange *X* chromosomes, both fourth chromosomes, and either the *Y* or the duplication. We further argue that such multivalents result from the same relaxation of homology dependent pairing postulated to account for *XX* ↔ *44* disjunction in *X/X* females.

Whether or not our proposed mechanism for the precise action of the *Axs* mutation is correct, it is clear that *Axs* defines a locus required for the proper disjunction of homologs within the distributive system. In light of previously described schemes of meiosis, we believe that the *Axs*$^+$ locus controls a crucial step in the differentiation of homologous *vs.* nonhomologous elements prior to disjunction. In addition, the effect on *X* chromosome disjunction revealed by the *Axs* mutation, taken together with the high degree of fourth chromosome interference that the mutation promotes, indicates a tight coupling of the *X* and fourth chromosomes within the distributive system that was also indicated by the *ald* and *mei-S51* mutations.

Perhaps the wild-type functions defined by these three mutations aid in the suppression of *X,4* associations which arise as a consequence of limited homology between the chromosomes. Thus, a mutation in one of these loci leads to high frequencies of nonhomologous disjunctions. If such a model is correct, then the analysis of the *Axs* mutation, along with the *ald* and *mei-S51* mutations, has revealed the existence of a novel component of distributive pairing in Drosophila which allows for the recognition of limited homologies between heterologous chromosomes.

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