GENETIC ANALYSIS OF SEX CHROMOSOMAL MEIOTIC MUTANTS IN *DROSOPHILA MELANOGASTER*[1]

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ABSTRACT

A total of 209 ethyl methanesulfonate-treated *X* chromosomes were screened for meiotic mutants that either (1) increased sex or fourth chromosome nondisjunction at either meiotic division in males; (2) allowed recombination in such males; (3) increased nondisjunction of the *X* chromosome at either meiotic division in females; or (4) caused such females, when mated to males heterozygous for Segregation-Distorter (*SD*) and a sensitive homolog to alter the strength of meiotic drive in males.——Twenty male-specific meiotic mutants were found. Though the rates of nondisjunction differed, all twenty mutants were qualitatively similar in that (1) they alter the disjunction of the *X* chromosome from the *Y* chromosome; (2) among the recovered sex-chromosome exceptional progeny, there is a large excess of those derived from nullo-*XY* as compared to *XY* gametes; (3) there is a negative correlation between the frequency of sex-chromosome exceptional progeny and the frequency of males among the regular progeny. In their effects on meiosis these mutants are similar to $In(1)sc^{4L}sc^{8R}$, which is deleted for the basal heterochromatin. These mutants, however, have normal phenotypes and viabilities when examined as *X*/0 males, and, furthermore, a mapping of two of the mutants places them in the euchromatin of the *X* chromosome. It is suggested that these mutants are in genes whose products are involved in insuring the proper functioning of the basal pairing sites which are deleted in $In(1)sc^{4L}sc^{8R}$, and in addition that there is a close connection, perhaps causal, between the disruption of normal *X*-*Y* pairing (and, therefore, disjunction) and the occurrence of meiotic drive in the male.——Eleven mutants were found which increased nondisjunction in females. These mutants were characterized as to (1) the division at which they acted; (2) their effect on recombination; (3) their dominance; (4) their effects on disjunction of all four chromosome pairs. Five female mutants caused a nonuniform decrease in recombination, being most pronounced in distal regions, and an increase in first division nondisjunction of all chromosome pairs. Their behavior is consistent with the hypothesis that these mutants are defective in a process which is a precondition for exchange. Two female mutants were allelic and caused a uniform reduction in recombination for all intervals (though to different extents for the two alleles) and an increase in first-division nondisjunction of all chromosomes. Limited recombination data suggest that these mutants do not alter coincidence, and thus, following the arguments of *Sandler et al.* (1968), are defective in exchange rather than a precondiiton for exchange. A single female mutant behaves in a manner that

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is consistent with it being a defect in a gene whose functioning is essential for distributive pairing. Three of the female meiotic mutants cause abnormal chromosome behavior at a number of times in meiosis. Thus, nondisjunction at both meiotic divisions is increased, recombinant chromosomes nondisjoin, and there is a polarized alteration in recombination.——The striking differences between the types of control of meiosis in the two sexes is discussed and attention is drawn to the possible similarities between (1) the disjunction functions of exchange and the process specified by the chromosome-specific male mutants; and (2) the prevention of functional aneuploid gamete formation by distributive disjunction and meiotic drive.

IN Drosophila, most of our knowledge of meiosis has come from a number of elegant genetic studies which have utilized structural and numerical rearrangements as well as the normal chromosomal constitution to gain insights into meiotic chromosome behavior. These studies, although they have given us a precise formal description of chromosome behavior during meiotic recombination and segregation, have shed little light onto the control of this behavior. One approach toward an understanding of the genetic control of meiosis in *Drosophila melanogaster* was suggested by SANDLER *et al.* (1968) who undertook the systematic isolation and characterization of mutants in which meiotic chromosome behavior was abnormal. The assumption upon which this approach is based is that the normal functions of genes governing meiotic processes can be inferred from the abnormal meiotic chromosome behavior caused by mutants in such genes.

With such a genetic approach, the detection of mutations affecting meiosis is based on the recovery of end-products of meiosis (eggs or sperm) that are abnormal either in their chromosome content (aneuploidy owing to chromosome nondisjunction, loss or breakage) or in the quality of the chromosomes they contain (abnormal patterns of recombination, coincidence, or unequal recovery of homologs). Hence, the types of genes in which meiotic mutants can be detected are limited by the screening method that is employed.

SANDLER *et al.* screened for meiotic mutants on chromosomes *2* and *3* isolated from natural populations of *D. melanogaster* by testing for increased rates of *X* or fourth chromosome nondisjunction or loss at either the first or second meiotic division in females and increased rates of fourth chromosome nondisjunction or loss at either meiotic division in males. By this procedure, they found 11 second and/or third chromosomes that had a detectable effect on chromosome segregation in females and 4 second and/or third chromosomes that had a detectable effect on chromosome segregation in males.

Their preliminary characterization of these mutants and the intensive characterization of several of these and other mutations affecting Drosophila meiosis (*ca*nd, G. DAVIS 1968; LINDSLEY *et al.* 1968; *c(3)G*, HALL 1971; *mei-S51*, ROBBINS 1971; *mei-S332*, B. DAVIS 1971; *mei-S282*, PARRY 1972) have shown that it is possible to delineate the control points of the genes defined by these mutations with respect to previously known genetic landmarks of meiosis (recombination, first and second division segregation, and distributive pairing). From these analyses, it has also been possible to make some inferences about the func-

tion of the wild-type alleles of these mutants in insuring a normal meiosis.

In this report, the results of a search for ethyl methanesulfonate (EMS)-induced meiotic mutants on the *X*-chromosome of *D. melanogaster* will be presented. The procedure used was designed to detect meiotic mutants that (1) increased sex- or fourth-chromosome nondisjunction or loss at either the first or second meiotic division in males hemizygous for the mutagenized *X* chromosome; (2) allowed recombination in such males (recombination is normally absent in *D. melanogaster* males); (3) increased nondisjunction or loss of the *X* chromosome at either meiotic division in females homozygous for the mutagenized *X* chromosome; or (4) caused such females to alter the amount of meiotic drive in males heterozygous for Segregation-Distorter (*SD*) and a sensitive homolog. (*SD* is a second chromosome that, when heterozygous in males with a sensitive chromosome *2*, is recovered much more frequently in the progeny than is its homolog. One hypothesis about the mechanism of meiotic drive (ZIMMERING, SANDLER and NICOLETTI 1970) is that females can distinguish *SD*-bearing sperm from sperm containing the homolog and cause selective fertilization by *SD*-bearing sperm. If this hypothesis is correct, it should be possible to isolate mutations which alter the females' ability to distinguish different sperm types.)

A screen for meiotic mutants on the *X* chromosome in which *X*-chromosome nondisjunction is used as one of the methods for detecting a mutant can theoretically detect not only mutations in meiotic controlling genes (that is, genes that affect the recombinational or disjunctional behavior of all chromosomes, such as *c(3)G* and *ca*nd) but, in addition, mutations that alter the ability of the chromosome carrying the mutation to respond to some normal control step of meiosis.

In tests of 209 mutagenized *X* chromosomes in males, at least 20 chromosomes were found in which meiotic chromosome segregation was abnormal; in tests of 189 of the mutagenized *X* chromosomes in females, 11 chromosomes were found to increase *X* nondisjunction. A preliminary characterization of these chromosomes with respect to their effects on recombination and disjunction will be presented; this characterization has allowed the times of action of these meiotic mutants to be specified with respect to the known genetic landmarks of meiosis, and some inferences to be made about the functions of the wild-type alleles of these loci.

EXPERIMENTAL PROCEDURES

To induce *X*-linked meiotic mutants, males of the constitution *y/Y; SM1, Cy; TM2, Ubx, e/T(2,3)S9, bw, e; spa*^{pol}*/spa*^{pol} (descriptions of chromosomes and markers are found in LINDSLEY and GRELL 1968) were fed on a 0.1% v/v solution of ethyl methanesulfonate (EMS) in 1% sucrose for twenty-six hours (LEWIS and BACHER 1968). In this laboratory, treatment of *Canton-S* males with this dose of EMS results in approximately 35% sex-linked lethals in a standard *Muller-5* test. The treated males were mated in mass to *C(1)RA, y f bb*$^{-}$ *[= C(1)DX of Muller]/y+Y; +/+; +/+; spa*^{pol}*/spa*^{pol} females (Figure 1, generation 1) for eight days after which the parents were discarded. This insures the utilization of only those sperm that were at meiotic or post-meiotic stages at the time of treatment. In generation 2, 394 $y_i/y+Y$; $SM1_i/+$; $TM2_i/+$; spa^{pol}/spa^{pol}_i males (where "*i*" indicates a mutagenized chromosome), each carrying a separately mutagenized chromosome complement, were mated singly to

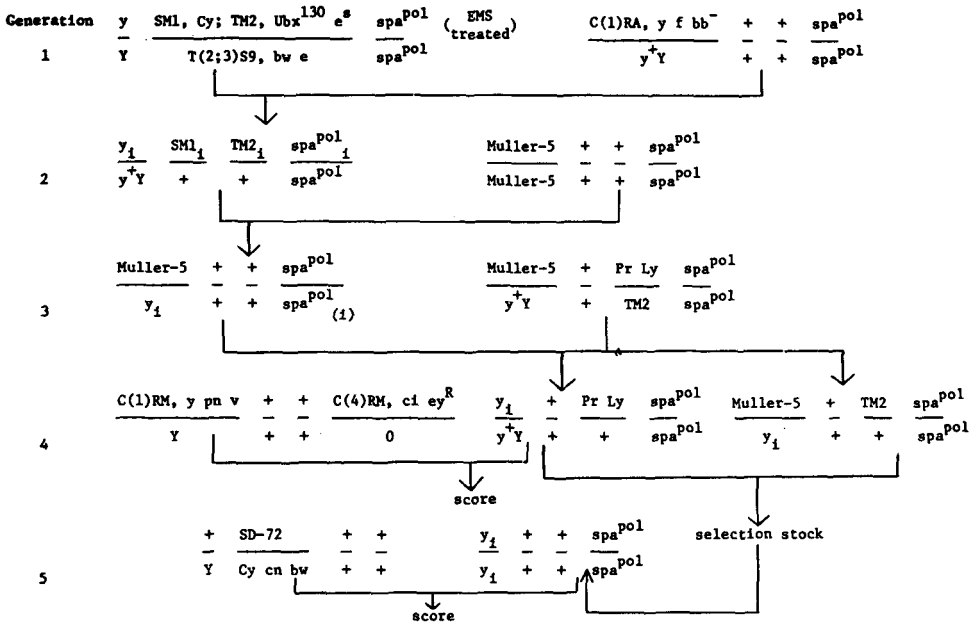

FIGURE 1.—The crossing scheme employed to examine the effects of mutagenized *X* chromosomes on meiotic chromosome behavior. Sub "i" denotes EMS-treated chromosome.

Muller-5/Muller-5; +/+; +/+; spa^{pol}/spa^{pol} females (Figure 1, generation 2) to establish the lines to be tested for meiotic mutants. Because the mutagenized *X* chromosomes, y_i, are recovered as hemizygous F_1 males, lethal-bearing *X* chromosomes are eliminated. Of these crosses, 160 were sterile; from the 234 fertile lines, *Muller-5/*y_i; +/+; +/+; $spa^{pol}/spa^{pol}{}_{(i)}$ virgins were collected in generation 3. In these females, the mutagenized second and third chromosomes have been replaced with untreated ones. No attempt was made to follow or eliminate the mutagenized fourth chromosomes.

The EMS mutagenesis procedure employed induces frequent half-chromatid mutations (JENKINS 1967). Half-chromatid mutations induced in this experiment would segregate so that the females collected in generation 3 could be a mixture of mutant and non-mutant females. To insure homogeneity within a line, the virgin females collected in generation 3 were mated singly with *Muller-5/*y^+Y; +/+; *Ly Pr/TM2;* spa^{pol}/spa^{pol} males. Only one of these sublines was used to establish generation 4.

In generation 4, y_i/y^+Y; +/+; *Ly Pr/++;* $spa^{pol}/spa^{pol}{}_{(i)}$ males were tested for the presence of a mutant affecting male meiotic behavior. For the test cross, at least five males from each line were mated singly to *C(1)RM, y pn v/Y;* +/+; +/+; *C(4)RM, ci* $ey^R/0$ females. This cross permits the recovery and detection of sperm that result from nondisjunction of the sex or fourth chromosomes at either the first or second meiotic division, as well as those that result from regular disjunction. Furthermore, recombination in these males between the dominant markers *Ly* and *Pr* is detectable.

Also in generation 4, a selection stock was established for each line by crossing y_i/y^+Y; +/+; *Ly Pr/++;* $spa^{pol}/spa^{pol}{}_{(i)}$ males to *Muller-5/*y_i; +/+; *TM2/+;* $spa^{pol}/spa^{pol}{}_{(i)}$ females. Females homozygous for the mutagenized chromosomes were obtained from these stocks in generation 5 and in subsequent generations, and their meiotic behavior was examined by mating 15 single y_i/y_i; +/+; +/+; $spa^{pol}{}_{(i)}/spa^{pol}{}_{(i)}$ females from each line to y^+/Y; *SD-72/Cy cn bw;* +/+; +/+ males. This cross allows the detection of *X*-chromosome nondisjunction (although eggs resulting from *X*-chromosome nondisjunction are recovered only

half as frequently as those resulting from regular disjunction) and maternal influences on the behavior of *SD-72* in the male. Maternal influences on meiotic drive in males were screened by looking for deviations from the control values for the relative recoveries of the *SD-72* and *Cy cn bw* chromosomes.

Of the 234 fertile lines in generation 3, twenty-five were unavailable for testing either because they were lost or proved to be homozygous lethal. The remaining 209 lines were tested for the presence of mutants affecting meiosis. In addition to the meiotic mutants described below, three lines showed abnormal meiotic behavior which, on subsequent testing, proved to be due to the presence of translocations .

MALE TESTS

In control crosses of y/y^+Y; *Ly Pr*/++; spa^{pol}/spa^{pol} males by *C(1)RM, y pn v*/*Y*; +/+; *C(4)RM, ci* ey^R/0 tester females, there were approximately 0.7 sex-chromosome exceptions and 1.6 fourth-chromosome exceptions per thousand progeny, and no recombinants between *Ly* and *Pr* among 4367 progeny. In similar crosses, males from 209 mutagenized lines were tested and an average of 220 progeny per line scored. A mutagenized line was retested as possibly having a meiotic mutant if either two or more exceptional progeny of two different types (*e.g.*, one derived from a nullo-*X*-bearing sperm and one from a diplo-*4*-bearing sperm), or three or more exceptional progeny of any one type, or any recombinants between *Ly* and *Pr* were found in the total progeny of all males tested from that line.

Two lines were retested because a single male from each line gave a few recombinants between *Ly* and *Pr*. On retesting, however, no further recombinants appeared, suggesting that the original events were spontaneous gonial exchanges which occur with a low frequency in *D. melanogaster* males.

On the basis of the disjunctional criteria, 69 of the 209 mutagenized *X* chromosomes were retested for the presence of an effect on sex-chromosome and fourth-chromosome disjunction. For retests, y_i/y^+Y; $spa^{pol}{}_{(i)}/spa^{pol}{}_{(i)}$ males were crossed to *y pn*/*y pn*; *C(4)RM, ci* ey^R/0 tester females. In the retests a number of these lines exhibited frequencies of sex chromosome, but not fourth chromosome, exceptional progeny that were significantly higher than the control frequency; however, there was no clear demarkation between those lines that were normal and those which had increased frequencies of sex-chromosome exceptional progeny (see Figure 2). If, arbitrarily, 1% or more sex-chromosome exceptional progeny is chosen as the criterion for accepting a line as having abnormal sex-chromosome disjunction, then 20 lines had high rates of sex-chromosome nondisjunction (Table 1) which were reproducible on further retesting.

These mutants are strikingly similar to one another in their effects. They all alter the disjunction of the *X* chromosome (the chromosome which they are on) from the *Y* chromosome, but do not affect fourth-chromosome disjunction. Among the recovered sex-chromosome exceptions, there is a large excess of those derived from nullo-*XY*, as compared to *XY*, gametes; between 70–95% of all recovered sex chromosome exceptions are from nullo-*XY* sperm. In addition, there is a negative correlation between the frequency of sex-chromosome excep-

TABLE 1

Sex and fourth chromosome disjunctional data from crosses of y mei-/y+Y; spa^{pol}/spa^{pol} *male to* y pn/y pn; C(4)RM,ci ey^{R}/0 *females*

Recovered male gametes:	*X;4*	*Y;4*	*X/Y;4*	*0;4*	*X;4/4*	*X;0*	*Y;44*	*Y;0*	*X/Y;4/4*	*X/Y;0*	*0;4/4*	*0;0*	Total	*XY*+0/10^{3} gametes	*44*+0/10^{3} gametes	*Y;4/X;4+Y;4*
X chromosome of male																
+	2973	2758	4	6	8	11	0	0	0	1	2	0	5763	2.3	3.8	.48
*sc^{4}sc^{8}**	3486	1771	149	1820	—	—	—	—	—	—	—	—	7226	272.5	—	.34
mei-23	1311	884	5	14	1	2	1	3	0	1	2	1	2225	10.2	4.9	.40
-99	3970	2830	16	55	8	9	3	3	0	1	3	1	6899	11.0	4.0	.41
-150	830	554	5	12	5	1	3	0	0	0	0	0	1410	12.1	6.4	.40
-243	1424	933	6	23	1	1	1	4	0	0	2	0	1873	12.9	3.8	.40
-390	1107	739	3	22	1	0	0	1	0	0	0	0	2396	13.3	1.1	.40
-10	873	536	3	16	2	1	0	2	0	0	0	0	1436	13.3	3.5	.38
-94	1123	757	1	23	1	0	0	0	0	1	1	0	1906	13.6	1.1	.40
-268	978	818	7	18	0	1	0	2	0	0	0	0	1824	13.8	1.6	.46
-183	1360	875	1	27	3	2	0	0	0	1	4	1	2274	15.0	4.8	.39
-75	371	274	2	8	0	0	0	0	0	0	0	0	655	15.5	0.0	.43
-334	784	469	1	19	0	0	1	0	0	0	0	0	1274	15.7	0.8	.37
-242	1184	903	4	33	1	2	2	2	0	0	0	0	2130	17.4	2.4	.43
-19	1307	708	1	36	0	2	2	0	0	0	0	1	2057	18.5	2.4	.35
-30	1084	774	9	26	2	0	1	0	0	0	0	0	1896	18.5	1.6	.42
-358	833	571	8	29	0	2	1	0	0	0	0	0	1414	24.9	2.1	.39
-346	1408	756	9	48	0	2	0	2	0	0	1	0	2226	26.1	2.3	.35
-36	1183	637	10	49	1	3	1	1	0	0	0	0	1885	31.3	3.2	.35
-126	437	283	2	24	2	0	0	1	0	0	1	0	750	36.0	5.3	.39
-256	293	182	2	35	0	0	0	0	0	0	1	0	513	74.0	2.0	.38
-269	804	464	14	118	2	1	2	1	0	0	3	0	1410	95.8	6.4	.37

* From SANDLER, Genetics **64,** 481 (1970), Table 4, page 486.

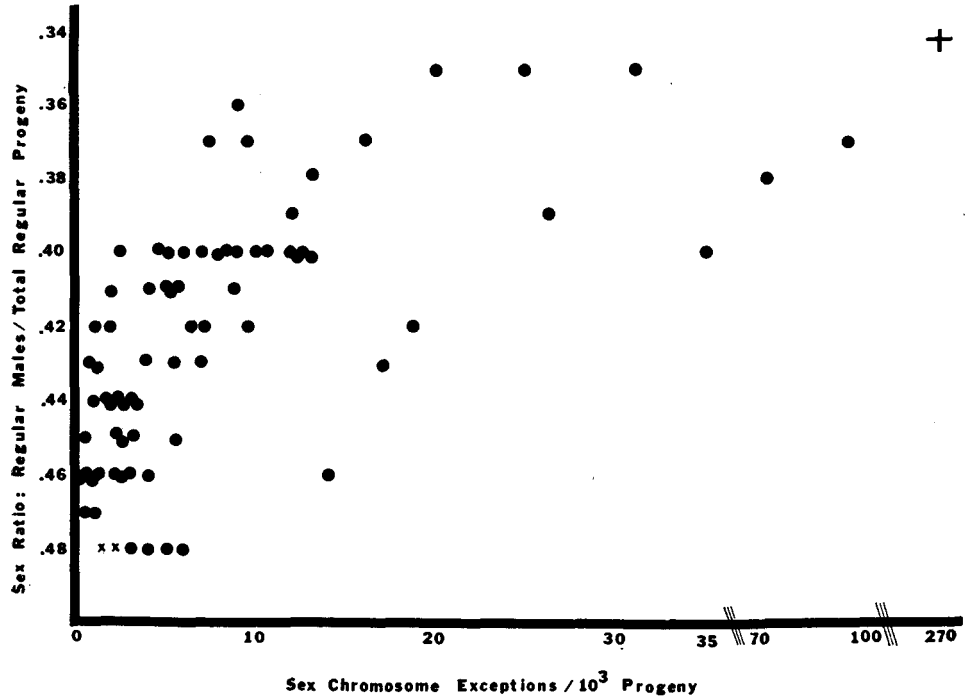

FIGURE 2.—Sex ratio among regular progeny of *y mei-/y+Y; spa^{pol}/spa^{pol}* males crossed to *y pn/y pn; C(4)RM, ci ey^{R}/0* females *vs.* the rate of sex chromosome exceptions produced by these males. ● denotes *y mei-* males, *X* denotes *y* control males, + denotes *In(1)sc^{4L}sc^{8R}* males.

tional progeny in these lines and the frequency of males among the regular progeny. In Figure 2, the sex ratio among regular progeny is plotted against the frequency of sex-chromosome exceptional progeny for all 69 lines originally retested as possibly having a male meiotic mutant. Finally, when homozygous in females, 19 of the 20 mutant chromosomes do not show any increase above wild-type controls in the frequency of *X*-chromosome exceptional progeny. In the case of the one chromosome which altered disjunction in both sexes (*mei-99*), the effects in the two sexes are quite different; this suggests that this chromosome has two meiotic mutants, one male-specific and one female-specific.

If nondisjunction at the first meiotic division were occurring in males in the crosses of *y mei-/y+Y; spa^{pol}/spa^{pol}* males by *y pn/y pn; C(4)RM; ci ey^{R}/0* females, then equal frequencies of progeny derived from nullo-*XY* and *XY*-bearing sperm would be expected (*y mei-* denotes an original mutagenized *X* chromosome marked with *y* and bearing one of the meiotic mutants). One possible source of the excess of nullo-*XY*-bearing sperm is nondisjunction at the second meiotic division because the reciprocal products (*XX* and *YY* gametes) result either in zygotic lethality (*XX* gametes) or in progeny phenotypically indistinguishable from regular male progeny (*YY* gametes). To test for the occurrence of second-division nondisjunction in the 20 mutant lines, *y mei-/y+Y* males were crossed to *C(1)RM, y pn v/0* females. This cross allows the recovery and

detection of diplo-*X* sperm, nullo-*XY* sperm, and *XY*-bearing sperm as well as the products of regular disjunction of the sex chromosomes. No progeny from diplo-*X* sperm were recovered in any of the 20 lines tested, establishing that the excess of nullo-*XY* gametes recovered from these mutants in crosses to free-*X*-bearing females is not due to the occurrence of second-division nondisjunction.

The disjunctional abnormalities observed in these lines are strikingly similar to those observed with *X* chromosomes that carry deficiencies in the basal heterochromatin (such as *In(1)sc^{4L}sc^{8R}*, SANDLER and BRAVER 1954, also see Table 1 and Figure 2). To see whether any of the mutant lines carried deficiencies for basal heterochromatin, *y mei-*/0 males from the 20 lines were examined. The phenotype and viability of *y mei-*/0 males were normal in all cases. However, a deficiency for basal heterochromatin does not necessarily lead to inviability or to phenotypic effects (BAKER 1971), so this matter perforce remains equivocal.

Two of these mutants (*mei-269, mei-346*) were mapped relative to *y^2 cv v wy car* by taking several hundred male progeny from females heterozygous for the *y mei-* chromosome and a *y^2 cv v wy car* chromosome, crossing them individually to *y/y* females, and scoring for sex-chromosome nondisjunction in the progeny. Because the number of exceptional progeny from any single meiotic-mutant-bearing male can overlap the number of exceptions produced by single control males, only those males which were clearly mutant ($\geq 1\%$ exceptional progeny) were used to map the mutants. In this mapping, *mei-269* was found to carry an inversion, *In(1)3AB-9E*, which suppressed nearly all recombination between *y* and *v*. The genotype and number of males in the mapping of *mei-269* which had 1% or more sex chromosome exceptional progeny are as follows: $y + + + + = 66$, $y + + + car = 12$, $y^2\ cv\ v\ wy + = 1$, $y^2\ cv\ v + + = 2$. This places *mei-269* in the euchromatin of the *X* chromosome between *wy* and *car* and close to *wy*. A similar mapping of *mei-346* gave *mei-346*-bearing male progeny of the following genotypes: $y + + + + = 33$, $y + + + car = 1$, $y^2\ cv\ v\ wy + = 3$, $y^2\ cv\ v + + = 2$, $y^2\ cv + + + = 3$. This places *mei-346* in the euchromatin of the *X* chromosome, between *wy* and *car*, but not necessarily at the same site as *mei-269*. Thus, though the number of recombinants is small in both cases, the mutants appear to map in the euchromatin and not in the basal heterochromatin.

At the time the above experiments were completed, further experimentation on these male mutants was interrupted for about nine months. When experiments were resumed with six of the stronger mutants, it was found that the frequencies of *X*-chromosome exceptional progeny were much lower than they had previously been and that the sex ratio among the regular progeny of these mutants approached that in control crosses. For example, *mei-269* in its original test produced 95.8 sex-chromosome exceptions per 10^3 recovered sperm (1410 progeny examined) and on retests one and five months later still exhibited a high level of nondisjunction (112 exceptions/10^3 sperm, 10963 total progeny; 115 exceptions/10^3 sperm, 747 total progeny), but when tested nine months subsequently produced only 25 exceptions/10^3 sperm (1778 total progeny). The other male mutants behaved similarly. Several attempts to restore the meiotic effects of these mutants by outcrosses to replace the autosomes have proved unsuccess-

ful. This would suggest that either the *Muller-5* chromosome failed to balance these mutants effectively and they were lost through crossing over, or they accumulated *X*-linked modifiers which suppress the disjunctional effects, or that mutants of this type revert frequently just as they are induced with high frequency.

DISCUSSION OF MALE MUTANTS

The behavior of these male meiotic mutants is very similar to that of *X* chromosomes, such as *In(1)sc^{4L}sc^{8R}*, which are deficient for basal heterochromatin (SANDLER and BRAVER 1954). Both *sc^{4}sc^8* and these mutants exhibit (1) increased nondisjunction of sex chromosomes (but not of the fourth chromosome); (2) a large excess of nullo-*XY* sperm compared to *XY* sperm among the recovered exceptional male gametes; and (3) a decrease in the sex ratio among regular progeny (measured as regular males/total regular progeny).

The behavior of *sc^{4}sc^8* has been extensively studied both genetically and cytologically. It has been found genetically that reciprocal products of meiotic segregations are not recovered equally ($X > Y$; $O > XY$). However, cytologically, reciprocal classes are found equally frequently at anaphase of the second meiotic division; furthermore, although frequent nondisjunction of the *sc^{4}sc^8* chromosome from the *Y* chromosome is observed cytologically, there is no evidence of chromosome loss (PEACOCK 1965). From the observation that equally-frequent products of meiosis were recovered very unequally as progeny, PEACOCK inferred that meiotic drive was occurring in *sc^{4}sc^8/Y* males. The nondisjunctional behavior of *sc^{4}sc^8* in males is understandable in that the sites at which the *X* normally pairs with the *Y* are located in the basal heterochromatin which is deleted in *sc^{4}sc^8* (GERSHENSON 1940; COOPER 1964).

Although the mutants reported here are similar to *sc^{4}sc^8* in their meiotic phenotype, the evidence cited above suggests that they are not mutations or deficiencies in the heterochromatic pairing sites of the *X*. Nevertheless, the very similar effects of these mutants and *sc^{4}sc^8* suggests that they are defective in the same process in male meiosis. If it is assumed that the primary defect in *sc^{4}sc^8* is the deletion of the heterochromatic pairing sites of the *X* chromosome, then it may be inferred that at least some of the mutants reported here are in genes whose products are involved in insuring the proper functioning of those pairing sites. Furthermore, if it may be inferred (from *sc^{4}sc^8*) that the unequal recovery of *X* and *Y* and also of *XY* and nullo-*XY* meiotic products in these mutants is due to meiotic drive, then it would appear that there is some close, perhaps causal, relationship between the disruption of normal *X-Y* pairing (and, therefore, disjunction) at meiosis I and the occurrence of meiotic drive in the male.

FEMALE TESTS

To test for female meiotic mutants, 15 or more *y$_i$/y$_i$*; *spa^{pol}/spa^{pol}* females from each line were crossed singly to *+/Y*; *SD-72/Cy cn bw* males. Of the 209 lines available for testing, six had *X* chromosomes that were homozygous lethal

and 14 were female sterile. To insure that the female steriles were not in fact meiotic mutants so strong that virtually all eggs were aneuploid, females from these lines were further tested by mass mating them to (1) males carrying attached-second and attached-third chromosomes (y^2; *C(2L)RM,dp*; *C(2R)RM, px*; *C(3L)RM,h rs*^{2}; *C(3R)RM*) which allows recovery of ova simultaneously nondisjunctional for the second and third chromosomes; (2) $y/Y{\cdot}sc^8$; *cn mei-S332/cn mei-S332*; *e/e*; *gvl/gvl* males which allows recovery of ova having anywhere from no chromosomes to the diploid chromosome complement (DAVIS 1972); and (3) to $Y^sX{\cdot}Y^L$, *In(1)EN, v f B*/0; *C(4)RM, ci ey*^{R}/0 males at both 18°C and 25°C to examine the possibility that the female sterility of these lines might be temperature sensitive. No progeny from aneuploid eggs were recovered from these females in any of these tests, suggesting that the sterility was not the consequence of extreme segregational anomalies.

The crosses of the 189 female-fertile lines by +/*Y*; *SD-72/Cy cn bw* males were examined for increased nondisjunction of the *X* chromosomes and for an effect of the females on the relative recovery of the two paternal second chromosomes. *SD* is a locus on the second chromosome which causes meiotic drive (SANDLER, HIRAIZUMI and SANDLER 1959). Control crosses of 24 females with wild-type *X* chromosomes all gave 99–100% *SD-72* progeny. The results of the tests for a female-dependent alteration in the recovery of *SD-72* were: 185 lines with 99–100% *SD-72* progeny; 3 lines with 98–98.9% *SD-72* progeny; one line with 96.9% *SD-72* progeny. The four experimental lines that produced less than 99% *SD-72* progeny all produced between 99 and 100% *SD-72* progeny on retesting.

Control crosses to measure *X*-chromosome nondisjunction in females produced approximately one *X*-chromosome exception per 1000 progeny. Therefore, a line was selected for retesting if two or more *X*-chromosome exceptional progeny were found (an average of 580 progeny were scored for each line). On this criterion, 23 lines were chosen for retesting and eleven of these had reproduceably high rates of *X*-chromosome nondisjunction. The characterization of these presumptive meiotic mutants is presented below.

CHARACTERIZATION PROCEDURES FOR MUTANTS AFFECTING FEMALE MEIOSIS

The presumptive female meiotic mutants were examined for their effects on the disjunction of all four chromosome pairs and on recombination. They were also examined as to their dominance and the division at which induced nondisjunction occurred. In this section we will present the experimental procedures and results and in addition the analysis of the data for one of these female meiotic mutants, *mei-218*, as an example of how these data have been analyzed. The results of similar analyses for all of the female meiotic mutants are presented in tabular form in Tables 6 and 7. In the next section all of the mutants will be discussed and the inferences that have been made as to the functions of the loci defined by these mutants will be presented.

Each mutant was tested for its effects on *X*- and fourth-chromosome disjunction by crossing *y mei-/y mei-*; spa^{pol}/spa^{pol} females to $Y^sX{\cdot}Y^L$, *In (1)EN, v f B*/0; *C(4)RM, ci ey*^{R}/0 males. This allows the detection of both *X*- and fourth-chromosome exceptions produced by the female. The results of such crosses for each of the female meiotic mutants is presented in Table 2. These data are the sum of at least two retests for each line. Neither the pattern nor the rate of *X*- and

TABLE 2

Progeny recovered from crosses in which the disjunction of the X *and fourth chromosomes was examined in the presence of homozygous and heterozygous female meiotic mutants*

Crosses are of *y mei-/y mei-; spa^{pol}/spa^{pol}* females by *Y^{S}X·Y^{L}, In(1)EN, v f B/0; C(4)RM, ci ey^{R}*/0 males.

Female gamete type:	*X;4*		*X;4/4*		*X;0*		*X/X;4*	*0;4*	*X/X;4/4*	*X/X;0*	*0;4/4*	*0;0*	
Male gamete type:	$\overline{XY};\overline{44}$	$0;\overline{44}$	$\overline{XY};0$	0;0	$\overline{XY};\overline{44}$	$0;\overline{44}$	$0;\overline{44}$	$\overline{XY};\overline{44}$	0;0	$0;\overline{44}$	$\overline{XY};0$	$\overline{XY};\overline{44}$	Total
X chromosome constitution of female													
+/+	6657	9167	3	2	3	5	0	4	0	2	1	0	15844
mei-218/mei-218	1134	1090	74	54	111	80	164	203	20	36	36	84	3086
mei-218/+	1501	1571	1	0	0	0	0	0	0	0	0	0	3073
mei-41/mei-41	252	548	2	7	3	5	15	23	0	0	0	1	856
mei-41/+	736	934	0	0	1	0	0	0	0	0	0	0	1671
mei-195/mei-195	592	1575	0	4	4	9	30	85	1	2	3	4	2309
mei-195/+	767	1083	0	0	0	1	0	1	0	0	0	0	1852
mei-352/mei-352	124	412	1	3	2	7	2	2	0	0	1	1	555
mei-352/+	1071	1362	0	1	0	0	2	1	0	0	0	0	2437
mei-251/mei-251	352	349	0	4	0	0	1	2	0	0	0	0	708
mei-251/+	790	900	0	0	0	0	0	0	0	0	0	1	1691
mei-9/mei-9	2471	1905	192	164	177	138	211	431	58	40	88	136	6011
mei-9/+	2722	3368	0	1	1	2	1	0	0	0	0	1	6096
mei-254/mei-254	568	702	128	103	1083	1337	93	107	17	130	35	259	4562
mei-254/+	1387	1607	1	0	0	1	2	0	0	0	0	0	2998
mei-38/mei-38	3638	4310	51	54	54	81	37	39	8	8	4	7	8291
mei-38/+	344	538	0	0	0	0	0	1	0	0	0	0	883
mei-160/mei-160	2508	2872	5	2	28	39	8	3	0	1	1	3	5470
mei-160/+	2817	4711	6	8	4	5	1	0	0	0	1	0	7553
mei-99/mei-99	3099	3752	5	3	5	15	6	16	2	1	1	1	6906
mei-99/+	803	901	0	0	0	0	0	1	0	0	0	0	1705
mei-152/mei-152	1846	1915	5	0	0	1	6	6	0	0	0	0	3789
mei-152/+	866	1171	3	0	0	1	0	0	0	0	0	0	2041

fourth-chromosome nondisjunctions differed significantly between tests of the same mutant. Among the progeny of these crosses and the other crosses to be discussed below the following types were observed but are not included in any of the tabulations presented here: haplo-*4*s, intersexes, triploids, metafemales, and metamales. The occasional gynandromorphs and diplo-*4*/haplo-*4* mosaics are entered as the genotype from which they were presumably derived. However, in this cross *X*-chromosome exceptional ova are potentially recoverable only half as frequently as *X*-chromosome regular ova, whereas fourth-chromosome exceptional and regular ova are recoverable equally frequently. Thus, in order to make the rates of *X*- and fourth-chromosome nondisjunction directly comparable, the numbers of *X*-exceptional progeny have been doubled to calculate the frequencies in Table 7 which are presented as exceptions per 1000 ova.

In *mei-218* females, nondisjunction of the *X* and fourth chromosomes is very frequent (Table 2). In addition, loss, as inferred from an excess of nullo exceptions for a given chromosome compare to the diplo exceptions for the same chromosome, is also frequent. For example, in the cross in which *X*- and fourth-chromosome nondisjunction was monitored (Table 2), 323 nullo-*X* ova were recovered from *mei-218* females as compared to 220 diplo-*X* ova, and 311 nullo-*4* ova compared to 184 diplo-*4* ova. Furthermore, nondisjunction of the *X* and fourth chromosome pairs is correlated in *mei-218* females. Thus, 176 *X*-chromosome–fourth-chromosome double exceptions were observed, whereas only 100 *X-4* double exceptions are expected on the assumption of independence (Table 7). However, among the *X-4* double exceptions, the four types are recovered in approximately the proportions expected if the *X* chromosome and fourth chromosome were segregating independently in those meioses in which they were simultaneously nondisjoining; specifically, there is no excess of diplo-*X*, nullo-*4* and nullo-*X*, diplo-*4* exceptions. We have assumed in these and subsequent calculations that tetra-*4* progeny are lethal. However, even if they do have an appreciable survival (GRELL 1961), this results in only a small increase in the real rate of fourth-chromosome nondisjunction and, for the data considered in this paper, leads to a small decrease in the expected number of *X-4* double exceptions. Thus, any survival of tetra-*4*'*s* leads to a greater discrepancy between the numbers of expected and observed *X-4* double exceptions.

The dominance of each of these mutants was examined by following *X*- and fourth-chromosome disjunction in females heterozygous for the mutants (Table 2). All mutants were

TABLE 3

Disjunction of the third and X *chromosomes in the presence of female meiotic mutants*

Crosses are *y mei-*/*y mei-*; +/+; +/+; spa^{pol}/spa^{pol} females by attached-third chromosome males (+/*Y*; +/+; *C(3L)RM, se* h^2 rs^2; *C(3R)RM, sbd gl* e^8; +/+).

Constitution of recovered ova	*X*;3/3	*X*;0	*X/X*;3/3	*X/X*;0	0;3/3	0;0	Total progeny	Number of female parents
MEIOTIC MUTANTS								
+	1	8	0	0	0	1	10	388
mei-218	165	152	20	51	58	15	461	250
-41	18	8	0	2	15	1	44	363
-195	24	40	0	7	13	6	90	541
-251	1	0	0	1	1	0	3	244
-9	156	137	3	56	88	10	450	260
-254	81	63	15	6	9	14	188	300
-352	1	2	1	0	0	1	5	163
-38	215	139	23	27	32	4	440	1104
-160	95	100	3	0	5	2	205	1037
-99	0	2	0	0	0	0	2	256
-152	3	0	0	0	0	5	8	196

TABLE 4

Disjunction of the second and X *chromosomes in the presence of female meiotic mutants*

Crosses are *y mei-/y mei-; pr cn/+ +; +/+; spa^{pol}/spa^{pol}* females by attached-second chromosome males (*+/Y; C(2L)RM, dp; C(2R)RM, px*; +/+; +/+).

Constitution of recovered ova	X;2/2,+	X;2/2, pr cn	X;0	X/X; 2/2,+	X/X;2/2, pr cn	X/X;0	0;2/2,+	0;0	Total progeny	Number of female parents
MEIOTIC MUTANTS										
+	1	0	12	0	0	0	0	1	14	1000
mei-218	427	0	383	21	0	306	370	35	1542	600
-41	25	0	38	0	0	17	46	4	130	1400
-195	12	0	38	0	0	18	20	0	88	600
-352	2	0	0	1	0	0	0	0	3	825
-251	7	1	11	1	0	9	17	1	47	2400
-9	427	1	408	7	0	257	293	21	1414	600
-254	227	0	190	21	0	17	52	34	541	850
-38	912(12)*	12	618	37	1	68	87	28	1779	4338
-160	135	6	226	3	0	12	11	11	404	3400
-99	15(2)*	2	219	2	0	2	8	9	260	4338
-152	10	1	24	2	0	4	8	4	53	1400

* Denotes exceptions which were recombinant between *pr* and *cn*.

completely recessive (except *mei-160* which was partially dominant, at least with respect to its effect on fourth-chromosome disjunction).

The effect of the mutants on disjunction of the second and third chromosomes was examined in mass matings of females from each line to attached-autosome-bearing males (either *+/Y; C(2L)RM, dp; C(2R)RM,px;* or *+/Y; C(3L)RM,se h^{2} rs^{2}; C(3R)RM,sbd gl e^{s}*, Tables 3 and 4). In a cross of attached-autosome-bearing males by free-autosome-bearing females, progeny are produced only when a gamete disomic for the chromosome in question from one sex unites with a gamete nullisomic for the same chromosome from the other sex. Since the only gametes recovered from the tested females are those nondisjunctional for a major autosome, it is possible to detect the occurrence of autosomal nondisjunction but not the absolute rate. A crude estimate of relative rates of nondisjunction is given by the number of progeny per mother (Table 7). The crosses to attached-second-chromosome-bearing males were arranged to establish not only whether second chromosome nondisjunction was occurring in these mutants, but also whether second chromosome nondisjunction occurred at the first or second meiotic division. The results of these crosses are given in Table 4. The second chromosome constitution of the female parents was *pr cn/+ +* (*pr* and *cn* are three map units apart and span the centromere). If nondisjunction occurs solely at the first meiotic division, all diplo-*2* ova recovered from the female will give rise to wild-type progeny (*pr cn/+ +*). If nondisjunction occurs exclusively at the second meiotic division, one-half of the diplo-*2* ova recovered will produce *pr cn* homozygotes and the other half wild-type homozygotes.

Nondisjunction of both the second and third chromosome pairs is very frequent in *mei-218* females (Tables 3 and 4). Furthermore, *mei-218*-mediated nondisjunction occurs at the first meiotic division as is evidenced by the observation that no ova resulting from equational nondisjunction (*pr cn*) were found among the 818 diplo-*2* ova recovered (Tables 4 and 7). In fact, all of the meiotic mutants caused first division nondisjunction, though three mutants (*mei-38, mei-160* and *mei-99*) also produced some second divisional exceptions. The observed second-division exceptions are probably real and not the result of the inadvertant use of a *pr cn/pr cn/+ +* triploid parent, since the putative second-division exceptions were all recovered from separate cultures, and there was no evidence of clustering of intersex or triploid progeny in those cultures which yielded the second-division exceptions.

Nondisjunction of the *X* chromosomes can also be detected in the crosses to attached-autosome-bearing males; it therefore was possible to determine the disjunctional behavior of the *X* chromosome when one of the major autosomes was nondisjunctional. In contrast, in the previous test in which *X*- and fourth-chromosome behavior was followed, it was possible to examine the disjunction of the *X* chromosomes only among gametes regular for the major autosomes.

In *mei-218* females, the nondisjunction of the *X* chromosome and the major autosomes is positively correlated as was the nondisjunction of the *X* and fourth chromosomes. Among ova regular for the second and third chromosomes, there were 299 *X*-chromosome exceptions per 10^3 ova, whereas among ova exceptional for the second chromosome, there were 644 *X*-chromosome exceptions per 10^3 ova, and among ova exceptional for the third chromosome there were 476 *X*-chromosome exceptions per 10^3 ova (Table 7). However, in contrast to the behavior of the *X* and fourth chromosomes, when both the *X* chromosomes and a pair of major autosomes nondisjoin, they do not segregate independently; there is a large excess of the diplo-*X*, nullo-major-autosome and nullo-*X*, diplo-major-autosome classes, indicating that the simultaneous nondisjunction of the *X* chromosomes and major autosomes in *mei-218* females is frequently the result of nonhomologous segregations. Thus, of the diplo-*3* exceptions, 58 were nullo-*X* and 20 were diplo-*X*, and of the nullo-*3* exceptions, 51 were diplo-*X* and 15 nullo-*X*. Similarly, of the diplo-*2* exceptions, 370 were nullo-*X* and 21 diplo-*X*, while of the nullo-*2* exceptions, 306 were diplo-*X* and 35 nullo-*X*.

The occurrence of nonhomologous pairing between the *X* chromosomes and the major autosomes may provide an explanation for the observed positive correlation between the nondisjunction of these chromosomes. Thus, if it is assumed that the *X* chromosomes and a pair of major autosomes, rather than nonhomologously pairing, nondisjoin independently of each other some fraction of the time to give rise to the observed nullo-*X*, nullo-major-autosome and diplo-*X*, diplo-major-autosome exceptional progeny and an equal number of nullo-*X*, diplo-major-autosome and diplo-*X*, nullo-major-autosome progeny, then the rate of *X* nondisjunction among ova nondisjunctional for a major autosome can be calculated for that fraction (Table 7). For the *X* chromosome and second chromosome this rate is $4(35 + 21)/[427 + 383 + 4(35 + 21)] = 216$ *X* exceptions per 10^3 second-chromosome nondisjunctional ova, and for the *X* chromosome and the third chromosome it is $4(15 + 20)/[165 + 152 + 4(15 + 20)] = 306$ *X* exceptions per 10^3 third-chromosome nondisjunctional ova. These two estimates of *X*-chromosome nondisjunction, in cells where a major autosome is also nondisjoining but not nonhomologously disjoining from the *X* chromosome, are in fairly good agreement with the estimates of *X* nondisjunction among ova regular for the major autosomes-299 *X* exceptions/10^3 ova (Table 7) and 250 *X* exceptions/10^3 ova (Table 5). This suggests that the positive correlation observed between the nondisjunction of the *X* chromosomes and the major autosomes in *mei-218* females is due solely to the occurrence of nonhomologous disjunctions.

Recombination on the second chromosome in the presence of homozygous meiotic-mutant-bearing-*X* chromosomes was examined for the regions *al*(0.0)–*dp*(13.0)–*b*(48.5)–*pr*(54.5)–centromere–*cn*(57.5) (numbers in parenthesis indicate standard map positions, LINDSLEY and GRELL 1968), by crossing *y mei-*/*y mei-*; *al dp b pr cn*/+ + + + +; spa^{pol}/spa^{pol} females by +/*Y*; *al dp b pr cn*/*al dp b pr cn*; +/+ males. The results are given in Table 5 and an analysis presented in Table 6. This cross also permits the detection of *X*-chromosome nondisjunction; exceptional progeny are also recorded in Table 5.

Recombination in *mei-218* females is drastically reduced. In control crosses, the total map distance for the *al–cn* region was 47.6 map units, whereas in homozygous *mei-218* females it was 3.8 map units. The reduction in recombination caused by *mei-218* is not uniform, being much more pronounced in distal regions. Thus the four regions in distal to proximal order and their map distances in *mei-218* as a fraction of the corresponding map distances in the control are: *al–dp*, 0.06; *dp–b*, 0.05; *b–pr*, 0.13; *pr*–centromere–*cn*, 0.55 (Table 6). Standard tetrad analysis (WEINSTEIN 1936) revealed that for the region of the second chromosome studied the frequency of no-exchange tetrads is increased in *mei-218* females to 0.92 as compared to 0.15 in the control, with concomitant reductions in the frequencies of single-exchange and double-exchange tetrads.

The effects of each of these mutants on the disjunction of the sex and fourth chromosomes in males was examined; in all cases except *mei-99* the rates of sex- and fourth-chromosome nondisjunction did not differ from control values. Nondisjunction of the fourth chromosome in *mei-99* males does not differ from control levels, though *X-Y* nondisjunction is several times higher than in the control cross (Table 1). From the observations that (1) *X*- but not fourth-chromosome nondisjunction is increased in *mei-99* males; (2) there is a large excess of nullo-*XY* relative to *XY* exceptional sperm recovered from these males; (3) there is a deficiency of males among the regular progeny of *mei-99* males, it would appear that *mei-99* is typical of the male meiotic mutants found in this mutant hunt. In *mei-99* females, however, nondisjunction of all chromosome pairs is increased, and, therefore, the *mei-99* chromosome probably has two meiotic mutants, one male specific and one female specific.

Finally, *X* chromosomes for each female meiotic mutant were examined in salivary gland squashes; no abnormalities were observed.

DISCUSSION OF FEMALE MUTANTS

mei-41, mei-195, mei-352, mei-251, mei-218: The meiotic mutants *mei-41, mei-195, mei-218, mei-251,* and *mei-352* are very similar in their effects, sufficiently similar to warrant considering them as a group at this stage in their analysis. Allelism tests indicate that, of these five mutants, only *mei-41* and *mei-195* are allelic.

Recombination is altered in a non-uniform manner by all of these mutants. Distal regions show marked decreases in recombination compared to controls, while the reduction in recombination is less pronounced in the more proximal regions examined. In fact, in *mei-195, mei-352,* and *mei-251,* recombination in the most proximal region (*pr–cn,* which spans the centromere of chromosome *2*) is increased significantly above control values. Tetrad analyses for the region of chromosome *2* studied shows that the fraction of no-exchange tetrads is increased with concomitant decreases in both single-exchange and double-exchange tetrads (with the exception of *mei-352,* which exhibits a decreased frequency of single-exchange but an increased frequency of double-exchange tetrads).

The mutants *mei-218, mei-41,* and *mei-195* increase first division nondisjunction for all chromosomes; the mutants *mei-251* and *mei-352* increase nondisjunction for at least the *X* and fourth chromosomes. (The failure to observe an increase in major autosome nondisjunction in *mei-251* and *mei-352* is probably the result of the combined effects of their semisterility and their relatively slight disruption of meiotic processes. That is, in the test employed to detect nondisjunction of the major autosomes, in which nondisjunction is measured as exceptional ova per female parent, an increase in the absolute rate of nondisjunction may be masked by a decrease in the number of ova per female.) In addition to nondisjunction, the mutants *mei-218, mei-195,* and probably *mei-352* exhibit chromosome loss. *mei-41* also exhibits apparent loss of the *X* chromosome; the *41* chromosome, however, carries a *bb* mutant which may explain the low recovery of diplo-*X* exceptions from homozygous *mei-41* females. In all these mutants, there is a positive correlation of nondisjunction of the *X* chromosomes and both of the major autosomes. In addition, segregation of the *X*-chromosome pair and either major autosome pair is not independent; when both chromosome pairs nondisjoin, there is a large excess of the non-homologous segregational types

TABLE 5

Recombination data from crosses of y mei-/y mei-; al dp b pr cn/+ + + + +; spa^{pol}/spa^{pol} *females by* +/Y; al dp b pr cn/al dp b pr cn; +/+ *males*

Meiotic mutant constitution of *X* chromosome	+	*-218*	*-41*	*-195*	*-352*	*-251*	*-9*	*-9^{b}*	*-254^{a}*	*-38*	*-160*	*-99*	*-152*
RECOMBINANT TYPE													
NONCROSSOVER:													
al dp b pr cn	2010	1841	264	393	782	1184	1399	1833	324	1038	880	1506	711
+ + + + +	4298	2490	709	841	1369	1994	1998	3001	434	1915	2090	2428	1614
SINGLE CROSSOVER:													
+ *dp b pr cn*	674	18	23	37	123	203	20	57	70	336	301	272	262
al + + + +	691	18	32	55	156	170	19	66	78	313	255	266	293
+ + *b pr cn*	1606	23	67	143	354	572	42	118	185	855	669	925	681
al dp + + +	1371	36	49	91	340	485	31	139	175	663	492	669	509
+ + + *pr cn*	237	13	24	57	182	111	8	26	19	177	145	256	128
al dp b + +	179	13	20	35	105	85	5	20	29	124	100	165	80
+ + + + *cn*	97	28	11	30	123	52	8	10	10	49	51	101	35
al dp b pr +	54	20	9	14	87	42	4	3	6	41	29	82	21
DOUBLE CROSSOVER:													
al + + + *cn*	11	0	0	2	11	5	0	0	2	13	8	13	2
+ *db b pr* +	13	0	1	1	7	9	0	0	2	13	4	12	5
al + + *pr cn*	32	1	2	2	9	3	0	0	5	21	7	10	6

+ *dp* *b* + +	29	0	0	2	8	4	0	0	0	24	20	7	18
al + *b* *pr* *cn*	43	0	4	2	7	11	0	0	4	34	19	18	21
+ *dp* + + +	41	0	2	6	13	28	0	1	4	44	24	15	21
al *dp* + + *cn*	19	0	1	4	27	7	0	1	0	23	15	24	12
+ + *b* *pr* +	31	0	0	2	24	14	0	0	1	31	18	42	21
al *dp* + *pr* *cn*	30	0	2	1	8	7	0	1	5	36	8	16	6
+ + *b* + +	18	0	7	9	23	15	0	0	2	43	21	22	17
al *dp* *b* + *cn*	5	0	1	1	6	1	0	0	0	1	2	2	0
+ + + *pr* +	4	0	0	3	7	2	0	0	0	6	9	6	2
TRIPLE CROSSOVERS:													
al + + *pr* +	0	0	0	0	1	0	0	0	0	2	1	0	0
+ *dp* *b* + *cn*	0	0	0	0	0	0	0	0	0	0	0	0	0
al *dp* + *pr* +	0	0	0	0	0	0	0	0	0	1	0	1	0
+ + *b* + *cn*	0	0	2	1	0	3	0	0	0	1	1	0	0
al + *b* *pr* +	1	0	0	3	2	0	0	0	0	2	1	1	0
+ *dp* + + *cn*	0	0	0	0	0	0	0	0	0	0	1	0	0
al + *b* + +	1	0	0	1	0	0	0	0	1	1	0	0	2
+ *dp* + *pr* *cn*	0	0	0	0	0	0	0	0	0	0	2	0	0
X EXCEPTIONAL PROGENY:													
X/X	0	258	7	64	3	1	131	144	7	16	2	17	0
nullo *X*	0	384	68	3	15	3	250	349	17	22	4	12	0
Total progeny	11495	4501	1230	1736	3776	5007	3534	5276	1356	5805	5173	6861	4467

TABLE 6

Analysis of recombination data presented in Table 5

	Total map	Map, individual regions				Map, relative to control				Tetrad distribution, frequency			
		al–dp	*dp–b*	*b–pr*	*pr–cn*	*al–dp*	*dp–b*	*b–pr*	*pr–cn*	E_0	E_1	E_2	E_3
MEIOTIC MUTANT													
+	47.56	13.36	27.50	4.65	2.04	1	1	1	1	0.146	0.759	0.094	0.001
mei-218	3.82	0.82	1.31	0.59	1.10	0.06	0.05	0.13	0.55	0.925	0.074	0.001	0.000
-41	22.84	5.20	10.89	4.72	2.03	0.39	0.40	1.0	1.0	0.615	0.327	0.045	0.013
-195	31.50	6.39	15.15	6.45	3.51	0.48	0.55	1.39	1.72	0.462	0.469	0.046	0.023
-352	47.11	8.93	21.13	9.24	7.81	0.67	0.77	1.99	3.83	0.219	0.625	0.149	0.006
-251	38.76	8.65	22.81	4.61	2.69	0.65	0.83	0.99	1.32	0.311	0.606	0.077	0.005
-9	3.85	1.10	2.06	0.36	0.33	0.08	0.07	0.08	0.17	0.923	0.078	0.000	0.000
-9[b]	8.44	2.35	4.91	0.91	0.27	0.18	0.18	0.19	0.14	0.834	0.164	0.002	0.000
-254[a]	46.09	12.24	27.80	4.50	1.55	0.91	1.01	0.96	0.78	0.155	0.774	0.065	0.006
-38	54.50	13.80	30.00	7.50	3.20	1.03	1.06	1.61	1.57	0.116	0.689	0.185	0.010
-160	44.69	12.33	23.70	6.03	2.63	0.92	0.86	1.30	1.29	0.210	0.670	0.120	0.000
-99	45.42	8.95	25.26	7.07	4.14	0.67	0.92	1.50	2.00	0.202	0.691	0.106	0.002
-152	50.93	14.10	28.82	5.79	2.19	1.06	1.05	1.25	1.07	0.100	0.785	0.112	0.004

TABLE 7

Summary and analysis of data from Tables 2, 3, and 4 on the disjunctional effects of female meiotic mutants

Meiotic mutant	Rates of nondisjunction*				Independence of nondisjunction‡			X nondisjunction among autosome exceptions due to other than nonhomologous disjunctions§		Independence of segregation of nonhomologous chromosome pairs when both pairs are nondisjunctional‖		
	$X/10^3$ ova	4th/10^3 ova	3rd/10^2 ♀ ♀	2nd/10^2 ♀ ♀	X-4th	X-2nd	X-3rd	$X/10^3$ 2nd exceptional ova	$X/10^3$ 3rd exceptional ova	X-4th	X-2nd	X-3rd
+[e]†	0.9	1.6	2.6	1.4	—	—	—	—	—	—	—	—
mei-218[d]	299.2	184.9	184.0	257.0	1.76	2.2	1.6	216	306	.7	12.1	3.1
-41[b]	87.1	21.2	12.1	9.3	1.21	7.1	6.1	195	—	—	15.8	17.0
-195[b]	102.7	15.2	16.6	14.8	5.26	4.9	4.4	—	272	1.0	3.3	∞
-251[c]	8.4	5.6	1.3	1.9	—	88.9	—	—	—	—	13.0	∞
-352[a]	21.4	30.3	3.1	0.3	11.00	—	—	—	—	—	—	—
-9[c]	276.4	188.5	173.0	235.6	1.71	2.1	1.9	117	333	.7	19.6	11.1
-254[d]	246.4	679.0	62.7	64.0	1.01	1.5	1.5	—	—	.6	.5	1.3
-38[e]	24.8	35.4	39.8	41.0	7.5	8.9	13.2	145	232	.8	2.3	2.2
-160[e]	5.8	15.3	19.7	11.6	21.70	29.0	16.0	132	93	.7	1.6	.5
-99[c]	7.8	5.5	0.8	5.1	34.3	19.2	—	155	—	.7	.9	—
-152[e]	11.5	1.6	4.1	3.8	—	44.1	66.9	—	—	—	—	—

* Expressed for X and fourth chromosome as exceptions per 1000 ova regular for second and third chromosomes, and for second and third chromosomes as exceptional progeny per 100 ♀ ♀ parents.

† Fertility of homozygous females in the cross to examine X and fourth chromosome disjunction reported in Table 3. a = < 1 progeny/female; b = 3–5 progeny/female; c = 10–25 progeny/female; d = 30–50 progeny/female; e = > 100 progeny/female.

‡ For X and fourth chromosomes expressed as observed X-4 double exceptions as a fraction of the X-4 double exceptions expected from independence. For the X and second chromosomes and the X and third chromosomes expressed as frequency of X exceptions among autosomal exceptions as a fraction of the frequency of X exceptions among autosomal regular ova (column one, this table).

§ Calculated as 4× (number of nullo;nullo + number of diplo;diplo exceptions)/Σ[number of major autosome exceptions regular for X chromosome + 4× (number of nullo;nullo + number of diplo; diplo exceptions)].

‖ Presented as recovered nonhomologous segregations (nullo;diplo and diplo;nullo) over the other types of segregations (diplo;diplo and nullo; nullo).

(diplo;nullo and nullo;diplo) compared to the diplo;diplo and nullo;nullo types. In at least three of the mutants, *mei-352*, *mei-195*, and *mei-218*, there is also a positive correlation in nondisjunction of *X* and fourth chromosomes, although here segregation of the heterologs is independent.

Finally, females homozygous for these mutants are more sterile than can be accounted for by the observed frequencies of aneuploid ova (with the probable exception of *mei-218*). However, the sterility is not correlated with either the strength of the recombinational effect or with the rates of nondisjunction.

From these results, it appears that these mutants specify genes whose functions are required at or before the time of recombination during the first meiotic division in females. SANDLER *et al.* (1968) have suggested, following an earlier formulation of BRIDGES (1915), that in conceptualizing the process of exchange it is useful to distinguish exchange itself from the array of preconditions (e.g. pairing) that must be fulfilled for exchange to occur. Further, they note that mutants which disturb preconditions for exchange may show altered interference, whereas in mutants that are defective in the exchange process itself interference should be unaltered. Since all of these mutants (except *mei-218* for which data are lacking) exhibit altered interference values for the three pairs of noncentromere spanning regions examined (Table 8), it is concluded that these mutants are defective in a precondition for exchange.

The effect of three of these mutants (*mei-195*, *mei-352*, *mei-251*) on recombination is characterized by a proximal increase in recombination above control values as well as the distal decrease. *A priori*, the proximal increases could represent either true increases in exchange or else preferential recovery of chromosomes that have exchanged proximally. The semi-sterility of these mutants makes it difficult to rule out selective recovery. However, if chromosomes which lack a proximal crossover are selectively eliminated, this elimination is not by nondisjunction, since even assuming that only non-proximal–exchange tetrads nondisjoin, there is not enough second-chromosome nondisjunction to give as large an increase in the map length of the proximal regions as is observed. That the proximal increase in recombination is real is suggested by the observation that a proximal increase in recombination occurs when a distally-located heterozygous

TABLE 8

Coincidence values for the control and the meiotic mutants that exhibit a nonuniform reduction in recombination

Region 1 = *al–dp*, region 2 = *dp–b*, region 3 = *b–pr*. Standard errors calculated following KOJIMA (1961).

Meiotic mutant	Coincidence values		
	Regions 1 & 2	Regions 2 & 3	Regions 1 & 3
+	0.20 ± .02	0.33 ± .05	0.87 ± .10
mei-41	0.86 ± .33	1.74 ± .45	0.66 ± .45
mei-195	0.71 ± .19	0.71 ± .19	0.70 ± .30
mei-352	0.31 ± .07	0.42 ± .07	0.58 ± .13
mei-251	0.40 ± .06	0.48 ± .09	0.35 ± .13

inversion is present on the same chromosome arm (GRELL 1962b). Such a heterozygous inversion and these mutants have the common property of causing a distal decrease in exchange, suggesting that this always causes proximal increases in recombination and that, therefore, only the decreases in recombination are the direct result of the meiotic mutants' effects. The proximal increase in recombination was not observed for two of these mutants—*mei-218 and mei-41*, and these are the mutants producing the greatest overall reduction in recombination. In fact, there is a strong negative correlation between the amount recombination is increased in the proximal regions of the chromosome and the overall reduction in recombination. A similar correlation was observed by GRELL (1962b) who found that the proximal increase in recombination which was observed with small distal inversions was not observed when large inversions were used. Thus, it would appear that the proximal increase in recombination is always associated with a distal decrease in recombination on the same chromosome but is observed only when the overall reduction in exchange is confined to distal regions so that the proximal increase is not obscured.

An alternative view of the effect of these mutants on recombination is suggested by a consideration of the observation that the probability of exchange in Drosophila is nonrandom with respect to the physical length of the chromosome. Thus basal heterochromatin, which comprises 15–20% of the metaphase length of the second chromosome (RUDKIN 1965, HINTON 1941), is contained within the region spanned by *pr* and *cn*, which is genetically about 3% of the second chromosome. A second example of this phenomenon is provided by the centromere effect: a physical region close to the centromere becomes genetically longer when relocated, via a rearrangement, to a position distant from the centromere. Thus, there would appear to be a process(es) in Drosophila that results in the nonrandom distribution of recombination with respect to physical length. Considering these mutants, it may be noted that the net result of their effects on recombination is to generate a pattern of recombination which is more reflective of physical length than is recombination in wild type. Thus all of the mutants show an increase in the fraction of all recombination which is in the region spanning the basal heterochromatin (*pr–cn*), as well as the region immediately adjacent (*b–pr*). For example, in *mei-218*, the strongest of these mutants, 29% of all recombination between *al* and *cn* is in the *pr–cn* region (as compared to 4% in the control). Thus recombination in *mei-218* much more closely reflects the relative physical distances of *al–pr* and *pr–cn* than does recombination in the control. (A very rough estimate of the relative physical lengths of these two regions can be obtained by taking the length of the basal heterochromatin of the second chromosome as 15% of its total metaphase length (HINTON 1941) and assuming that numbered salivary regions correspond to equal metaphase lengths (2% of the total metaphase length per numbered salivary region). Then, using the cytological location of the markers we employed (LINDSLEY and GRELL 1968; LINDSLEY and SANDLER *et al.* 1972), the relative physical lengths of these regions can be calculated as 35–45 for *pr–cn* and 65–55 for *al–pr*.) Thus, under this view we imagine that these mutants are de-

fective in genes that specify a precondition(s) for exchange that has as its functions (1) increasing the probability of exchange (since these mutants decrease exchange) and (2) delimiting where exchange may occur along the chromosome, and in so doing making the probability of exchange nonrandom with respect to physical length.

The properties of the distributive pairing system, as elucidated by GRELL (1962a, reviewed in GRELL 1969), have proved extremely useful in interpreting the disjunctional effects of meiotic mutants. In a normal meiosis, if a chromosome has exchanged it will disjoin from its homologue, but if it has not exchanged, then it is available to pair with and disjoin from any other non-exchange chromosome, via what she calls the distributive pairing system. However, the major chromosomes (*X*, second and third) almost always recombine and, thus, are not available for distributive pairing, whereas the fourth chromosome, which does not recombine, always distributively pairs. By using structural and numerical rearrangements to make different pairs of nonhomologous chromosomes simultaneously available for distributive pairing, it has been shown that when two pairs of chromosomes are in the distributive pool they will frequently disjoin from each other to give rise to nullo;diplo and diplo;nullo exceptional ova indicative of nonhomologous disjunctions. The probability that a chromosome will distributively disjoin from a nonhomologous chromosome (as opposed to its normal homolog) is governed primarily, if not exclusively, by the relative sizes of the chromosomes involved. The approximate sizes of the chromosomes in mitotic metaphases are $X = 1.8\mu$, second $= 2.6\mu$, third $= 3.2\mu$, fourth $= 0.24\mu$ (COOPER 1950). Thus, considering a diploid meiosis in which all chromosomes are structurally normal, it appears that the great disparity in size between the fourth chromosomes and any other pair of chromosomes makes a fourth chromosome virtually unable to distributively disjoin from any chromosome other than the other fourth chromosome under these circumstances (GRELL 1964). The second chromosome is closer to the size of the *X* than is the third chromosome, and therefore *X-2* distributive disjunction should be more frequent than *X-3* distributive disjunction.

With regard to the analysis of nondisjunction in these five mutants, there are a number of ova simultaneously nondisjunctional for two chromosome pairs, and the patterns of segregation in these cases is consistent with a normal distributive pairing system with respect to nonhomologous pairing and size-recognition. Thus, there is no evidence of distributive disjunction among *X-4* double exceptions, but among *X-2* and *X-3* double exceptions, the vast majority are of the nonhomologous types. Furthermore, the rate of *X*-chromosome nondisjunction among second-chromosome exceptions is greater than the rate of *X*-chromosome nondisjunction among third-chromosome exceptions as expected under the assumption that the *X* chromosome and the major autosomes are, in these mutants, entering the distributive pool independently. Thus, the second and third chromosomes have nearly the same genetic length and should therefore have approximately the same frequency of no-exchange tetrads. If recombination on the *X* chromosome and the major autosomes is independent in these mutants, as

it is in wild-type and in a meiotic mutant, *mei-S282*, which has a nonuniform reduction in recombination similar to that observed in these mutants (PARRY 1972), then the frequency with which the *X* and second chromosomes or the *X* and third chromosomes are simultaneously in the distributive pool should be nearly equal. In the distributive pool, there is some probability, *p*, that the *X* chromosome will pair distributively with the autosome present. Since the *X* chromosome is more similar in size to the second chromosome than it is to the third chromosome, *p* for *X-2* pairing should be greater than *p* for *X-3* pairing and, hence, *X-2* nonhomologous double exceptions should be more frequent than *X-3* nonhomologous double exceptions, which is what is observed. Furthermore, the positive correlation between the occurrence of nondisjunctions of the *X* chromosomes and the major autosomes would appear to be due to nonhomologous disjunctions of the *X* chromosomes and the major autosomes. Thus, if the rate of *X* exceptions among second or third chromosome exceptions is calculated from those *X* exceptions not attributable to nonhomologous disjunctions, that is, the diplo-*X*, diplo-major autosome and nullo-*X*, nullo-major autosome exceptions, then this rate of *X* nondisjunction is in fair agreement with the rate of *X* exceptions among ova regular for the second and third chromosomes (Table 7). Though the data from which these calculations are made are small, they do suggest that the nonindependence observed between the nondisjunctions of the *X* chromosomes and the major autosomes is attributable primarily, if not exclusively, to the nonhomologous disjunctions. Therefore, the segregational as well as the recombinational aspects of the data on these mutants indicates that they act early in meiosis I, at or before the time of exchange.

However, one observation is troublesome under this point of view—namely the occurrence of nondisjunction of the fourth chromosomes. Fourth chromosomes do not recombine and they always enter the distributive pool and disjoin by the distributive pairing system (GRELL 1969). Thus, it would be expected that mutants, such as these, which cause defects at or before exchange, should not affect the disjunctional behavior of the fourth chromosomes. The occurrence of fourth chromosome nondisjunction in these mutants suggests either that all chromosome pairs, including chromosome *4*, go through the meiotic processes specified by the normal alleles of these mutants and that the anomalies in these processes caused by the mutants results in the fourth chromosomes occasionally failing to enter the distributive pairing pool; or, alternatively, that the nondisjunction of the fourth chromosomes in these mutants is a secondary effect resulting from a disturbance in distributive pairing owing to the abnormal behavior of the other chromosomes (e.g. the major chromosomes may associate nonhomologously with the fourth chromosomes and therefore interfere with *4–4* distributive pairing, but these associations are sufficiently unstable that they fail to give rise to nonhomologous disjunctions). If the second alternative is correct, it would explain the excess of *X-4* double exceptions observed in these mutants, over the number expected under a hypothesis of independence (Table 7), and the lack of evidence for nonhomologous segregations in the *X-4* double exceptions. In fact, for those mutants for which the data are substantial (*mei-218* and *mei-*

195), it is possible that the observed fourth chromosome exceptions come only from those meioses in which the *X* chromosomes also nondisjoin. That is, among gametes nondisjunctional for chromosome *4*, approximately half are regular and half nondisjunctional for the *X* chromosome. Furthermore, detailed analysis of two alleles of the meiotic mutant, *c(3)G*, has provided a body of data strongly suggesting that chromosomes can alter the disjunctional behavior of chromosome *4* in the distributive pool without actually disjoining from it (HALL 1971).

Therefore, the data are consistent with the proposition that the mutants *mei-41*, *mei-195*, *mei-251*, *mei-21*^{s} and *mei-352* disrupt a process(es) that is a precondition for exchange. At least some of the nondisjunction caused by these mutants is attributable to the increases in no-exchange tetrads and thus to an increased probability of distributive segregation.

mei-9, *mei-254:* The chromosome *254* has been found to carry two strong meiotic mutants. One of these is an allele of *mei-9*, called *mei-9*^{b}. The second mutant on the *254* chromosome has been designated *mei-254*^{a}. Recombinational and *X*-chromosome disjunctional data for these two mutants are presented in Tables 5 and 6.

The meiotic mutant *mei-9* and its allele *mei-9*^{b} have essentially the same effects on recombination and disjunction; the primary difference between them is that *mei-9* is slightly stronger. Thus, the map distance for the *al–cn* region is reduced to 8.1% of the control value in *mei-9* (3.82 map units) and to 17.7% of control value in *mei-9*^{b} (8.44 map units). For both alleles, recombination is reduced to the same extent in all intervals. Tetrad analysis for the region of the chromosome *2* studied shows that the fraction of no-exchange tetrads is increased with concomitant decreases in the single-exchange and double-exchange tetrads.

A uniform reduction in recombination suggests several alternative models for the function of the wild-type allele of *mei-9*. Most directly, *mei-9*$^{+}$ could be a gene which signals some general precondition for exchange. In the mutant, this signal is faulty such that most chromosomes do not get the signal and thus fail to exchange; chromosomes that do receive the signal behave normally and have the normal amount of exchange. The most obvious prediction of this model is that the ratio of single crossovers to double crossovers in the mutants should be identical to that in the control, or, equivalently, by the choice of the proper number of noncrossover chromosomes from the total noncrossover chromosomes recovered, plus the single crossover and double crossover chromosomes from *mei-9*, it should be possible to derive the same tetrad distribution as in the control. Both of these tests yield a negative result. Thus, the ratio of double crossovers to single crossovers in *mei-9*^{b} is approximately ten-fold lower than it is in the control; three double crossovers were recovered from *mei-9*^{b}, whereas 24 would have been expected if the single-crossover/double-crossover ratio were the same as the control. A second test of a more general formulation of this model, namely that the exchange machinery in *mei-9* is normal, but that some precondition of exchange is faulty, is as follows: SANDLER *et al.* (1968) have shown theoretically that a mutation that alters the preconditions of exchange can be distinguished from a mutation in the exchange process itself by the fact that the coefficient of coinci-

dence should be altered in the former, but unchanged in the latter. From *mei-9^b*, two double crossovers were recovered from non-centromere-spanning regions, one *al–dp, dp–b* double crossover and one *dp–b, b–pr* double crossover. The coefficient of coincidence for these two intervals is (for *al–dp, dp–b*) $C = 0.16$ in *mei-9^b* and 0.20 for the control, and (for *dp–b, b–pr*) $C = 0.43$ for *mei-9^b* and 0.33 for the control. This close agreement between the coincidence values for *mei-9^b* and the control suggests that the locus defined by *mei-9* and *mei-9^b* functions in the exchange process itself rather than in specifying one of the preconditions for exchange. Confirmation of this will have to await the collection of a much larger amount of recombination data from *mei-9*, so that more reliable coincidence values can be calculated.

The disjunctional effects of *mei-9* and *mei-9^b* (in the absence of the other mutant on the *254* chromosome) are very similar to those of the mutants *mei-41*, *mei-195*, *mei-352*, *mei-218*, and *mei-251* previously discussed. Thus, by arguments identical to those used in the previous section, distributive pairing would appear to be normal in females homozygous for *mei-9* and *mei-9^b* and probably accounts for the positive correlation observed between the nondisjunction of the *X* chromosomes and the major autosomes. In addition to nondisjunction, there is apparent loss of *X*, but not fourth, chromosomes from *mei-9* females. The apparent loss of *X* chromosomes is probably due to poor viability of the *mei-9/mei-9* diplo-*X* exceptions since *X*/0 males bearing the *mei-9* chromosome relative to *mei-9/$\overline{XY}$* females are only half as frequent as +/0 males relative to +/$\overline{XY}$ females (Table 2).

In *mei-9*, as in the case of the other mutants, the occurrence of frequent nondisjunction of the fourth chromosome is troublesome; this is especially so in the case of *mei-9*, since we have suggested on the basis of the recombination data that the defect is in the exchange process itself, and the fourth chromosomes do not recombine. Thus, either this model for the defect in *mei-9* is incorrect, or else it must be supposed that abnormal behavior of the *X* chromosomes and the major autosomes, due to lowered exchange, disturbs distributive pairing so that nondisjunction of the fourth chromosome is increased. As before, the latter alternative is perhaps supported by the observations that the frequency of fourth chromosome exceptions among *X* chromosome exceptions is much greater than would be expected if they were nondisjoining independently, so much so that it is possible that the recovered fourth chromosome exceptions come only from meioses in which the *X* chromosomes are also nondisjoining, though there is no evidence for nonhomologous segregations in the *X-4* double exceptions.

Therefore, we propose that the gene defined by the meiotic mutants *mei-9* and *mei-9^b* functions to specify a component of the exchange process in female meiosis, and that mutants in this gene lead to a decreased frequency of exchange without altering coincidence, and as a concomitant, leads to an increased rate of nondisjunction for all chromosomes.

Also present on the chromosome carrying the meiotic mutant *mei-9^b* was a second meiotic mutant, *mei-254^a*. This mutant has been extensively characterized and it behaves as if it were in a gene essential for distributive disjunction (A.

CARPENTER in preparation). That is, in a number of experimental situations where in control crosses nonhomologous chromosomes regularly disjoin from one another, *mei-254*^{a} results in nonhomologs segregating independently of one another. For example, in crosses of *mei-9*^{b} (or *mei-9*, Table 7) to attached-second or attached-third chromosome-bearing males, nearly all *X-2* and *X-3* double exceptions are the result of nonhomologous disjunction. In the double mutant *mei-9*^{b} *mei-254*^{a}, however, the *X* and *2* or *X* and *3* segregate nearly independently when both nondisjoin (Table 7). Alone, *mei-254*^{a} has no effect on recombination, a low rate of *X*-chromosome nondisjunction, and a very high rate of fourth-chromosome nondisjunction. The rate of *X*-chromosome nondisjunction (3%, Table 5) is, in fact, approximately half the usual frequency of no-exchange tetrads for the *X* chromosome, as if no-exchange tetrads fell apart and segregated at random to the poles at meiosis I, instead of disjoining by the distributive pairing process as normally occurs.

mei-38, *mei-99*, *mei-160:* Though there are some differences between *mei-38*, *mei-99*, and *mei-160*, the general similarities of their effects on disjunction and recombination warrant considering them as a group at this stage in their analysis.

All three mutants cause an increased rate of nondisjunction for all chromosome pairs. In addition, the mutants *mei-99* and *mei-160*, but not *mei-38*, exhibit some chromosome loss. Nondisjunction occurs predominantly at the first meiotic division, though some, approximately 2–22% of the total, occurs at the second meiotic division. For at least two of these mutants, *mei-99* and *mei-38*, some nondisjunction of recombinant chromosomes occurs, although the data are insufficient to identify at which meiotic division this occurs. Nondisjunction of different chromosome pairs is positively correlated. Thus, the observed *X-4* double exceptions are 8 to 50 fold more frequent than would be expected if *X*- and fourth-chromosome nondisjunction were independent. Similarly, *X*-chromosome nondisjunction in second-chromosome or third-chromosome exceptional ova is much more frequent than among ova regular for the second and third chromosomes. In the case of two of the mutants, *mei-99* and *mei-160*, the excess of *X*-chromosome exceptions among exceptions for the major autosomes is not attributable to nonhomologous segregations; in fact, the data for these two mutants are consistent with the *X* chromosome and the autosomes segregating independently when they are simultaneously nondisjoining. In *mei-38*, there is a significant, but not very great, excess of the types of double exceptions expected from distributive pairing. Thus, in these three mutants the correlations between nondisjunction of nonhomologous chromosome pairs is not attributable to distributive disjunction as it was in the mutants previously considered. This suggests the possibility that the mutants *mei-38*, *mei-99* and *mei-160* are in genes which specify meiotic processes that occur at a cellular, as opposed to chromosomal or sub-chromosomal, level and that the failure of these processes in the mutants results in correlated abnormal behavior of nonhomologous chromosome pairs.

Although total map distances are similar to control values, recombination in all three of these mutants is altered in a nonuniform manner. Thus, recombination in the proximal region, *pr-cn*, is 1.3–2 times the control value, whereas the

map distance for the distal-most region, *al–dp*, is only 0.67–1.0 times that of the control. Tetrad analyses reveal an increase in the frequency of no-exchange tetrads for the region of chromosome *2* studied from 15% in controls to approximately 20% in the mutants *mei-99* and *mei-160* and a decrease in no-exchange tetrads to approximately 11% in *mei-38*.

Perhaps the most striking fact about these mutants is that they cause abnormal chromosome behavior at a number of different stages of meiosis. Thus, they all alter the pattern of recombination, cause nondisjunction at both the first and second meiotic division, allow nondisjunction of recombinant chromosomes (at least for *mei-99* and *mei-38*), and for *mei-38*, there is an excess of nonhomologous segregations of *X* and major autosomes. *A priori*, this could be seen as implying either that these mutants are in genes whose products are required at several different times in meiosis, or that the genes identified by these mutants are required at only one stage in meiosis, and that if they fail to function properly then, abnormal chromosome behavior may occur at several subsequent times. Precedent for the latter is provided by the work of SEARS (1952) who showed that in wheat a chromosome that fails to pair in meiosis I frequently shows abnormal behavior in prometaphase I, metaphase I, anaphase I, or anaphase II.

Since recombination is altered in these mutants, it must be that either the mutants act at or before the time of exchange, or that they act after exchange in which case the observed alteration in recombination is due to the differential recovery of recombinant types. A precedent for exchange interacting with a meiotic mutant that acts later in meiosis is provided by *mei-S332*, in which an exchange is associated with a decreased probability of a reductional nondisjunction (DAVIS 1971). Assuming a selective loss of recombinants, a rough calculation can be made of the number of second chromosome nondisjunctional ova per female that would be needed to change a map identical to that in the control into the map that is observed in each of these mutants. The number of second-chromosome nondisjunctional ova per female required under this model is much greater (*e.g.* 50-fold for *mei-160*) than is observed in the cross of these females to compound-second-chromosome-bearing males. This suggests that selective loss of recombinant types through nondisjunction is not sufficient to explain the recombination results, but this calculation is based on a number of untested assumptions concerning the probability of recovery of a second chromosome nondisjunctional ovum. Furthermore, the non-independence of nondisjunction of nonhomologous chromosome pairs in these mutants may well lead to a number of second chromosome exceptional ova types that would not be recoverable in the cross to attached-autosome-bearing males. The data at hand, therefore, appear insufficient to distinguish between these alternatives for the time of action of the genes specified by these mutants. A detailed study of the non-independence of the disjunctional behavior, perhaps in crosses to *mei-S332* males from which frequencies of all ova types can be obtained, and of the relationship between exchange and nondisjunction using a fully-marked chromosome, would help in distinguishing between the alternatives.

mei-152: Though the effects of *152* are reproducible, they are so weak as to

make it impossible to draw any conclusions as to the nature of the defect in this mutant.

GENERAL COMMENTS

In their considerations of the control of chromosome behavior, SANDLER *et al.* (1968) and LINDSLEY *et al.* (1968) suggested that the control of cell division in males and females in pre-meiotic (gonial) mitosis was probably the same, but that at the first meiotic division the control of chromosome behavior in the two sexes diverged. Thus, male, unlike female, *D. melanogaster* do not exhibit exchange, interchromosomal effects, or nonhomologous segregation. In addition, of the 13 meiotic mutants recovered by SANDLER *et al.* (1968), all but one affected meiosis in one sex only and at the first meiotic division. The one mutant they found that acted in both sexes, *mei-S332*, acts late in the first meiotic division and/or early in the second meiotic division, and thus suggests that the control of meiosis in the two sexes has converged by the time (DAVIS 1971).

The conclusion that the genetic control of meiosis I is different in the two sexes has been strengthened by the discovery and characterization of additional meiotic mutants. Thus, SANDLER (1971) has reported the discovery of two additional autosomal meiotic mutants, both of which act in only one sex, and we have found 30 *X*-chromosome meiotic mutants, all of which are sex-specific and act at the first meiotic division.

A substantial number of meiotic mutants have been partially characterized in *D. melanogaster*, and it is striking that nearly all of these seem to act early in the first meiotic division (in females at, or before, the time of exchange and distributive pairing). This may simply reflect the fact that this would seem, in females at least, to be the most complex part of the meiotic cycle. Nevertheless, we know of many processes and structures involved in other stages of meiosis, which on cytological grounds would appear to be common to the two sexes (spindles, centrioles, centromeres, the entire second meiotic division) and yet mutants in the genes controlling these processes are not among those found. This suggests the possibility that mutants in these processes are not being recovered by the screening procedures in use, perhaps because the products specified by these genes also function in mitosis (*e.g.* spindle, centromeres, centrioles), in which case mutants in these genes strong enough to be detected by our tests would probably be lethal. This possibility is perhaps strengthened by the observation that the one second divisional meiotic mutant that has been isolated and characterized, *mei-S332* (DAVIS 1971), is in a gene concerned with insuring that sister centromeres stay together between the first and second meiotic divisions, a process unique to meiosis.

A consideration of the types of abnormalities induced by the known meiotic mutants suggests that the processes used in the two sexes to bring about a normal first meiotic division may be very different, though in both cases the processes function to insure the regular pairing and disjunction of homologs. All known female meiotic genes are involved in insuring the proper recombinational or disjunctional behavior of all chromosome pairs, whereas many of the male meiotic

mutants appear to be chromosome specific. Thus, all of the *X*-chromosome male meiotic mutants reported in this study cause nondisjunction of the sex chromosomes only. Of the three first divisional male meiotic mutants found by SANDLER *et al.* (1968), two were allelic and affected the disjunction of the fourth chromosome only, and one caused increased nondisjunction of both sex and fourth chromosomes, and thus probably all chromosomes, as does the male meiotic mutant *mei-W5* (SANDLER 1971). Finally, there are the cases of Segregation-Distorter (SANDLER, HIRAIZUMI and SANDLER 1959) and Recovery-Disrupter (NOVITISKI and HANKS 1961) which act during meiosis I in males and are also chromosome specific. Thus, it appears that the genic control of disjunction at meiosis I in males is often chromosome specific, whereas control of disjunction at meiosis I in females is by genes affecting all chromosome pairs. A possible explanation for this difference is suggested by the observation that there is no recombination in males, whereas in females proper disjunction of homologs, once they have paired, appears to be provided for by the occurrence of exchange or, failing an exchange, by the distributive pairing system and its property of size recognition (GRELL 1969). Males, though they lack recombination and distributive pairing, must have some process functionally equivalent to exchange in that homologs pair and disjoin from one another. Perhaps it is such a process that is specified by the chromosome-specific male meiotic mutants. In attempting to specify the nature of this process more fully, it must be kept in mind that cytologically the only regular association of homologs seen in male meiosis takes place between the basal heterochromatin of the homologs (COOPER 1964). Furthermore, for the *X* and *Y* chromosomes, the only pair of homologs for which the question has been examined, the pairing of homologs during male meiosis occurs at a specific set of pairing sites (COOPER's collochores) in the basal heterochromatin (LINDSLEY and SANDLER 1958, COOPER 1964). If these pairing sites are deleted, as in $In(1)sc^{4L}sc^{8R}$, then the *X* and *Y* fail to pair and disjoin (SANDLER and BRAVER 1954; PEACOCK 1965). Thus, if it is assumed that the chromosome-specific male meiotic mutants are involved in specifying a process that results in the formal equivalent of an exchange in that it ensures that homologs stay paired and disjoin properly, then it would seem to be necessary to conclude that this process was carried out at the pairing sites in the basal heterochromatin. Conceptually one can imagine that the normal alleles of these genes specify substances which recognize specific pairing sites on a chromosome and bind these to the equivalent sites on the chromosome's homolog, thus providing the functional equivalent, in males, of an exchange.

To extend the above analogy between the male and female meiotic processes a bit further, it appears that there may exist a parallel between the function of meiotic drive in males (see "discussion of male meiotic mutants," above) and distributive pairing in females. Thus, in the absence of an exchange in a female meiosis the distributive pairing system functions to pair non-exchange chromosomes so that following disjunction a euploid ovum is frequently formed. In the case of males, the available data on meiotic drive (*cf.* ZIMMERING, SANDLER and NICOLETTI 1970) would appear to be consistent with the notion that it is the

absence of proper pairing (or the male's equivalent of an exchange) that results in the occurrence of meiotic drive, which in turn blocks the maturation of the resulting potentially aneuploid spermatids into functional sperm. Thus, meiotic drive, if viewed in this manner, can be considered the male's functional equivalent to distributive pairing in the female in that both are "failsafe" processes that: (1) come into operation when pairing or "exchange" at meiosis I is faulty or absent and (2) function so as to reduce the probability that a functional aneuploid gamete will be formed.

There are several objections to viewing meiotic drive in this manner. First, it is not obvious how a disruption of pairing in male meiosis would lead to meiotic drive. However, for the one case in which we know the mechanism of meiotic drive, *SD*, it has been shown that *SD* turns its homolog into a gametic lethal (SANDLER and CARPENTER 1972). Could it be that all chromosomes in the male enter meiosis I with an "armed bomb" which is defused if they pair properly, but which, if pairing is faulty, is not disarmed and detonates to prevent sperm maturation? For example, under this hypothesis, we can ascribe functions to the two components of the *SD* chromosome described by SANDLER and CARPENTER as follows. The *SD* chromosome contains *insensitive-receptor*, a region which confers immunity to the action of *SD*, and *SD*, a region which causes drive; the non-*SD* (sensitive) homolog contains *sensitive-receptor*, a region which responds to the action of *SD* by causing the chromosome bearing it to become a gametic lethal. Then *sensitive-receptor* is the region of the "armed bomb", *SD* acts by preventing the "pairing" which is requisite for its disarmament, and *insensitive-receptor* is a faulty bomb which does not become armed and therefore does not require disarmament; the net result is that the *SD insensitive-receptor* chromosome is the sole survivor.

A second difficulty with viewing meiotic drive as a normal part of male meiosis is that the basal level of first division nondisjunction in males is sufficiently low that the selective advantage to having meiotic drive as a part of the normal meiotic machinery would appear to be miniscule. However, at some point in the evolution of Drosophila, males lost the ability to recombine and at that time there must have been many aneuploid sperm being produced and consequently much stronger selection for a mechanism which would eliminate aneuploid sperm before they could compete with euploid sperm for fertilization. Thus, selection may have been strong enough to allow for the evolution of meiotic drive as a part of the normal meiotic apparatus.

The above speculations suggest that the very great differences observed between meiosis I in males and females are at the level of the processes that are used to ensure a normal first meiotic division, but that if these processes are viewed in terms of their functions then there is a very striking similarity between what males and females do to ensure regular disjunction at the first meiotic division and the production of euploid gametes.

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