THE EFFECT OF RECOMBINATION-DEFECTIVE MEIOTIC MUTANTS ON FOURTH-CHROMOSOME CROSSING OVER IN *DROSOPHILA MELANOGASTER*[1]

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Manuscript received May 4, 1978
Revised copy received July 5, 1978

ABSTRACT

Crossing over was measured on the normally achiasmate fourth chromosome in females homozygous for one of our different recombination-defective meiotic mutants. Under the influence of those meiotic mutants that affect the major chromosomes by altering the spatial distribution of exchanges, meiotic fourth-chromosome recombinants were recovered irrespective of whether or not the meiotic mutant decreases crossing over on the other chromosomes. No crossing over, on the other hand, was detected on chromosome *4* in either wild type or in the presence of a meiotic mutant that decreases the frequency, but that does not affect the spatial distribution, of exchange on the major chromosomes. It is concluded from these observations that (a) in wild type there are regional constraints on exchange that can be attenuated or eliminated by the defects caused by recombination-defective meiotic mutants; (b) these very constraints account for the absence of recombination on chromosome *4* in wild type; and (c) despite being normally achiasmate, chromosome *4* responds to recombination-defective meiotic mutants in the same way as do the other chromosomes.

RECOMBINATION-defective meiotic mutants affect the frequency, the spatial distribution along the chromosome, or both, of meiotic exchanges in females. Among the 21 loci thus far identified by recombination-defective meiotic mutants in Drosophila, all but one (*mei-352*) reduce exchange; also all but one (*mei-9*) alter the spatial distribution of exchanges. Moreover, the alteration in the spatial distribution is, in every case, of such a nature that the distribution of exchanges generated by a meiotic mutant is much more nearly proportional to physical distance than is the distribution characteristic of the wild type. This is seen as relatively drastic decreases in recombination in distal regions, with proximal regions being much less affected; indeed, the most proximal regions may show control levels, or even above, of crossing over. The evidence supporting these generalizations and references to the original reports are found in the review by BAKER *et al.* (1976a).

From the analyses of recombination-defective meiotic mutants, it has seemed reasonable to infer that there are normally (that is, in the absence of meiotic

[1] Research supported by Public Health Service Grant RG-9965.

mutants) genetically controlled spatial constraints on recombination. The meiotic defects caused by most recombination-defective meiotic mutants, in addition to reducing exchange, apparently weaken or obliterate these constraints. It is, therefore, the absence of normal control that results in the observed alterations in the spatial distribution of exchanges.

A puzzling observation about the effects of recombination-defective meiotic mutants, one that has been commented upon by many workers, is that under the influence of such mutants both the major chromosomes and chromosome *4* exhibit elevated levels of primary nondisjunction. In the case of the major chromosomes, numerous lines of evidence agree that the chromosomes fail to disjoin only because they have failed to undergo exchange and, hence, have disjoined distributively from a nonhomologous chromosome (see GRELL 1976 for a discussion of the distributive system); the primary effect of the meiotic mutant is thus viewed as exclusively that of reducing exchange. That is, a mutant-induced decrease in exchange causes an increase in the frequency of no-exchange tetrads, which, in turn, results in distributive disjunction of nonhomologs and hence an increase in nondisjunction. In the case of chromosome *4*, however, virtually all tetrads are no-exchange in wild type [for a discussion of recombination in chromosome *4*, see the review by HOCHMAN (1976)], so that an overall reduction in exchange ought not affect fourth-chromosome disjunction. Despite this argument, recombination-defective meiotic mutants, in fact, increase fourth-chromosome nondisjunction and do so approximately proportionately to the decrease in overall exchange [for literature and discussion, again see BAKER *et al.* (1976a)].

The proposition that we advance in this report is that chromosome *4* does not cross over in wild type because of the same genetically controlled regional constraints on recombination that are revealed on the other chromosomes by recombination-defective meiotic mutants. The evidence for this is that meiotic fourth-chromosome crossing over is here shown to occur under the influence of those meiotic mutants that alter the spatial distribution of exchanges, even though most decrease exchange on all chromosomes other than chromosome *4*. As a corollary, this result implies a direct effect of recombination-defective meiotic mutants on the meiotic behavior of chromosome *4* of the same nature as that observed on the other chromosomes. Whether, however, this effect is observed here for the first time or has often revealed itself as the elevated rate of fourth-chromosome nondisjunction noted in the preceding paragraph is not obvious and will be discussed below.

MATERIALS AND METHODS

Crossing over on chromosome *4* was measured in wild type and under the influence of four recombination-defective meiotic mutants. The meiotic mutants included: the third-chromosome *mei-S282* and the sex-linked *mei-218*, both of which markedly reduce exchange on all the major chromosomes and alter the distribution along the chromosome of those exchanges that do occur (SANDLER *et al.* 1968; LINDSLEY *et al.* 1968; BAKER and CARPENTER 1972; PARRY 1973; CARPENTER and SANDLER 1974); *mei-9*^{b}, on the *X* chromosome, which reduces the amount of exchange on all the major chromosomes but does not affect the spatial distribution of the remaining exchanges (BAKER and CARPENTER 1972; CARPENTER and SANDLER 1974); and *mei-352*, also sex-linked,

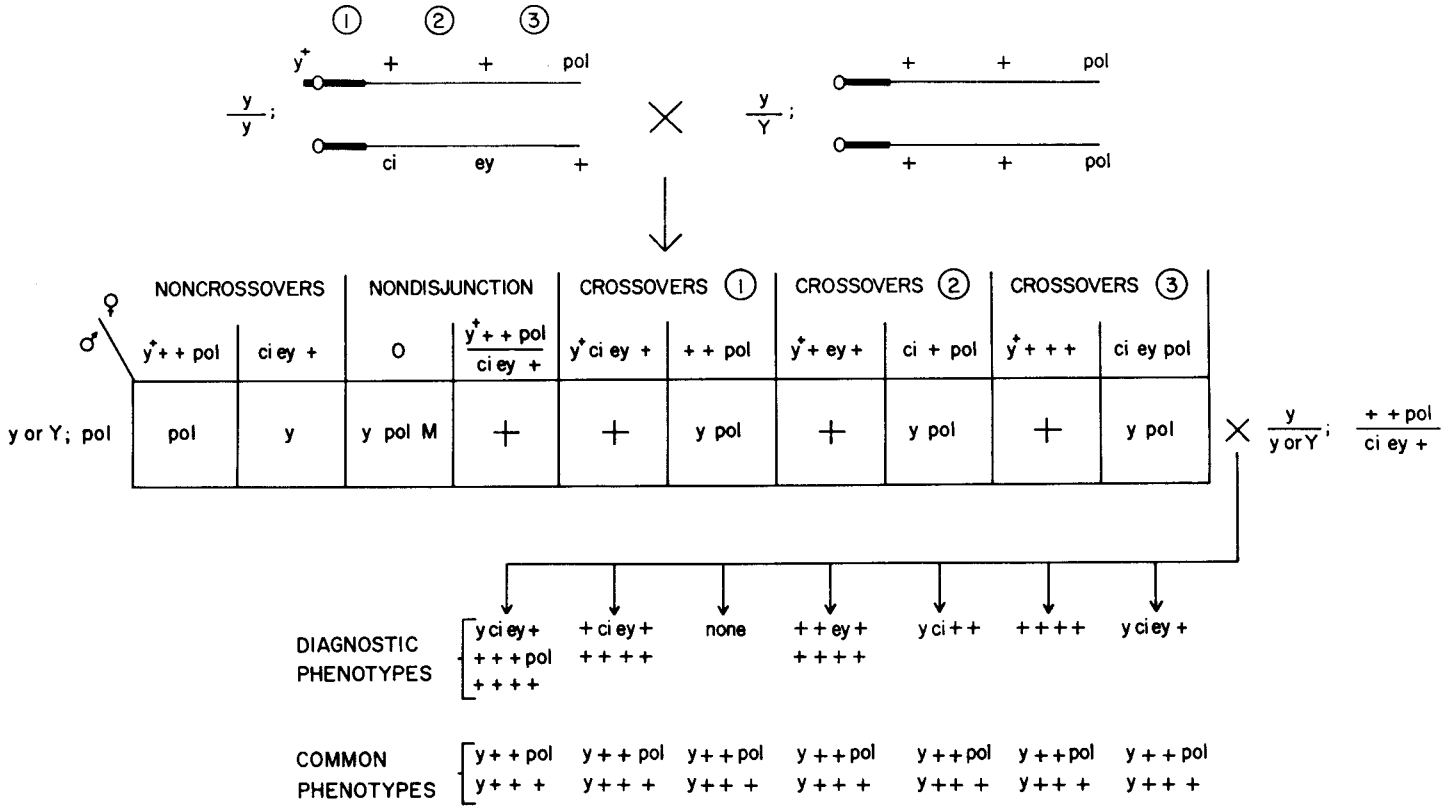

FIGURE 1.—The mating scheme used to measure recombination on chromosome *4*. The top line gives the cross (note that the parental female may be homozygous for a meiotic mutant that is not indicated in the diagram); the second line shows both the progeny types recovered and the progeny-test cross; the bottom line shows the phenotypes in the offspring of the progeny-test crosses that discriminate between nondisjunctional and recombinant types produced by the original parental female.

which exhibits near-normal levels of exchange but causes an abnormal spatial distribution of those exchanges (BAKER and CARPENTER 1972; BAKER *et al.* 1976a).

The females in which fourth-chromosome exchange was monitored were heterozygous for a structurally normal chromosome *4* marked by the recessive mutants *ci* and ey^R (hereafter *ey*), and a homolog carrying the recessive marker spa^{pol} (hereafter *pol*) and also *y*+ translocated from the tip of the *X* chromosome to the left arm of chromosome *4* (PARKER 1969); these chromosomes are pictured in the top left portion of Figure 1. Because all of the *X* chromosomes used in this study carry a recessive *y* allele, these markers define three adjacent crossover regions on chromosome *4*—(1) an entirely or mostly heterochromatic region from the centromere to *ci*, (2) a proximal euchromatic region from *ci* to *ey*, and (3) a distal euchromatic region from *ey* to *pol*.

The cross, diagrammed in the top line of Figure 1, allows direct scoring of the two nonrecombinant gamete types (*pol* or *y* progeny) and of the nullo-*4* nondisjunctional gametes (*y pol* and, because they result in haplo-*4* progeny, Minute). Diplo-*4* exceptional gametes and gametes carrying crossovers in any of the three regions will all appear in either wild-type or *y pol* individuals. This is shown in the center section of Figure 1. Accordingly, as many as practicable of the *y pol M*+ and wild-type offspring were progeny tested by crossing them to *y*; *ci ey/pol* flies of the opposite sex. From the types of offspring produced in such progeny tests, the nature of the *y pol* and wild-type individuals can be ascertained. The phenotypes by which the ascertainment is accomplished are shown in the bottom section of Figure 1.

RESULTS

The progeny from the cross diagrammed in the top line of Figure 1, for the control and the four meiotic mutants, are enumerated in Table 1. The results of the progeny tests of a sample of the "exceptional" offspring recorded in Table 1 are presented in Table 2. It can be seen that (a) crossing over occurs in both euchromatic regions of the fourth chromosome in *mei-S282*, *mei-218* and *mei-352* homozygotes, (b) no such recombination occurs in either wild-type or $mei\text{-}9^b$ homozygotes, and (c) reciprocal classes are generally recovered.

The heterochromatic region 1 is atypical in two respects: only one recombinant class is observed, and this class is recovered from $mei\text{-}9^b$, as well as from the other meiotic mutants, despite the absence of crossing over in the euchromatic regions

TABLE 1

The results of crosses of females homozygous for y *and the indicated meiotic mutant, by* y *males*

Chromosome 4*	Phenotype of progeny	Meiotic mutant involved				
		+	*mei-9*	*mei-S282*	*mei-218*	*mei-352*
Regular	*y*+ *pol*	28729	16375	47404	17763	5972
	y pol+	29973	14992	48382	18362	6459
	y+ *pol*+	7†	820	511	2517	151
Exceptional	*y pol*	0	126	84	79	59
	y pol M	0	7	125	185	10

The fourth chromosomes carried by the parental females are *y*+. + + *pol*/ (*y*−). *ci ey* +; the parental males are homozygous for *pol*. This cross is diagrammed in the top line of Figure 1.

* "Regular" refers to nonrecombinant, normally segregating, fourth chromosomes; "Exceptional" implies either putative nondisjunctional or putative recombinant fourth chromosomes.

† Four of these seven exceptions were members of a single cluster.

TABLE 2

The results of progeny testing a sample of the "exceptional" progeny recorded in Table 1

	Meiotic mutant involved									
	+		*mei-9*		*mei-S282*		*mei-218*		*mei-352*	
	+ +	*y pol*	+ +	*y pol*	+ +	*y pol*	+ +	*y pol*	+ +	*y pol*
Total number	7	0	820	126	511	84	2517	79	151	59
Number tested	6	0	382	39	179	71	712	45	115	42
Crossover in 1	0	–	0	39	0	69	0	38	0	18
Crossover in 2	0	–	0	0	0	1	1	1	20	20
Crossover in 3	0	–	0	0	0	1	3	6	5	4
Nondisjunctional	6	–	382	–	179	–	708	–	90	–

The basis of the determinations recorded here is shown in the lower part of Figure 1.

under the influence of *mei-9*^{b}. This suggests that the apparent recombinants in region 1 in fact result from something other than meiotic exchange; their origin will be considered below.

As it was impractical to progeny test all "exceptional" offspring, the data in Table 2 were used to determine the fraction of each recombinant and nondisjunctional class among the tested exceptions; these fractions were then used to calculate the expected number of each class among the total exceptional progeny. The data in Tables 1 and 2, converted in this sense, are given in Table 3. Because of the peculiarities noted above, the apparent exchanges in region 1 (*y pol* exceptions) are recorded as cases of "y^{+} loss" in Table 3.

TABLE 3

The results, after progeny testing, of the crosses diagrammed in Figure 1 and recorded in Table 1

Type of oocyte	Genotype of oocyte	Meiotic mutant involved				
		+	*mei-9*	*mei-S282*	*mei-218*	*mei-352*
Noncrossover	+ + + *pol*	28729	16375	47404	17763	5972
	y ci ey +	29973	14992	48382	18362	6459
Diplo-exception	+ + + *pol/ci ey* +	7	820	511	2503	118
Nullo-exception*	Nullo-*4*	0	7	125	185	10
Crossover in 1	y^{+} *ci ey* +	0	0	0	0	0
	+ + *pol*	0	0	0	0	0
Crossover in 2	y^{+} + *ey* +	0	0	0	3	26
	ci + *pol*	0	0	1	2	28
Crossover in 3	y^{+} + + +	0	0	0	11	7
	ci ey pol	0	0	1	10	6
y^{+} loss	+ + *pol*	0	126	82	67	25
Recombinants (regions 2 + 3) per 10^{3} regulars		0.00	0.00	0.021	0.72	5.39
y^{+} loss per 10^{3} regulars		0.00	4.02	0.86	1.85	2.01

The basis of the conversion of the numbers in Tables 1 and 2 to those shown here is given in text.

* The resultant exceptions, being haplo-*4*, are Minute.

To determine whether each of the meiotic mutants in these experiments, exhibited its characteristic meiotic behavior, the frequency of fourth-chromosome nondisjunction caused by each was calculated from the data in Table 3. The frequency is given by twice the number of triplo-*4* progeny divided by the total number of progeny (counting the triplo-*4* progeny twice and excluding *Minutes*). The resultant frequencies are: *mei-9*^{b} = 5.0%; *mei-S282* = 1.1%; *mei-218* = 12.3%; *mei-352* = 1.9%; wild type = 0.024%. These frequencies may be compared with those published previously, which are: *mei-9*^{b} = 6.6% and *mei-218* = 12.8% (CARPENTER and SANDLER 1974, personal communication); *mei-218* = 13.3% and *mei-352* = 1.8% (BAKER and CARPENTER 1972); *mei-S282* = 0.65 to 1.0% (PARRY 1973; SANDLER *et al.* 1968). It is clear that all of the meiotic mutants exhibit the expected meiotic abnormalities. It is, in addition, noteworthy that *mei-S282* has a noticeably weaker meiotic phenotype than either *mei-218* or *mei-352*, and it also results in a much lower level of fourth-chromosome recombination.

In view of the mitotic instability often associated with recombination-defective meiotic mutants (BAKER *et al.* 1976b, 1978), it is necessary to consider whether the fourth-chromosome recombinants recovered in these experiments result from meiotic recombination or from germ-line premeiotic mitotic recombination. There are three lines of evidence bearing on the distinction between these alternatives. First, the relative frequencies of mitotic recombination in the somatic cells reported for the different meiotic mutants may be compared to the frequencies of fourth-chromosome recombination observed here to see if they are correlated. Second, because meiotic recombination does not occur in Drosophila males, while germ-line mitotic recombination occurs in both sexes (BECKER 1976), a mitotic contribution to fourth-chromosome recombination might be detected in males. Finally, premeiotic mitotic recombination in the female germ line can result in clusters of recombinants in the progeny of single females; meiotic recombination, on the other hand, will result in a binomial distribution of recombinants among the progeny of the females tested. All three lines of evidence suggest that the euchromatic fourth-chromosome recombination observed here under the influence of recombination-defective meiotic mutants occurs during meiosis. The analysis of clusters, on the other hand, indicates that the y^{+} loss events are mostly, or exclusively, premeiotic.

First, there is an elevated frequency of mitotic recombination and chromosome breakage in the somatic cells of *mei-9*^{b} and *mei-S282* females, whereas the chromosomes in somatic cells of *mei-218* females are as stable as are those in wild-type females (BAKER *et al.* 1978). Therefore, if meiotic-mutant-induced mitotic chromosome instability in the germ line were an important source of fourth-chromosome recombinants, *mei-9*^{b} and *mei-S282* females should exhibit more recombination than *mei-218* females. The data in Table 3, however, show the reverse to be true.

Second, three chromosome *4* crossover tests were conducted in males of the genotype *y/Y*; y^{+}*.pol/ci ey*, which were, in addition, *mei-9*^{b}, *mei-352*, or wild

type. In the control, 17,295 *pol*, 17,427 *y*, and nine wild-type progeny were recovered; upon progeny testing, the latter proved to be triplo-*4* nondisjunctional exceptions. From *mei-9*^{b} males, there were 23,917 *pol*, 23,701 *y*, and 42 wild-type progeny; from *mei-352* males, there were 20,878 *pol*, 21,172 *y*, and 47 wild-type progeny. All wild-type offspring, upon progeny testing, proved to be nondisjunctional exceptions. Thus, fourth-chromosome mitotic recombinants are either absent or generated very infrequently in males carrying a meiotic mutant that induces substantial numbers of fourth-chromosome recombinants in females, suggesting that the fourth-chromosome recombination observed in females is meiotic.

Finally, the data from the progeny tests presented in Table 2 can be examined to determine whether fourth-chromosome recombinants are recovered in accordance with binomial expectations. With respect to the euchromatic recombinants that are recovered from homozygous *mei-S282*, *mei-218*, and *mei-352* females, there were, overall, 58 cultures that produced at least one such recombinant in a total of 933 cultures examined. On the assumption of independence, therefore, 3.6 cultures should have yielded two recombinants; in fact, four did. Thus, in the case of euchromatic recombinants, there is no evidence of clustering. On the other hand, with respect to the heterochromatic region 1 (that is, the y^{+}-loss events), clustering is evident, as may be seen in the distribution of y^{+} losses among cultures shown in Table 4.

We conclude from these three analyses that fourth-chromosome recombination in the euchromatic regions 2 and 3 occurring under the influence of *mei-218*, *mei-S282*, and *mei-352*, is mostly or entirely meiotic recombination. On the other

TABLE 4

The clustering of y^{+}*-loss events* per vial† induced by the indicated meiotic mutant*

Events per vial	Number of vials with y^{+} loss events				
	mei-9	*mei-9*‡	*mei-S282*	*mei-218*	*mei-352*
0	174	368	537	227	125
1	7	31	11	13	8
2	4	5	1	1	1
3	3	1	0	0	0
4	0	4	1	0	0
5	3	3	0	1	0
6	0	3	1	1	0
7	0	1	0	0	0
8	0	3	0	0	1
9	0	0	1	0	0
≥10	0	12	2	1	0

* Only $y+$ loss events confirmed by progeny tests are included, except as noted below‡.

† The vials in this test contained a number of parental females: ten for *mei-9*, one for *mei-S282*, ten for *mei-218*, and 20 for *mei-352*.

‡ All *y pol* progeny recovered, irrespective of whether progeny tested or not, from three separate *mei-9* experiments, including the one recorded in the first column; because no recombination is observed in the case of *mei-9*, it seems reasonable to assume that these progeny all result from $y+$ losses.

hand, y^+-loss events occurring under the influence of *mei-9*^{b}, *mei-218*, *mei-S282*, and *mei-352*, are unlikely to be the consequence of meiotic recombination. Instead, y^+ losses appear to result from premeiotic events that occur in the germ line of females, but not (curiously) of males, homozygous for recombination-defective meiotic mutants.

We shall now consider, and test for, four possible premeiotic events that could result in the recovery of *y pol* progeny. Two events yielding y^+ losses both require the generation of translocations between the heterochromatic right arm of the *X* chromosome and the heterochromatin of chromosome *4* (*4L* or *4R*). If the translocation involves *4R*, one of the elements will be an *X* chromosome with the right arm replaced by *4R* (*XL.4R*), while the other element will be a fourth chromosome bearing the y^+ duplication on the left arm, with the right arm replaced by *XR* (y^+ *4L.XR*). A formally analogous event would be a translocation involving the heterochromatin of *XL* and *4L*; in this case, the centromere of the *XL.4R* chromosome would be derived from chromosome *4* rather than from the *X* chromosome. The resulting *XL.4R* chromosome would probably disjoin no differently from one carrying an *X*-chromosome centromere (SANDLER 1956). Following fourth-chromosome nondisjunction or loss, it is possible to generate ova bearing the *y pol X* chromosome (*XL.4R*). The *y pol* exceptions would result from fertilization of such ova by *y*; *pol* or *Y*; *pol* sperm. This explanation predicts that the *pol* locus should display sex linkage in the progeny of *y pol* exceptions. Accordingly, 22 *y pol* males were obtained from a cross of *y mei-9/y mei-9*; y^+*.pol/ci ey* females by *y/Y*; *pol/pol* males. These 22 *y pol* males, obtained from 13 different vials, were crossed individually to *C(1)DX, y f/Y* females. The *y* males produced were collected, and 8 to 12 of these were crossed individually to *y/y*; *pol/pol* females. In all cases, both *y* and *y pol* males and females were observed in the progeny. It appears, therefore, that the *y pol* exceptions do not result from *XR-4R* or *XL-4L* translocations.

If the *X-4* translocation involves *4L*, one of the elements will be an *X* chromosome with the right arm replaced by *4L* (*XL.4L* y^+), while the other element will be a fourth chromosome with the left arm replaced by *XR* (*XR.4R*). A formally analogous event would be a translocation involving the heterochromatin of *XL* and *4R*; in this case, the centromere of the *XL.4L* chromosome would be derived from chromosome *4* rather than from the *X* chromosome. If the two elements of the translocation segregate from each other, *y pol* and wild-type progeny will be recovered from *XR.4R* and*XL.4L* ova, respectively. This explanation predicts that some of the wild-type progeny will carry the *XL.4L* chromosome, and that in the progeny test shown in Figure 1, some wild-type males will yield sons of the phenotypes *y*, *y pol*, and *y ci ey* and females of the phenotypes *pol* and wild type. From the cross described in the preceding paragraph, 187 wild-type males were recovered, of which 104 were *XY*. When 93 of these males, from 59 different vials, were progeny tested as shown in Figure 1, all 93 yielded *y*, *y pol*, *pol*, *y ci ey*, and wild-type males and females. Since no y^+ *X* chromosomes were obtained, wild-type progeny result neither from *XL.4L* chromosomes nor from y^+ *X* chromosomes obtained from reciprocal exchanges between the *y* locus on *XL* and the y^+ locus on *4L* (see below). Therefore, the *y pol* exceptions do not result from *XR-4L* or *XL-4R* translocations.

While these results show that y^+ loss in *mei-9*^{b} does not result from *X-4* translocations, five wild-type exceptions recovered independently in the experiment with *mei-218* yielded a result suggesting a translocation event. These wild-type exceptions produced *y*, *y pol*, *y ci ey*, *pol*, *ci ey*, and wild-type offspring in the progeny test shown in Figure 1. The vials producing these offspring were not included in the totals given in Tables 1 and 2. Because no exceptions of this kind were observed in the experiments with the other meiotic mutants, we conclude that *y pol* exceptions do not in the main result from reciprocal translocations between the major autosomes and the fourth chromosome.

A third explanation for y^+ loss requires a reciprocal exchange between the *y* region on *XL* and the corresponding y^+ region on *4L*. Such an event will produce an *X* chromosome carrying y^+, and a *y.pol* fourth chromosome. The *y pol* exceptions would then result from the fertiliza-

tion of *y*; *y.pol* ova by *y*; *pol* or *Y*; *pol* sperm. In this case, the *y.pol* fourth chromosome should still carry the wild-type alleles of the other *X* chromosome loci on the duplication. To test this possibility, a number of independent lines were established from *y pol* exceptional progeny recovered from *y mei-9^b/y mei-9^b*; *y+ pol/ci ey* females mated with *y/Y*; *ci ey pol/ci ey pol* males. These lines were maintained by selecting *y pol* progeny. Males from each line were crossed to *y l(1)J1/Muller-5* females; *y* male progeny from this cross demonstrate the presence of *l(1)J1+* (which is distal to *y*) on the fourth chromosome. Males from each line were also crossed to *y ac v/y ac v* females; *y v* male progeny from this cross would demonstrate the presence of *ac+* (which is just proximal to *y*) on the fourth chromosome (PADILLA and NASH 1977). Of the ten independent lines of *y+*-loss exceptions recovered from *mei-9^b* females, none carried *l(1)J1+* or *ac+*. An additional line resulting from spontaneous *y+* loss in a *y+.pol/y+.pol* stock was also tested and found to lack *l(1)J1+* and *ac+*.

The fourth explanation for *y+* loss requires either a sister-strand exchange between *4L* and *4R* or centromere misdivision, either of which would result in the generation of a compound chromosome *4* consisting of two right arms attached to the same centromere. The *y pol* exceptions would then result from the fertilization, by *y*; *pol* or *Y*; *pol* sperm, of *y*; *C(4)RM, pol* ova. The compound-fourth chromosome may be detected by crossing *y pol* exceptional progeny to *y/y*; *C(4)RM, ci ey* females; diplo-*4*, *y pol* progeny of this cross result from either nondisjunction or the presence of a compound-fourth chromosome. If the *y pol* progeny themselves yield diplo-*4*, *y pol* progeny when the cross is repeated, then the presence of a compound-fourth chromosome is demonstrated. Accordingly, males from each line established from the exceptions described in the preceding paragraph were crossed to *y/y*; *C(4)RM, ci ey* females. Of the ten independent lines of *y+*-loss exceptions recovered from *mei-9^b* females, none carried a compound chromosome *4*. The line resulting from spontaneous *y+* loss in a *y+.pol/y+.pol* stock also lacked a compound chromosome *4*. As an additional observation, progeny of this cross that were *C(4)RM, ci ey/pol* were crossed to each other to produce *pol/pol* progeny; in all cases, homozygotes for the fourth chromosomes from *y+* losses were found to be viable, fertile, and morphologically normal.

In summary, then, in the premeiotic gonia of females homozygous for recombination-defective meiotic mutants, an event occurs that results in a class of progeny that appear to carry a normal (*i.e.*, homozygotes are viable and phenotypically wild type) fourth chromosome from which the entire translocated *y+* region has been lost. None of the events for which we have been able to test has provided a mechanism for this loss. Other possibilities that come readily to mind involve recombination within repeated telomeric sequences. For example, if such sequences were present between the *y+* region and the heterochromatin of *4L*, then recombination between those sequences and the normal telomeric sequences of *4R* would result in the loss of the entire *y+* region from a monocentric, genetically complete chromosome *4* that, however, would be ringshaped. A crossover involving, instead of *4R*, the normal telemere of the translocated portion of the *X* chromosome would yield a rod, rather than a ring, chromosome *4*. While tests for such possibilities are not obvious, examples of analogous events are known [NEWMEYER and GALEAZZI (1978), see the review of PERKINS and BARRY (1977) for references].

Finally, in this context, it is worth noting that, whatever their origin, the *y+* losses are demonstrably premeiotic and occur only under the influence of recombination-defective meiotic mutants. They thus represent an example of a meiotic-mutant-induced mitotic chromosome instability of the general type that has been exhibited in many cases in Drosophila (BAKER *et al.* 1976a,b; BOYD *et al.* 1976a,b;

BOYD and PRESLEY 1974; NGUYEN and BOYD 1976; SMITH 1976, 1973; SMITH and SHEAR 1974), as well as in other organisms (see review by BAKER *et al.* 1976a). The y^+ losses differ, however, from all previously reported instances in that (a) although mitotic, they do not occur in males, and (b) they are induced by *mei-218*, which does not induce any of the other types of mitotic instability (BAKER *et al.* 1978). Whether these differences between y^+ losses and other types of mitotic instability are the consequence of differences in the nature of the events being monitored or differences in the way premeiotic germ-line cells in females respond to meiotic mutants remains to be determined.

DISCUSSION

Crossing over does not ordinarily occur in the fourth chromosome of *Drosophila melanogaster* even though homologous fourth chromosomes pair with, and segregate from, one another at first meiosis. Two lines of evidence suggest that this absence of recombination is not merely a stochastic consequence of the small size of the fourth chromosome. In the first place, although fourth chromosomes are associated with synaptonemal complex, that complex is morphologically of the type located near centromeres (heterochromatic complex) that is not ordinarily associated with genetic exchange (CARPENTER 1975). On the other hand, under any of a variety of special circumstances, fourth-chromosome crossing over does occur—in diplo-*4* triploids (MORGAN, STURTEVANT and MORGAN 1945; STURTEVANT 1951), when heat-induced (GRELL 1971), and possibly when induced by interchromosomal effects (see APPENDIX). It seems, therefore, that fourth chromosomes are physically capable of recombining, but are ordinarily constrained from doing so.

This regionally localized constraint on exchange is but one example of a general class of such constraints, the overall result of which is the marked regional difference in the amount of recombination per unit of chromosome length (for a recent discussion, see LINDSLEY and SANDLER 1977). It has been repeatedly observed (see BAKER *et al.* 1976a for general discussion and documentation, and BAKER and HALL 1976 for a detailed consideration) that many recombination-defective meiotic mutants attenuate or eliminate these localized constraints, so that the distribution of exchanges per unit of chromosome length occurring under their influence exhibits much less regional variation than obtains in the wild type. The result reported here, that fourth-chromosome meiotic crossing over occurs under the influence of meiotic mutants that attenuate this general class of localized constraints but not under the influence of the recombination-defective *mei-9*^{b}, which does not, strongly suggests that (a) the normal absence of crossing over in chromosome *4* is the result of the same regional constraints that operate on all of the chromosomes of the Drosophila genome, and (b) despite being normally achiasmate, chromosome *4* is directly affected by recombination-defective meiotic mutants in the same way as are the major chromosomes.

The fourth-chromosome recombination permitted by meiotic mutants is restricted to the euchromatin (our regions two and three) of chromosome *4*, even

though an analysis of X-ray-induced recombination showed that a majority of those events occurred in the centric heterochromatin (WILLIAMSON, PARKER and MANCHESTER 1970). This implies that the centric heterochromatin is achiasmate for some reason other than the regional recombination restriction revealed by meiotic mutants. Unpublished observations, kindly supplied by A. T. C. CARPENTER and B. S. BAKER, imply that this conclusion is not limited to chromosome *4*, but applies generally. They examined crossing over in the *car-bb* (partly euchromatic) and *bb*-centromere (entirely heterochromatic) regions of the *X* chromosome under the influence of a set of recombination-defective meiotic mutants and observed that the meiotic effects of these mutants did not include allowing recombination to occur between *bb* and the centromere.

Little can be said about the frequency with which recombination in chromosome *4* occurs under various circumstances, except that it varies widely. Thus, in triploids there were some 26 recombinants recovered per thousand progeny (STURTEVANT 1951), whereas about five for *mei-352*, near two (maximally) for heat-induction (GRELL 1971), approximately one under the influence of *mei-218*, and only 0.02 in the case of *mei-S282*. Similarly, there appear to be regional differences in recombination caused by the different meiotic mutants that also have no ready explanation.

The final matter that must be considered is whether the elevated frequency of fourth-chromosome nondisjunction that occurs in females homozygous for recombination-defective meiotic mutants is a direct consequence of the same defect that results in the recombinational abnormalities. For the genome in general, we may entertain three possibilities about the relationship between mutant-induced anomalies in crossing over and disjunction (for a detailed consideration of the arguments and relevant data, see BAKER and HALL 1976): (1) recombination-defective meiotic mutants have separate recombinational and disjunctional effects; (2) recombination-defective meiotic mutants affect some single property of meiosis that is neither recombination nor disjunction, but that secondarily affects both; and (3) recombination-defective meiotic mutants affect only exchange, the reduction in which secondarily (*e.g.*, by distributive disjunction) produces nondisjunction.

All existing data argue for the rejection of the first possibility; possibilities two and three are both tenable, although the third accommodates existing observations rather more easily than does the second. The chromosome *4* results presented here demand one additional assumption whichever of these possibilities is adopted. Under hypothesis two, *mei-9*^{b} must be assumed to affect a single process whose recombinational secondary effect is different from that of other recombination-defective meiotic mutants in that it does not result in fourth-chromosome crossing over, but whose disjunctional secondary effect is exactly like that of all other recombination-defective meiotic mutants. Under hypothesis three, on the other hand, the direct effect of recombination-defective meiotic mutants on fourth-chromosome crossing over exhibited here must be assumed to be causally unrelated to the effect of these same mutants on fourth-chromosome disjunction.

For a great deal of valuable assistance at various stages of this work, the authors are grateful to AVERIL ROSENFELD, LIZ HAMILTON-BYRD and DORI KOHAN and also to M. BUTLEY and R. TRENT. For reading the manuscript and making many useful suggestions, we are pleased to acknowledge our colleagues IAN DUNCAN, LARRY GOLDSTEIN, SCOTT HAWLEY and most especially ADELAIDE CARPENTER, BRUCE BAKER and BEN HOCHMAN.

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Corresponding editor: G. LEFEVRE

APPENDIX

The experiment described in this report and diagrammed in Figure 1 has been used to inquire whether inversion heterozygosity, long known to increase crossing over on chromosomes other than the inverted pair (the interchromosomal effect; for a recent review see LUCCHESI 1976),

promotes recombination on chromosome *4*. Accordingly, test females were constructed that were heterozygous for *In(2LR)SM1* and *In(3LR)TM2* (LINDSLEY and GRELL 1968), and crossing over on chromosome *4* was monitored.

The cross of *y/y*; *SM1/+*; *TM2/+*; *y+.pol/ci ey* females by *y/Y*; *pol/pol* males yielded 9174 *pol*, 9588 *y*, 12 wild-type, and 1 *y pol* progeny. Ten of the 12 wild-type offspring were successfully progeny tested and proved to be nondisjunctional exceptions. The *y pol* individual, on the other hand, resulted from a crossover in region 2 (between *ci* and *ey*); homozygotes for the recombinant chromosome were viable, fertile, and without morphological abnormalities.

Several previous studies have examined the effect of inversion heterozygosity on fourth chromosome crossing over. CURRY (reported by BRIDGES 1935) observed no recombination on chromosome *4* despite the presence of five heterozygous inversions in the parental females. SCHULTZ and REDFIELD (1951) also reported no fourth-chromosome crossing over in inversion heterozygotes. In another study, THOMPSON (1954) reported four crossovers between *ci* and *ey* among 392 progeny from females heterozygous for three inversions. However, no crossovers were recovered among 2361 progeny from a supposedly identical set of matings done later. In addition, no crossovers between *ci* and spa^{cat} were recovered among 5554 progeny from a related set of matings in which the parental females were heterozygous for three multiply inverted chromosomes (THOMPSON and SHUET-FAI WEI 1963). Finally, M. WILLIAMSON (unpublished) found several progeny that may have been recombinants among 19,601 progeny of *y+.pol/ci ey* females heterozygous for one or two multiply inverted chromosomes. These experiments were complicated by a possible translocation of *y+*, the appearance of a cluster of recombinants from a single female, and a lack of complete progeny testing.

In summary, although several of these experiments are suggestive that the interchromosomal effect promotes fourth-chromosome recombination, none is conclusive so that the matter must remain equivocal. This elusiveness is in part a consequence of the technical problems involved in these experiments, in part the result of the obviously very low frequency with which fourth-chromosome recombination occurs (if it occurs at all), and in part because chromosome *4* does recombine premeiotically which provides an inevitable confounding element in the experimental system.