MEIOSIS IN MALE *DROSOPHILA MELANOGASTER* I. ISOLATION AND CHARACTERIZATION OF MEIOTIC MUTANTS AFFECTING SECOND CHROMOSOME DISJUNCTION[1]

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ABSTRACT

Two second chromosome, EMS-induced, meiotic mutants which cause an increase in second chromosome nondisjunction are described. The first mutant is recessive and causes an increase in second chromosome nondisjunction in both males and females. It causes no increase in nondisjunction of the sex chromosomes in either sex, nor of the third chromosome in females. No haplo-*4*-progeny were recovered from either sex. Thus, it appears that this mutant, which is localized to the second chromosome, affects only second chromosome disjunction and acts in both sexes.——The other mutant affects chromosome disjunction in males and has no effect in females. Nondisjunction occurs at the first meiotic division. Sex chromosome disjunction in the presence of this mutant is similar to that of sc^4sc^8, with an excess of *X* and nullo-*XY* sperm relative to *Y* and *XY* sperm. In some lines, there is an excess of nullo-*2* sperm relative to diplo-*2* sperm, which appears to be regulated, in part, by the *Y* chromosome. A normal *Y* chromosome causes an increase in nullo-*2* sperm, where B^8Y does not. There is also a high correlation between second and sex chromosome nondisjunction. Nearly half of the second chromosome exceptions are also nondisjunctional for the sex chromosomes. Among the double exceptions, there is an excess of *XY* nullo-*2* and nullo-*XY* diplo-*2* gametes. Meiotic drive, chromosome loss and nonhomologous pairing are considered as possible explanations for the double exceptions.

IN Drosophila, insights into the genetic control of meiosis have been made through the analyses of structurally rearranged genomes, aneuploid genomes, and, more recently, mutants of genes which regulate chromosome behavior during the two meiotic divisions. Most of these studies have been confined to female Drosophila, primarily because analysis of the female system has been more amenable to the above techniques. In females, alterations in the meiotic process can be recognized by abnormal patterns of chromosome segregation (either nondisjunction or nonrandom segregation) and by changes in the frequency or distribution of crossing over. In males, however, crossing over is absent and nonrandom chromosome segregation has not been observed (HOLM, DELAND and CHOVNICK 1967).

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That the female and male meiotic systems are different has been known for many years. However, it has recently been suggested that these differences are restricted to the first meiotic division (SANDLER *et al.* 1968). It has been found that mutants which affect the first meiotic division act in only one of the two sexes (SANDLER *et al.* 1968; DAVIS 1969; ROBBINS 1971; HALL 1972; BAKER and CARPENTER 1972; PARRY 1973), whereas mutants that act during the second meiotic division affect both sexes (SANDLER *et al.* 1968; DAVIS 1971). GRELL (see R. F. GRELL 1969, for a review of exchange and distributive pairing) has demonstrated that in females, chromosomes which fail to enter into an exchange can enter into nonhomologous associations which, in turn, can lead to nondisjunctional gametes. Thus, in females, as compared to males, we have a more complete picture of the sequence of events that occur in meiosis, as well as inferences as to the mechanism and type of gene regulation that occurs.

In males, BAKER and CARPENTER (1972) isolated several *X*-linked meiotic mutants that were specific to sex chromosome disjunction. These mutants behaved genetically like $In(1)sc^{4L}sc^{8R}$, which is a deletion of the basal heterochromatin and contains the *XY* pairing sites. SANDLER *et al.* (1968) found three autosomal meiotic mutants. Two were allelic and caused an increase in nondisjunction of chromosome four but had no effect on *XY* disjunction. The other caused nondisjunction of both the sex chromosomes and the fourth chromosomes. The following is a report on the isolation of several presumptive meiotic mutants which cause an increase in second chromosome nondisjunction in males and a preliminary characterization of two of them.

ISOLATION AND RECOVERY OF THE MEIOTIC MUTANTS

Males of the genotype *cn*; e^s were fed EMS (0.2% in 1% sucrose) for twenty hours (LEWIS and BACHER 1968). The frequency of sex-linked, recessive lethals was 33% (8/24). The treated second and third chromosomes were screened for meiotic mutants by The Free Recombination Scheme of LINDSLEY, outlined in Figure 1. Briefly, treated males were mated *en masse* to females of the genotype *SM1/102*; *TM2/Sb-PC* for 48 hours (see footnote to Figure 1 for explanation of symbols). Single daughters ($SM1/cn_i$; $TM2/e^s_i$) were crossed to single sons of the genotype $SM1/cn_j$; $TM2/e^s_j$ (cross G2). In the G3 cross, all cinnabar ebony-sooty progeny were inbred and the sons (cross G4) were crossed to *XXY* females carrying compound second chromosomes.

The rationale for this procedure is as follows: By a conventional mutagenesis scheme, mutagenized chromosomes are isolated and stocks are established prior to any testing for a mutant. Since the most frequent class of EMS-induced mutants is recessive lethals, a low dose of the mutagen must be used such that lethal-free chromosomes can be recovered. This, of course, also lowers the probability of recovering any other type of mutant. Thus, one must establish many stocks, several of which will contain a recessive lethal and cannot be tested for any other recessive mutant.

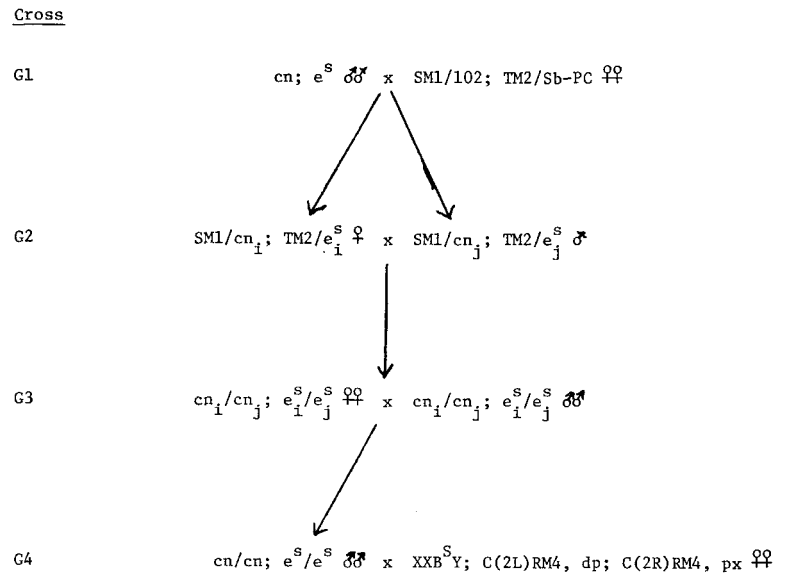

FIGURE 1.—The free-recombination scheme of LINDSLEY used in isolating and recovering male meiotic mutants. Symbols used in this Figure: *SM1 = In(2LR)SM1, al² Cy cn² sp²; 102 = In(2LR)102, ds^W sp²; TM2 = In(3LR)Ubx^130, Ubx^130 e^s; Sb-PC = In(3L)P + (3R)C, Sb e. C(2L)RM4* and *C(2R)RM4* are compound autosomes which are two left arms or two right arms of the same chromosome attached to a single centromere. See LINDSLEY and GRELL (1968) for complete descriptions of all mutants and rearrangements used in these experiments.

The Free Recombination Scheme attempts to eliminate these problems by (1) selectively eliminating those regions of a chromosome that carry a recessive lethal, and (2) reducing the number of stocks established, as stocks are established only after a presumptive mutant has been identified. An additional feature of this scheme is that each sequence of crosses screens two independently treated genomes.

The crucial cross in this scheme is the G3 cross which allows crossing over to occur between the two independently treated chromosomes. If a lethal and a meiotic mutant were simultaneously induced in the same chromosome, they can be separated by crossing over and progeny homozygous for the meiotic mutant (and heterozygous for the lethal) can be recovered.

Since many of the G4 males are heterozygous for the two treated genomes, it is necessary to have some type of a selective screen to recognize a meiotic mutant. The G4 cross is such a cross, as the only viable progeny from this cross will be those which receive a diplo-*2* complement from one parent and a nullo-*2* complement from the other parent. Thus, the only viable progeny in this cross are those that are the result of second chromosome nondisjunction in the male parent. A *Y* chromosome was added to the female genome to increase the frequency of diplo-*2* and nullo-*2* gametes (GRELL 1970).

Finally, the presumptive meiotic mutant is recovered from the exceptional progeny. In theory, only four types of offspring should be found: males and

females homozygous for the treated paternal second chromosome and heterozygous for a treated third chromosome, and males and females carrying the compound second chromosomes, but heterozygous for a treated third chromosome. Phenotypically, the former flies are *cn* and the latter one are *dp px*. Both treated autosomes can be isolated from the exceptional *cn* males by crosses to *SM1/102*; *TM2/Sb-PC* females. The treated second chromosomes can also be isolated from the exceptional females, as these females should be homozygous for a recessive meiotic mutant. The treated third chromosomes were isolated from the compound-*2* males by crosses to *C(2L)RM4, dp*; *C(2R)RM4, px*; *TM2/+* females.

In the G4 crosses, males were tested in groups of 3–5, and each cross was transferred every four days for six transfers.

RESULTS

One hundred and forty pair matings were made in cross G2 (Figure 1). Of these, 22 were sterile. Thirty-eight of the G3 crosses were either sterile or produced too few male progeny for testing. Thus, eighty lines representing 160 treated second and third chromosomes were screened for male meiotic mutants by the G4 crosses. The number of males tested for each line varied according to the number available. Whenever possible, approximately 90 males were tested for each line. This was possible for about half of the lines. The results of these crosses are given in Table 1. The lines are grouped according to the number of males tested. A total of 74 exceptional diploid offspring were recovered from the 4993 males tested.

Although the frequency of nondisjunction cannot be directly measured in crosses to compound-autosome females, the frequency of exceptional offspring per tested male can be used as a relative measure of nondisjunction. Thus, in the crosses listed in Table 1, the frequency of exceptional offspring was 0.0148 (74/4993), as compared to a spontaneous frequency of 0.0152 (see Table 2). Therefore, many of the exceptional offspring recovered were the result of spontaneous nondisjunction, rather than the result of any genetic defect in the male meiotic system.

TABLE 1

Distribution of exceptional offspring recovered from each line tested
Lines grouped according to number of males tested

Number of males tested	Number of lines	Number of exceptional diploid offspring						
		0	1	2	3	4	5	6
1–10	12	11		1				
11–20	8	6	2					
21–50	10	7	3					
51–70	10	4	2	1	1	1	1	
71–90	3	1	1	1				
91–100	28	12	8	4	1	2		1
>101	9	3		5			1	

TABLE 2

Nondisjunctional offspring and frequency of nondisjunction from presumptive meiotic mutants
Males were crossed to *C(1)RM, y/B^{s}Y; C(2L)RM4, dp; C(2R)RM,* + females

Line	Chromosome	No. exceptions / No. males tested	Frequency
G13	3	0/40	——
G17	2	20/26	0.769
	3	0/44	——
G22	2	0/38	——
G27	2	0/36	——
G31	3	1/40	0.025
G39	2–3	0/4	——
	2	0/34	——
	3	1/20	0.050
G68	2	0/42	——
G78	2	0/42	——
G87	2–3	8/34	0.235
	2	5/34	0.147
	3	0/28	——
G89	2	0/40	——
G121	2	1/40	0.025
G123	3	0/42	——
G126	2	0/42	——
S332	2	5/20	0.250
cn;e^{s}	2–3	1/66	0.015

In addition to the *cn* and *dp px* diploid offspring, two other classes were recovered. These were triploid females and intersexes. A total of 240 such flies were found. Triploids and intersexes have been regularly recovered from crosses to compound-*2* females, and are believed to be the result of a regular haploid sperm fertilizing a diploid, or unreduced egg (GETHMANN 1972).

Thirty-four chromosomes were isolated from the exceptional progeny, 15 second chromosomes and 19 third chromosomes. Of these, five of the second-chromosome stocks are now homozygous for a lethal, twelve of the third-chromosome stocks are homozygous for a lethal and one third-chromosome stock is male sterile. All of the non-lethal, non-sterile chromosomes have been tested for a meiotic mutant. The results of these tests are given in Table 2. The tests were made by crossing two males to four *C(1)RM, y/B^{s}Y; C(2L)RM4, dp; C(2R)RM,* + females. The crosses were transferred every four days, for a total of five transfers. Two types of control crosses were made in these experiments; the first was a test with the original, untreated *cn; e^{s}* stock and the second was a test of a known meiotic mutant that acts in males, *mei-S332* (DAVIS 1971). Under these conditions, *mei-S332* produced five exceptional offspring from twenty males, and the *cn; e^{s}* males gave only one offspring from 66 tested males. The latter figure was taken as the frequency of spontaneous exceptions from males. An examination of the data in Table 2 indicates the presence of meiotic mutants in lines G17 and G87, both on the second chromosome.

mei-G87: In the original tests of line G87, a total of 95 males were tested in 23 sets of crosses. A single exceptional *cn B* male was recovered from one of the crosses, and both the second and the third chromosome were isolated from this male. Both chromosomes were tested for the presence of a meiotic mutant, and from Table 2 it can be seen that the second chromosome carries a meiotic mutant.

In males, the mutant behaves as a recessive (Table 3). Nine exceptional offspring were recovered from 72 males tested as homozygotes (Cross 1). Heterozygous males (*mei-G87/+*) produced only one exception from 50 tested males (Cross 2), which is the same as the control frequency (Cross 6). The mutant has no effect on sex chromosome disjunction (Crosses 9 and 10).

Tests for nondisjunction were also made in the females (Table 3). For the second chromosome, 13 exceptions were recovered from 58 tested females (Cross 3). Heterozygous females (Cross 4) and control females (Cross 7) produced two exceptions out of 44 tested females and three exceptions out of 48 tested females, respectively. Thus, *mei-G87* acts as a recessive meiotic mutant in both males and females.

For the third chromosome, there was no increase in nondisjunction in females (Crosses 5 and 8). There was no effect on sex chromosome disjunction in females; one exception was recovered out of 625 offspring from crosses to Bar males. Furthermore, neither homozygous males nor females produced any haplo-*4* progeny, suggesting that fourth chromosome disjunction is regular. Thus, it seems reasonable to conclude the *mei-G87* is a chromosome-specific mutant that causes only second chromosome nondisjunction in both sexes.

TABLE 3

Second and sex chromosome nondisjunction for mei-G87

Cross number	Cross	Autosomes: Number tested	Diplo	Nullo	Frequency
1	*G87/G87* ♂ × *C(2)* ♀*	72	3	6	0.125
2	*G87/SM1* ♂ × *C(2)* ♀	50	0	1	0.020
3	*G87/G87* ♀ × *C(2)* ♂†	58	6	7	0.224
4	*G87/+* ♀ × *C(2)* ♂	44	1	1	0.045
5	*G87/G87* ♀ × *C(3)* ♂‡	42	1	0	0.024
6	*cn; e*^{s} ♂ × *C(2)* ♀	66	1	0	0.015
7	*cn; e*^{s} ♀ × *C(2)* ♂	48	2	1	0.063
8	*cn; e*^{s} ♀ × *C(3)* ♂	40	0	1	0.025
		Sex chromosomes: X	Y	XY	Nullo
9	*+/B*^{s}*Y; G87/G87* ♂ × *C(1)RM, y v bb* ♀	239 (.497)	242 (.503)	0 —	0 —
10	*+/B*^{s}*Y; G87/SM1* ♂ × *C(1)RM, y v bb* ♀	271 (.498)	272 (.500)	0 —	1 (.002)

* — C(2) ♀ = *C(1)RM, y/B*^{s}*Y; C(2L)RM4, dp; C(2R)RM, +.*
† — C(2) ♂ = *+/B*^{s}*Y; C(2L)RM4, dp; C(2R)RM, +.*
‡ — C(3) ♂ = *+/Y; C(3L)RM, se h*^{2} *rs*^{2}*; C(3R)RM, sbd gl e*^{s}.

Further tests with this mutant have been complicated by the fact that the frequency of exceptional offspring has varied greatly from experiment to experiment. In some lines, males no longer exhibit a high rate of nondisjunction, whereas females still do. In other lines, both sexes are now normal. The reasons for this variability are not known. The variability of this mutant, along with the apparent separation of its effect on nondisjunction in females and males, suggests it may not be a simple mutant. It is possible that this could be either a double mutant or it could be a synthetic mutant. There is some evidence that nondisjunction in the female may occur at the second meiotic division. Several new lines of this mutant have been isolated and these lines appear to be stable. A further analysis is under way.

mei-G17: In the original G17 line, 95 males were tested in 23 sets of crosses. A total of four exceptional offspring were recovered, all from the same cross. Of the four exceptional offspring, one was from a diplo-*2* sperm and the other three were from nullo-*2* sperm. The second chromosome was isolated from the diplo-*2* exception and a third chromosome was isolated from one of the nullo-*2* exceptions. The mutant was localized to the second chromosome. Further properties of *mei-G17* are given in Table 4.

From Table 4, it can be seen that *mei-G17* is a recessive mutant that acts only in males (Crosses 1, 2, and 3). The mutant also affects sex chromosome disjunction (Crosses 6 and 7). In these crosses to attached-*X* females, first division nondisjunction in the males can be distinguished from second division nondis-

TABLE 4

Second and sex chromosome nondisjunction for mei-G17

Cross number	Cross	Second chromosomes			
		Number tested	Diplo	Nullo	Frequency
1	*G17/G17* ♂ × *C(2)* ♀ *	50	15	18	0.660
2	*G17/SM1* ♂ × *C(2)* ♀	36	0	0	0
3	*G17/G17* ♀ × *C(2)* ♂ †	48	2	0	0.042
4	*cn; e*^{s} ♂ × *C(2)* ♀	66	1	0	0.015
5	*cn; e*^{s} ♀ × *C(2)* ♂	48	2	1	0.063
		Sex chromosomes			
		X	Y	XY	Nullo
6	+/*B*^{s}*Y; G17/G17* ♂ × *C(1)RM, y v bb* ♀	384 (.591)	227 (.349)	1 (.002)	38 (.058)
7	+/*B*^{s}*Y; G17/SM1* ♂ × *C(1)RM, y v bb* ♀	302 (.504)	297 (.496)	0 —	0 —
8	+/*B*^{s}*Y; G17/G17* ♂ × *w*^{a}/*w*^{a} ♀	412 (.584)	264 (.374)	5 (.007)	24 (.034)
9	+/*B*^{s}*Y; G17/SM1* ♂ × *w*^{a}/*w*^{a} ♀	307 (.484)	326 (.514)	0 —	1 (.002)

* — C(2) ♀ = *C(1)RM, y*/*B*^{s}*Y; C(2L)RM4, dp; C(2R)RM,* +.
† — C(2) ♂ = +/*B*^{s}*Y; C(2L)RM4, dp; C(2R)RM,* +.

junction. Nondisjunction at the first meiotic division will yield two types of exceptional gametes, nullo-*XY* and *XY*. Second division nondisjunction will yield three types: nullo-*XY*, double-*Y* and double-*X*. The *XY* gamete will produce a Bar-eyed son and the double-*X* gamete will yield a wild-type daughter. Thirty-nine exceptional offspring were recovered and all but one were from nullo-*XY* gametes. The single diplo exception was a Bar-eyed male, which is a first division exception. In the preliminary mapping experiments (to be discussed), a total of eight exceptions from diplo-*2* sperm were recovered from males heterozygous for at least one dominant marker. All of these were first division exceptions. Thus, the mutant causes nondisjunction at the first meiotic division.

There was also an effect of this mutant on the sex ratio. Homozygous males produced an excess of *X* and nullo-*XY* sperm, as compared to *Y* and *XY* sperm (Cross 6). Heterozygous males did not (Cross 7). This pattern of recovery of the four sex chromosome classes is identical to that observed by BAKER and CARPENTER (1972) with their male meiotic mutants.

A second series of tests were made to confirm the sex chromosome behavior of *mei-G17* (Crosses 8 and 9). In the Cross 8 progeny, there was again an excess of nullo-*XY* exceptions, although a higher proportion of the exceptional progeny were from *XY* gametes. The frequency of nondisjunction was 4.1% and as before, there was an excess of *X*-bearing sperm.

Preliminary mapping experiments indicate that *mei-G17* maps to the distal part of 2R, most likely the distal third. These tests were conducted by selecting recombinant males from the cross of *S Tft* bw^D/*cn mei-G17* ♀ ♀ × *cn mei-G17*/*cn mei-G17* ♂ ♂ (*S* = Star, 1.3; *Tft* = Tuft, 53.2; bw^D = brown-Dominant, 104.5 on the standard map) and testing them for second chromosome nondisjunction. None of the bw^D recombinants yielded any exceptions, whereas the bw^+ recombinants did produce exceptional offspring.

The frequency of second chromosome nondisjunction is dependent, in part, on the *Y* chromosome present in the genome. This is shown by the crosses listed in Table 5. Lines 2 and 3 are the two original lines which showed a marked difference. Line 3 was derived from line 2 by outcrossing to introduce B^sY into the stock and then by backcrossing to line 2. As shown in Table 5, line 2 has a much higher frequency of exceptional offspring than does line 3. The difference between these two lines is due to an increase in the nullo-*2* gametes, as the frequency of diplo-*2* gametes remains the same for the two lines.

The two lines were crossed to each other to generate the line A and B males. Both line A and line B males are identical for their autosomes and differ only in their sex chromosome constitution. Line A males carry the *X* chromosome from the low line and the *Y* chromosome from the high line, whereas line B males carry the *X* from the high line and the B^sY from the low line. As can be seen, the line B males which carry the B^sY have a lower frequency of nondisjunction than do the line A males. Again, as with the two original lines, the difference is in the number of nullo-*2* gametes produced. Diplo-*2* gametes are produced by both lines at about the same frequency, and it is the same as the parental lines. The frequency of nondisjunction in line A is approximately 80% of the differ-

TABLE 5

The frequency of nondisjunction in different lines of mei-G17
All males were crossed to *C(1)RM, y/0; C(2L)RM4, dp; C(2R)RM,* + females

Line	Genotype	Exceptions Diplo-2	Exceptions Nullo-2	Total	Number males tested	Frequency
2	$+_2/Y;\ cn_2/cn_2$	6	48	54	50	1.08
3	$+_3/B^sY;\ cn_3/cn_3$	8	5	13	52	0.25
A*	$+_3/Y;\ cn_2/cn_3$	7	29	36	40	0.90
B†	$+_2/B^sY;\ cn_2/cn_3$	8	8	16	40	0.40
C‡	$+/Y;$	13	33	46	80	0.58
D§	$+/B^sY;$	5	19	24	80	0.30

* Generated by crossing line 3 ♀ ♀ (+/+; *cn/cn*) to line 2 ♂ ♂ (+/*Y*; *SM1/cn*).
† Generated by crossing line 2 ♀ ♀ (+/+; *SM1/cn*) to line 3 ♂ ♂ (+/B^sY; *cn/cn*).
‡ Generated by backcrossing F_1 ♂ ♂ from line A (+/*Y*; *SM1/cn*) to parental line 2 ♀ ♀ or to parental line 3 ♀ ♀.
§ Generated by backcrossing F_1 ♂ ♂ from line B (+/B^sY; *SM1/cn*) to parental line 2 ♀ ♀ or to parental line 3 ♀ ♀.

ence between lines 2 and 3, and the frequency of nondisjunction in line B is about 20% of the difference.

F_1 males from both lines A and B were backcrossed to parental females from lines 2 and 3. Of the four backcrosses, the nondisjunction frequencies in the progenies of the A males were similar, as were the frequencies of nondisjunction from the progenies of the B males. These have been pooled as line C and line D males in Table 5. The effect of the two different *Y* chromosomes is shown again in these backcrosses. It would appear, from a consideration of all four crosses, that a minor component of the differences between line 2 and line 3 is autosomal. However, the major difference appears to be due to the *Y* chromosome, which is expressed as an increase in the frequency of nullo-*2* gametes.

Since the parental females used in the crosses in Table 5 did not carry a *Y* chromosome, nondisjunction of the sex chromosomes in males could be followed for those lines with B^sY. Half (27/53) of the exceptional-*2* progeny were also nondisjunctional for the sex chromosomes. Some of the exceptional males from the unmarked *Y* crosses were progeny tested, and four out of 17 of these were nondisjunctional for the sex chromosomes.

Because of the nature of this cross, only four of the eight possible types of exceptional male gametes could be recovered. The parental females produced two types of recoverable gametes, *C(1)RM, y*; *nullo-2* and *nullo-X*; *C(2L)RM4, dp*; *C(2R)RM,* +. The former would give rise to viable offspring when fertilized by either *Y;22* or *0;22* sperm, and the latter when fertilized by an *X;0* or *XY;0* sperm. The *X;22, Y;0, XY;22* and *0;0* sperm gave rise to lethal sex chromosome aneuploids. Nevertheless, these results indicate that there is a high correlation of nondisjunction for the second and sex chromosomes.

This aspect of the behavior of *mei-G17* was investigated by crosses to females carrying a new compound-second chromosome, *C(2)EN*. This chromosome was generously supplied by DR. E. NOVITSKI, and is an attachment of two entire

TABLE 6

Progeny recovered from crosses of males homozygous for mei-G17 *to* y w^a/y w^a; C(2)EN, c bw *females*

Male gamete	Sex chromosome constitution of parental male $+/B^sY$	y^2/Y*	y^2/y^+Y
X;0	186	94	136
Y;0	79	49	45
X;22	71	18	27
Y;22	19	10	3
XY;0	63	36	32
0;22	185	38	101
XY;22	0	1	0
0;0	22	10	15

* Each fly was progeny tested to determine presence or absence of a *Y* chromosome; males were tested for fertility by appropriate crosses and females were crossed to appropriate *XY/0* males, and a minimum of 5 F_1 males were tested for fertility.

second chromosomes to one centromere. The chromosome is a reversed metacentric with the order of *2R2L·2L2R* (NOVITSKI, personal communication). This chromosome permits recovery of all eight types of exceptional male gametes. Tests were made with males of three different sex chromosome constitutions, $+/B^sY$, y^2/Y and y^2/y^+Y. The results are given in Table 6 and summaries and comparisons of the data are given in Table 7.

TABLE 7

A summary of the data given in Table 6

Row number and comparison	Sex chromosome constitution of the parental male $+/B^sY$	y^2/Y	y^2/y^+Y
1. Total exceptions	625	256	359
2. Number males tested	400	100	100
3. Frequency	1.56	2.56	3.59
4. Nullo-2 offspring	350	189	228
5. Diplo-2 offspring	275	67	131
6. Nullo-2/male tested	0.88	1.89	2.28
7. Diplo-2/male tested	0.68	0.67	1.31
8. Nullo/diplo	1.27	2.82	1.74
9. X/Y	2.62	1.90	3.40
10. Doubles/total progeny	0.43	0.33	0.41
11. Frequency among nullo-2 exceptions			
X	0.53	0.50	0.60
Y	0.23	0.26	0.20
XY	0.18	0.19	0.14
0	0.06	0.05	0.07
12. Frequency among double exceptions			
XY;0	0.23	0.42	0.22
0;22	0.69	0.45	0.68
XY;22	0	0.01	0
0;0	0.08	0.12	0.10

Several points emerge from a consideration of the data in Tables 6 and 7. First, from Table 6, all classes of exceptional progeny are not recovered in equal frequency, even though all diplo-*2* male gametes are recovered by the same nullo-*2* female gamete, and all nullo-*2* male gametes are recovered by the same diplo-*2* female gamete.

Each line is characterized by a different frequency of nondisjunction (row 3, Table 7). The B^sY line is the lowest, the Y line is intermediate and the y^+Y line is the highest. The difference between the B^sY line and the Y line is in the frequency of nullo-*2* gametes (.88 *vs.* 1.89, row 6). The frequency of diplo-*2* gametes is the same (.68 *vs.* .67, row 7). This is similar to the results reported in Table 5, although the magnitude of the difference is not as great. The y^+Y line seems to be characterized by a higher frequency of both diplo- and nullo-*2* types. The ratio of nullo-*2* to diplo-*2* (row 8) is intermediate in the y^+Y line (1.74), as compared to the B^sY line (1.27) and the Y line (2.82).

There is an increase in all four types of nullo-*2* gametes in the Y line, as compared to the B^sY line. This can be seen by comparing the relative frequencies of X, Y, XY and nullo-XY types among the nullo-*2* types (row 11). Thus, the increase in nullo-*2* gametes in the Y line does not appear to be related to the sex chromosome constitution of the gamete.

Among the progeny with regular sex chromosome disjunction, there is an excess of X-bearing gametes relative to Y-bearing gametes. This is true for all three lines, although the X/Y ratio varies for each line (row 9). It is highest for the y^+Y line (3.40) and lowest for the Y line (1.90). For the B^sY line, the X/Y ratio among the exceptional-*2* progeny can be compared with the ratio in the regular progeny (Table 4). The X/Y ratio is 2.62 when the second chromosomes nondisjoin and 1.62 when second chromosome disjunction is regular. Since the bias towards X-bearing gametes is the same regardless of whether second chromosome disjunction is regular or not, it seems reasonable to conclude that the mechanism leading to this bias is the same for haplo-*2*, diplo-*2* and nullo-*2* gametes.

It may be the case that the selection against the Y-bearing gametes is greater when the second chromosomes nondisjoin than when they do not, although this point cannot be resolved until simultaneous measurements of sex chromosome and second chromosome nondisjunction can be made.

In all three lines, the frequency of sex chromosome nondisjunction is high when the second chromosomes nondisjoin (row 10). For the B^sY line, it can be shown that sex chromosome nondisjunction is higher when the second chromosomes nondisjoin than when second chromosome disjunction is regular. When the seconds nondisjoin, the frequency of sex chromosome nondisjunction is 43%. But, when second chromosome disjunction is regular, the frequency of sex chromosome nondisjunction is about 5% (Table 4). Thus, for B^sY, there is a high correlation of sex and second chromosome nondisjunction. In the stocks of the y^2/y^+Y line, y^2 males and y^+ females are found, but not at a frequency that would suggest 41% nondisjunction. Male sterility is low in the Y line, suggesting that XO males are rare and that XY nondisjunction is not high. Thus, it seems

reasonable to conclude that in all three lines, there is a high correlation of nondisjunction with respect to the sex and second chromosomes.

All three lines have a non-random distribution of double exceptional offspring which is essentially the same (row 12). The B^sY and the y^+Y lines are virtually identical. The *0;22* progeny make up nearly 70% of the total double exceptions, *XY;0* comprise about 23%, *0;0* are 8–10% and there were no *XY;22* progeny recovered. In the *Y* line, *0;22* and *XY;0* progeny occurred with equal frequency (42–45%), *0;0* represented 12% of the total, and one *XY;22* exception was found. This single fly could have resulted from either an *XY;22* male gamete, or it could have been the result of secondary nondisjunction from either parent, since both parents carried an unmarked *Y*. Regardless of the source of this questionable fly, it is clear that *XY;22* gametes either occur very infrequently or they cannot be recovered.

DISCUSSION

First division meiotic mutants are sex-specific, a fact which has led to the speculation that the genetic control of the first meiotic division is different for the two sexes (SANDLER *et al.* 1968; BAKER and CARPENTER 1972). Furthermore, it has been noted that whereas female meiotic mutants affect all chromosome pairs, male meiotic mutants are often chromosome-specific (BAKER and CARPENTER 1972). For example, the 20 *X*-linked male meiotic mutants found by BAKER and CARPENTER (1972) were specific to *XY* disjunction and had no effect on the fourth chromosomes, whereas the eleven female meiotic mutants they found affected all chromosome pairs. It is not known how many loci are represented by the 20 male mutants. Two of the mutants were mapped and were localized to the euchromatin.

There are at least three, and possibly four, male meiotic mutants which are not chromosome-specific. *mei-081* causes nondisjunction of both the sex and fourth chromosomes (SANDLER *et al.* 1968), as well as of the second chromosomes (GETHMANN, unpublished). *mei-G17* causes nondisjunction of the sex and second chromosomes. *mei-W5* or *pal* causes loss of parental chromosomes during meiosis (SANDLER 1971; BAKER, in preparation). *mei-S8* has no effect on sex chromosome disjunction but causes nondisjunction of the fourth chromosomes (SANDLER *et al.* 1968). This mutant was not tested for second or third chromosome disjunction. Thus, it could be associated with disjunction of all of the autosomes, or it could be a gene concerned with only fourth chromosome disjunction.

Part of the apparent specificity of male meiotic mutants might be due to the unique nature of *XY* pairing, as compared to autosomal pairing. Cytologically, *XY* pairing occurs at the base of the *X* chromosome (COOPER 1964), whereas autosomes are paired along their entire length (COOPER 1950). At metaphase, the autosomes show only a tip-to-tip association (COOPER 1950), while the *XY* bivalent still shows only a basal association (COOPER 1950, 1964). Thus, one might expect to find a class of meiotic genes associated with only *XY* pairing. Such meiotic genes would be represented by the *X*-linked mutants found by BAKER and CARPENTER.

Of the remaining first division male meiotic mutants, three cause nondisjunction of two or more pairs of chromosomes (*mei-081*, *mei-G17*, *pal*) and one (*mei-S8*) may or may not be chromosome-specific. Thus, it is possible that the apparent specificity of male meiotic mutants is related to *XY* pairing, and that there are two major classes of male meiotic genes: (1) those associated with just *XY* pairing and disjunction and (2) those associated with pairing and disjunction of all chromosome pairs. A third possible category would be meiotic genes concerned with just autosomal pairing. *mei-S8*, depending on its behavior with respect to the second and the third chromosomes, could be a representative of this class. Characterization of further mutants will resolve this question of the chromosome specificity of male meiotic genes.

With respect to *mei-G17*, there are three interesting properties of this mutant: (1) the unequal recovery of the different sex chromosome classes, (2) the effect of the *Y* chromosome on second chromosome disjunction and (3) the high correlation of sex and second chromosome disjunction.

The behavior of the sex chromosomes in *mei-G17* is identical to that of $In(1)sc^{4L}sc^{8R}$ and the male meiotic mutants of BAKER and CARPENTER (1972). An excess of *X*-bearing and nullo-*XY*-bearing gametes are recovered, relative to *Y*-bearing and *XY*-bearing gametes. PEACOCK (1965) has interpreted the behavior of sc^4sc^8 as a case of meiotic drive, and BAKER and CARPENTER (1972) extended the argument to include their male meiotic mutants. They argued further that unpaired chromosomes are subject to meiotic drive. The unequal recovery is believed to be the result of dysfunction of those sperm cells bearing a particular chromosome. For *mei-G17*, it would appear that meiotic drive and dysfunction are responsible for the unequal recovery of the sex chromosome classes.

Different lines of *mei-G17* are characterized by different frequencies of nondisjunction. It has been shown that the higher frequency of nullo-*2* gametes in the *Y* line is due mainly to the *Y* chromosome. The *Y* chromosome could be causing changes in the frequency of dysfunction for different gametic classes, or it could be influencing, in some way, the actual event of nondisjunction. Since the distribution of *X*, *Y*, *XY*, and nullo-*XY* gametes does not change among the nullo-*2* gametes in the *Y* and B^sY lines (row 11, Table 7), it would appear that the *Y* chromosome does not change the relative frequency of dysfunction with respect to the sex chromosome constitution. If the *Y* does change the frequency of dysfunction, then it is acting on the probability of dysfunction of nullo-*2* gametes and is acting independently of the sex chromosome constitution.

The most interesting property of *mei-G17* is the high correlation of double nondisjunction. Some of the differences in the distribution of the double exceptional classes can be explained by meiotic drive, but, as will be shown, not all of it can be explained by drive. Firstly, in all three lines, there is an excess of nullo-*2* progeny. Secondly, with respect to the sex chromosomes, nullo-*XY* is preferentially recovered relative to *XY*. Therefore, if the second and the sex chromosomes were nondisjoining at random, and there was meiotic drive with respect to the sex chromosome constitution, one would predict that the largest class recovered

would be nullo-*XY*; nullo-*2*. However, this is clearly not the case. Nullo-*XY*; nullo-*2* progeny make up only about 10% of the total double exceptions for all three lines. Invoking meiotic drive with respect to the second chromosome does not resolve the problem, as the results cannot be explained by any simple system of double drive. For example, sex chromosome drive is toward dysfunction of *XY* gametes; thus second chromosome drive would have to be toward dysfunction of nullo-*2* gametes if they were nullo-*XY*, but not if they were *XY*. By any model of double drive, one of the two drive systems has to be reversible to explain these results.

Another possible interpretation would be random nondisjunction coupled with an increase in the probability of chromosome loss for nonhomologs. For example, consider those gametes which have undergone nondisjunction for chromosome *2* but where sex chromosome disjunction is regular. There would be an array of four types: *X;22*, *Y;22*, *X;0* and *Y;0*. If, because of the nondisjunctional event, the probability of sex chromosome loss were increased, then this would lead to the production of *0;22* and *0;0* gametes. For those meiocytes where second chromosome disjunction was regular but where the sex chromosomes underwent nondisjunction (*XY;2* and *0;2*), chromosome loss of the second chromosomes would lead to an increase in *XY;0* and *0;0* gametes. Thus, this model would predict that the majority of the double exceptions would be the *0;22*, *0;0* and *XY;0* types, with the *0;0* type expected to be the largest class. However, since the *XY;0* and *0;22* gametes make up 85–90% of the double exceptions, one would have to assume that loss occurred only in the diplo-*2* and *XY;2* gametes to make this model consistent with the data.

A third alternative is nonhomologous pairing between the sex and second chromosomes and meiotic drive with respect to the sex chromosomes. If homologous pairing were either blocked or disrupted in some way, and the chromosomes were permitted to pair nonhomologously, one would expect to find a high correlation of nondisjunction between the two sets of chromosomes and an excess of the nullo diplo and diplo nullo types of gametes. This is essentially what is found with *mei-G17*. Meiotic drive would be expected to select against the *XY;0* and *XY;22* classes, which are both deficient relative to their reciprocal classes.

If *mei-G17* is causing nonhomologous pairing, it could be acting at any stage of chromosome pairing. For example, it could disrupt pairing by affecting the specific recognition of a chromosome for its homolog. Thus, the chromosomes could either remain unpaired and segregate as univalents, or they could pair nonhomologously. Since the frequency of double exceptions is high, and of these there are only a few *XY;22* and *0;0* gametes, it would appear that most of the chromosomes will disjoin from some other chromosome rather than segregating as a univalent.

Alternatively, homologs could pair initially, but because of the mutant, they would be unable to maintain synapsis until anaphase I. Thus, they would separate prematurely. This model would require a second pairing which could involve nonhomologous associations. It also suggests the possibility that nonhomologous pairing can occur in males as a consequence of a failure to pair

initially. Thus, there could also be a second pairing stage in males similar to that found in females, although the situations necessary to invoke this second pairing stage in males would have to be quite different than in females.

Further studies with this mutant are under way to determine its properties with respect to the simultaneous nondisjunction of two pairs of chromosomes. Additional experiments are also being designed to investigate the possibility of nonhomologous pairing in males.

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