THE ORIGIN OF MULTIPLE CROSSOVER CHROMATIDS IN SHORT GENETIC INTERVALS IN *DROSOPHILA MELANOGASTER*[1,2]

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INTERFERENCE in higher organisms such as Drosophila has been generally considered to be complete within genetic intervals of less than ten map units. The recovery of apparent double crossovers within shorter regions of the X chromosome in *Drosophila melanogaster* (REDFIELD 1955, 1957; GREEN 1960; BAILLIE, ASTELL and SCHOLEFIELD 1966) would appear to indicate that interference is not complete. However, such rare double crossovers could be generated by a mechanism which is independent of the degree of interference.

In Drosophila, pairing between homologs during mitosis may result in crossing over in somatic and gonial cells (STERN 1936; KAPLAN 1953; WHITTINGHILL 1955). WHITTINGHILL (1955) suggested that a single crossover occurring during meiosis in an oocyte derived from a cell in which a mitotic crossover had occurred previously could generate an apparent double crossover chromatid. This will be called a Two Step Mechanism for the production of double crossovers whereas doubles generated from genuine double exchange tetrads will be said to result from a One Step Mechanism. The two mechanisms are illustrated in Figure 1. The Two Step Mechanism has been proposed recently to explain high negative interference and gene conversion in microorganisms (HARTLEY and WHITTINGTON 1966).

The production of apparent double crossovers by the Two Step Mechanism predicts that such doubles would be associated with a cluster of single crossovers if the mitotic exchange occurred several divisions prior to meiosis (Figure 1). Initial attempts to verify this prediction were unsuccessful (SUZUKI 1958). Subsequently, W. M. HEXTER and his students (personal communication) were able to recover doubles at the tip of the X chromosome which appeared to be associated with clusters of single crossovers although the data were not convincing. The recovery of half tetrads by the use of attached-X chromosomes permits greater resolution of exchange events and has provided the evidence for the Two Step Mechanism presented in this report.

MATERIALS AND METHODS

The experiments were carried out in two parts: (1) crossing over was measured in the distal

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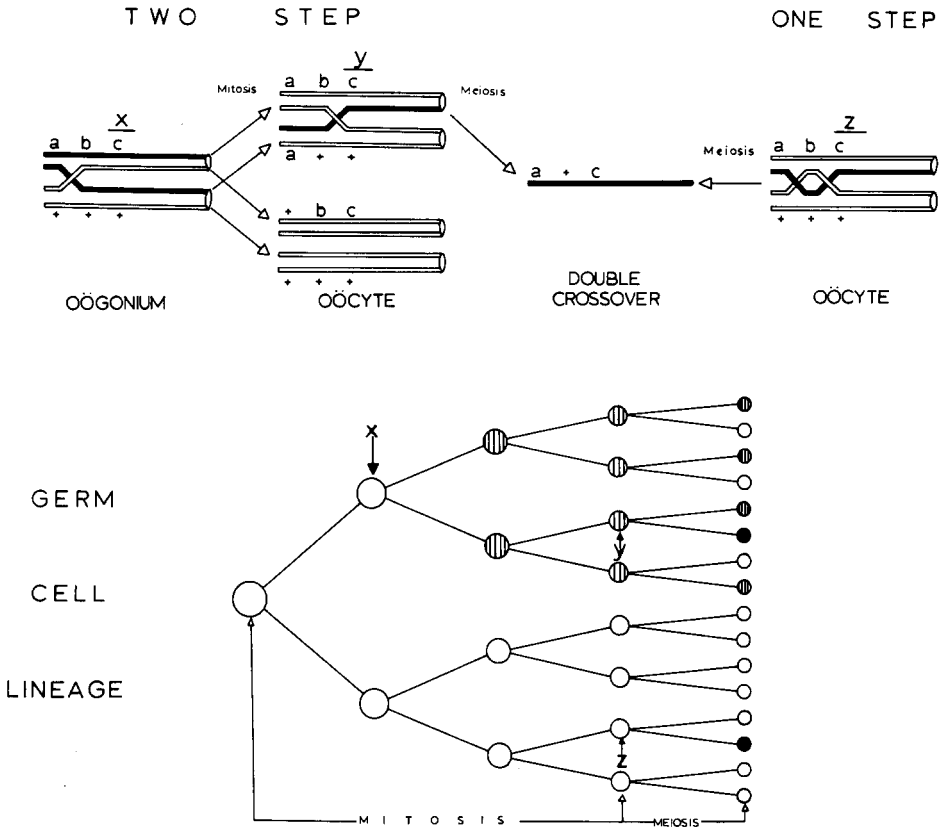

FIGURE 1.—Mechanism of generating double crossover chromosomes by One or Two Steps. ○-noncrossover, cross-lined circle-single crossover, ●-double crossover.

regions of rod X chromosomes in order to establish the genetic intervals between the markers tested, the effect of autosomal inversions on the crossover values and whether double crossovers can be recovered in that region; (2) crossing over was measured in attached-X chromosomes bearing the same markers tested in the rod X study.

The following X chromosome mutants followed by their map positions as listed by BRIDGES and BREHME (1944) were used to mark the chromosomes: *l(1)J1*—lethal (1) first of Jacobs-Muller 0.0, *y*—yellow 0.0+, *w*—white 1.5, *sp-w*—spotted-white 1.5, N^{264-40}—Notch$^{264-40}$ 3.0. The inseparable combinations *l(1)J1* y^+ and *l(1)J1*+ *y* will be referred to as y^+ and *y*, respectively. Thus, homozygosity for y^+ will be lethal. Note that *w* and *sp-w* are position pseudoalleles and interact in the *trans* combination to produce a dark brown eye, a phenotype easily distinguishable from the homozygous state of either allele.

Part 1—Rod X chromosomes: Single virgin females of the genotype *y w N*/+ *sp-w* + were crossed to three *y w spl*/Y males in vials for six days. Progeny of each female were recorded separately and the pooled data of female progeny only were used to calculate the standard crossover values. Crossing over in sibling females heterozygous for different combinations of the autosomal inversions *Cy* and Ubx^{130} was also measured.

Part 2—Attached-X chromosomes: The mutant combination selected for these studies allows the determination of genotype of every attached-X chromosome by the phenotype of the female since the phenotype of each combination of each pair of alleles is different. The laborious test mating of each female in order to determine the exact genotype of each arm by homozygosis

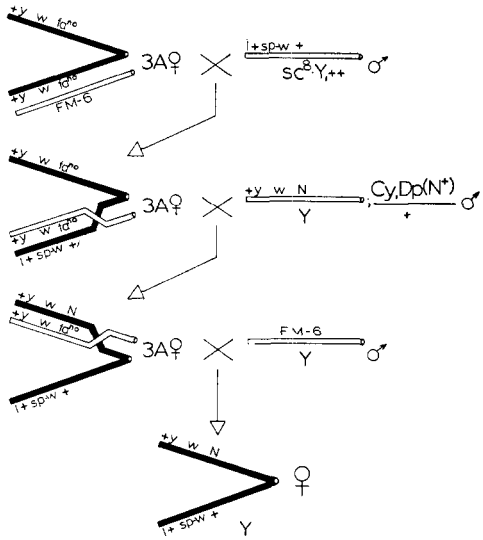

FIGURE 2.—Mode of synthesis of the attached-X chromosomes with the required markers. 3A-triploid, *l-l(1)J1*.

(WELSHONS 1955) is eliminated. Attached-X chromosomes with the same gene order in each arm as in the rod X chromosomes were generated from triploid females as shown in Figure 2.

Each attached-X-bearing female was mated singly to three Oregon-R males and her progeny recorded separately. All female offspring with wild-type eyes were mated and found to be detachments. Each female bearing an apparent multiple exchange chromosome was tested to verify the genotype. All sisters of such females were tested to detect any gonadal mosaicism in their mother which would be predicted by the Two Step Mechanism (WHITTINGHILL 1955).

The marker system used has the additional feature of constituting a selector system which eliminates all nonreciprocal exchanges between *N* and the centromere (region 3) owing to homozygosis for the lethal markers (Figure 3). Reciprocal exchanges between *y* and *w* (region 1) and *w* and *N* (region 2) produce chromosomes (transpose) phenotypically similar to noncrossover chromosomes but readily distinguishable by the high frequency of homozygosis when tested (Figure 4). Homozygosis values in transpose chromosomes permit an estimate of exchange frequencies in region 3.

RESULTS

The crossover values in regions 1 and 2 of rod X chromosomes are shown in Table 1. BAILLIE's (1966) values of 1.16 and 0.64 are in reasonable agreement with the values of 0.93 and 0.69 obtained by REDFIELD (1957). PARRY's (1965) crossover analysis was carried out shortly after the synthesis of the marked chromosomes and gave values significantly higher than those of REDFIELD and BAILLIE. Double crossovers were detected by REDFIELD (1957) and PARRY (1965) in the presence of autosomal inversions which increase crossing over (Table 1). BAILLIE (1966), on the other hand, recovered one double crossover in the control series and none in the presence of inversions. The heterogeneity of these results must reflect the sensitivity of the double crossover events to genotypic differences. Nevertheless, the tests indicated that apparent double crossovers can be detected with the markers tested.

The number of progeny of females carrying attached-X chromosomes with

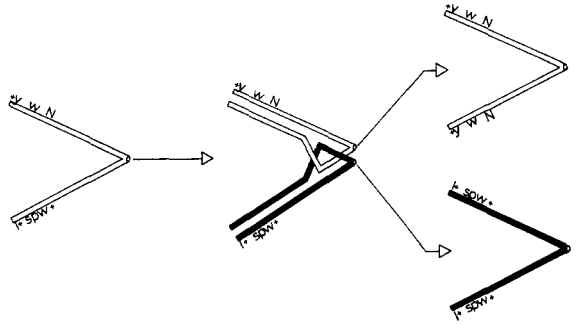

FIGURE 3.—Consequences of a nonreciprocal exchange between *N* and the centromere in attached-X chromosomes.

TABLE 1

Crossover values in the y-w-N *region of rod X chromosomes*

Investigator	Autosomal inversions		Crossover regions: *y-w* No.	*y-w* %	*w-N* No.	*w-N* %	*y-w-N* No.	Total progeny
D. PARRY (1965)	+	+	221	1.67	162	1.22	0	13,180
	Cy	+	151	2.39	123	1.95	2	6,393
	+	*Ubx*	659	3.79	159	0.91	1	17,432
	Cy	*Ubx*	162	4.68	138	3.94	1	3,512
D. BAILLIE (1966)	+	+	435	1.16	241	0.64	1	37,489
	Cy	+	398	2.50	185	1.16	0	15,932
	+	*Ubx*	470	2.16	219	1.00	0	21,802

the proper gene order is listed in each phenotypic class in the top row of Table 2. The total of 49,640 viable females scored represents a portion of the total gametic sample since *l(1)J1* and *N* homozygotes are not detected. In order to calculate

TABLE 2

Analysis of simple exchange tetrads in the attached-X chromosome

Tetrad type and crossover region	Phenotypic classes: NCO*	Lethal	*y sp-w/w N*	*y w N*	*+ sp-w +*	*+ sp-w/w +*	Multiple	Totals
Offspring scored:	48,224	...	483	289	406	225	13	49,640
Phenotypic ratios for each tetrad type:								
No exchange	1	..	..	..	..	..	..	0
Single exchange 1	2	1	1	..	..	..	..	1,120
Single exchange 2	2	1	..	1	..	..	..	708
Single exchange 3	1	1	..	..	..	..	..	90,576
Double exchange 1,3	2	3	1	..	2	..	..	1,624
Double exchange 2,3	2	3	..	1	..	2	..	900
				Total gametes sampled				94,928

* NCO = noncrossover.

crossover values in the attached-X chromosome, the total number of gametes sampled must be estimated. A tetrad analysis of the attached-X data was performed (WELSHONS 1955) and the ratios of chromosomes in each phenotypic class produced from tetrads of each simple exchange type are shown in Table 2. These estimates assume an absence of both sister chromatid exchange (BAKER and SWATEK 1965) and chromatid interference (WELSHONS 1955). This analysis provides an estimate of the number of lethal gametes and therefore the total number of gametes sampled from tetrads of each type (last column, Table 2). Nonreciprocal and reciprocal exchanges in region 3 produce lethal and noncrossover phenotypes, respectively. In order to determine the number of tetrads with a single exchange in region 3, the data from transpose chromosomes can be used. Homozygosis for one of the transposed arms by a nonreciprocal exchange in region 3 (Figure 4) yields viable offspring which provide a means of estimating the exchange frequency by a tetrad analysis. It is to be noted that only ¼ of all chromosomes from single exchange tetrads in region 3 are detected as viable exchange chromosomes since ¼ die and the remaining ½ is phenotypically indistinguishable from noncrossovers. From a total of 2,465 offspring of type a (Figure 4) transpose chromosomes, 858 were *y sp-w* + and of 2,054 offspring of type b transposes, 698 were *y w* +. A tetrad analysis of the transpose data indicates that the frequencies of nonreciprocal exchanges are 0.522 and 0.504 in transposes a and b, respectively. Thus it may be assumed that all noncrossover chromosomes in regions 1 and 2 undergo an exchange in region 3. On the basis of rod X crossover data, WEINSTEIN (1936) estimated that 94% of all X chromosome tetrads have an exchange.

The total number of gametes sampled (last column, Table 2) is estimated to be 94,928. Crossover values for each region were estimated by the simple formula:

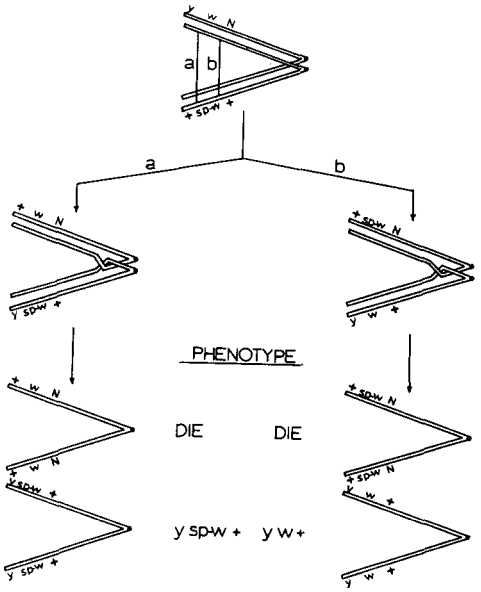

FIGURE 4.—Products of reciprocal exchanges in regions 1 and 2 and their homozygosis patterns.

TABLE 3

Offspring of females with gonads mosaic for noncrossover and crossover chromosomes

Mother	Number of each F_1 phenotype		Genotype of F_1 phenotypic noncrossovers
A	19 noncrossover 2 *y sp-w* +	—	2 transpose, 7 noncrossover (10 not fertile)
B	20 noncrossover 4 *y sp-w* +	—	4 transpose, 6 noncrossover (10 not fertile)
C	15 noncrossover 1 *y w* +	—	6 transpose, 2 noncrossover (7 not fertile)
D	13 noncrossover 1 *y w* +	—	6 transpose, 2 noncrossover (5 not fertile)

$\frac{SET/2 + DET/2}{Total\ Gametes} \times 100$, where SET and DET stand for the number of single and double exchange tetrads for each region. The crossover values in the attached-X chromosome were calculated to be 1.38 and 0.81 for regions 1 and 2, respectively, and are in remarkably good agreement with the values of 1.16 and 0.64 obtained in the rod X tests (Table 1). This agreement provides evidence that the basic assumptions underlying the tetrad analysis are valid.

Four females had progeny with apparent multiple crossover and noncrossover phenotypes (Table 3). The two crossover classes, *y sp-w* + and *y w* + are usually generated in females carrying transpose chromosomes and would require four-strand double exchanges in regions 1 and 2, respectively, if generated from non-transpose chromosomes by the One Step Mechanism. However, tests of the siblings of the crossover classes indicated that each female parent was actually a gonadal mosaic yielding gametes bearing transpose and nontranspose chromo-

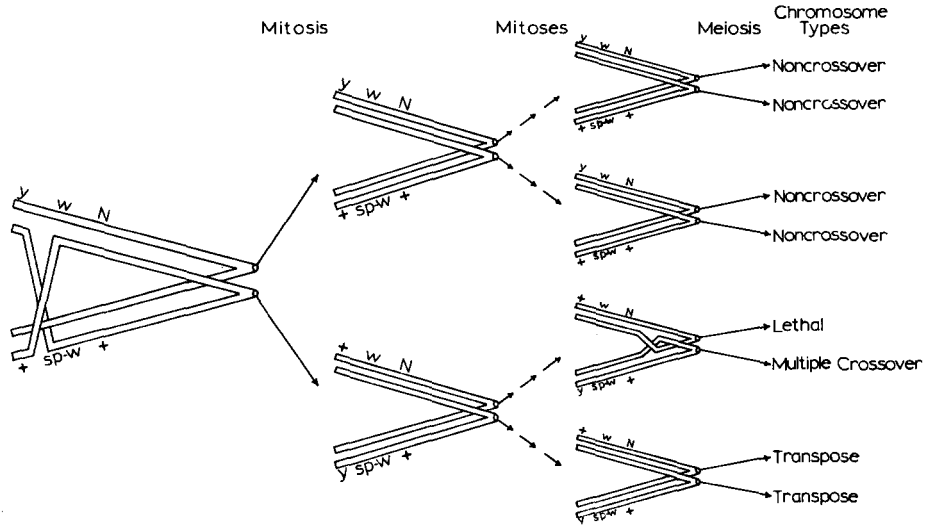

FIGURE 5.—The Two Step Mechanism for the formation of a mosaic gonad.

TABLE 4

Multiple exchange phenotypes recovered from nonmosaic females

Phenotype	Number
y sp-w +	3
y w +	3
y sp-w N	2
+ *w* +	1
+ *sp-w N*	2
+ *w N*	2
Total	13

somes (column 2, Table 3). This is precisely the prediction of a Two Step Mechanism (Figure 1) and indicates that an initial mitotic crossover in regions 1 or 2 of an oogonium was duplicated several times and produced a gonad which was mosaic for transpose and non-transpose chromosomes. A nonreciprocal crossover

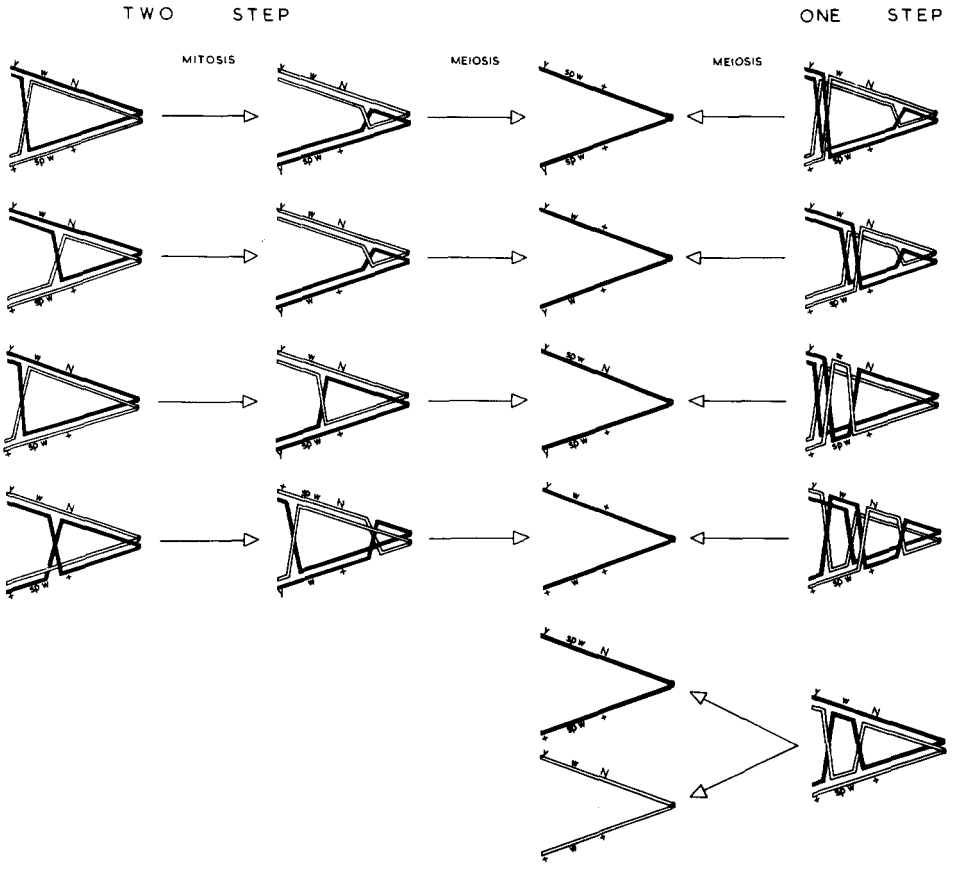

FIGURE 6.—Examples of the One and Two Step Mechanisms for generating the multiple exchange chromosomes recovered.

in region 3 in chromosomes carrying the original mitotic exchange will generate a chromosome with a multiple exchange phenotype. This mechanism is illustrated in Figure 5. Females which gave rise to multiple exchange classes and one genuine noncrossover to several transpose chromosomes were excluded from consideration since the mother could have inherited a transpose chromosome and a reciprocal exchange during meiosis could regenerate the noncrossover chromosome.

The phenotypes of 13 additional multiple crossover chromosomes in regions 1 and 2 are listed in Table 4. Each chromosome was recovered from a different parent in a brood in which all other fertile sisters were shown by further mating to be noncrossovers. The mode of generation of the 13 chromosomes by the One and Two Step Mechanisms is shown in Figure 6. The multiple exchange chromosomes fall into two categories: (1) the + *sp-w N* and + *w N* phenotypes (bottom lines, Figure 6) can only be generated by the One Step Mechanism and represent products of a genuine double exchange tetrad; and (2) the other four phenotypes can be generated by either mechanism, the Two Step comprising successive single crossovers in mitosis and meiosis, and the One Step requiring two or three crossovers within the *y-N* region. If it is indeed assumed that all of the chromosomes in Table 4 resulted from a One Step Mechanism, an analysis of the types and ratios of products from tetrads generating each crossover class can be made (Table 5). Since analysis of data from non-transpose (Table 2) and transpose chromosomes indicates that over half of all tetrads having an exchange in either region 1 or 2 have a second exchange in region 3, it will be assumed for simplicity that half of all double and triple exchange tetrads in regions 1 and 2 will also have an exchange in region 3. The analysis indicates that the *y sp-w* + and *y w* + phenotypic classes are unique to 1,1 and 2,2 double exchange tetrads.

TABLE 5

Analysis of multiple exchange tetrads presumed to generate multiple crossovers by the One Step Mechanism

	Tetrad types and crossover regions*				
	Doubles			Triples	
Phenotypic classes	1,1	1,2	2,2	1,1,2	1,2,2
Noncrossover	18	6	18	18	18
Lethal	8	8	8	18	18
y sp-w/w N	3	3	..	3	9
y w N	..	..	3	3	..
+ *sp-w* +	2	..	..	..	2
+ *sp-w/w* +	..	2	2	6	2
+ *sp-w N*	..	6	..	6	6
+ *w N*	..	6	..	6	6
y sp-w/w +	..	1	..	1	1
y w +	..	..	1	..	..
y sp-w +	1	..	..	..	..
y sp-w N	..	..	..	3	..
+ *w* +	..	..	..	..	2

* The ratios are based on the assumption that half of the tetrads have an exchange in region 3.

However, the + *sp-w N* and + *w N* classes may be generated from 1,2 double and 1,1,2 and 1,2,2 triple exchange tetrads. For every two *y sp-w N* chromosomes recovered, eight + *sp-w N* and + *w N* chromosomes should be detected and for every one + *w* +, six more are expected. Thus, from 1,1,2 and 1,2,2 triple exchange tetrads alone, 14 + *sp-w N* and + *w N* are expected whereas only four were actually recovered. The assumptions on which the tetrad analysis (Table 5) are based render a statistical comparison between the observed number of 4 and the expected number of 14 impossible. It does seem reasonable, however, in view of the high positive interference in short intervals to assume that the + *sp-w N* and + *w N* chromosomes came from 1,2 double exchange tetrads rather than tetrads of higher rank. The failure to detect an excess of these 1,2 double crossover chromosomes as predicted by the tetrad analysis can be taken as further evidence that the rare multiple exchange chromosomes shown in rows 1 to 4 (Figure 6) result from a Two Step Mechanism.

As a final point, it should be noted that no *y sp-w/w*+ chromosomes were recovered even though they would be expected to result from 1,2 double and 1,1,2 and 1,2,2 triple exchange tetrads at a low frequency (Table 5). The *y sp-w/w* + chromosome can also be generated by a Two Step Mechanism by a mitotic exchange in region 1 followed by a 2,3 double in meiosis. Undoubtedly the failure to detect the chromosome reflects the low frequency of its generation as predicted by either mechanism.

DISCUSSION

It has been shown that the multiple crossover phenotypes recovered in the attached-X chromosomes studied fall into two distinct classes. The first class (+ *sp-w N* and + *w N*) can only be meiotic in origin and cannot be generated by a Two Step Mechanism (Figure 6). The recovery of four chromosomes in this class (the number predicted on the assumption of no interference is 21.2) conclusively demonstrates that interference is not complete over short intervals but is highly positive. HEXTER (personal communication) has pointed out that this phenotypic class could also be generated by the mutation of *w* to *sp-w* or vice versa in noncrossover chromosomes. While this possibility has not been ruled out, it is not considered likely since it requires the mutation of one allele to another specific state with a high frequency. The occurrence of such mutations would further the Two Step Mechanism as a more likely explanation for the generation of multiples.

The second class of multiple exchange phenotypes can be generated by either the One or the Two Step Mechanisms (lines 1 to 4, Figure 6) and merits close inspection in order to determine whether its mode of origin can be defined. Two lines of evidence favor the Two Step Mechanism for the production of this class of chromosomes. (a) Some *y w* + and *y sp-w* + classes were recovered in clusters and in broods of offspring bearing transpose and noncrossover chromosomes (Table 3). This observation indicates mosaicism of the maternal gonad which is readily explained by the assumption of an oogonial crossover early in the cell lineage of the ovary (Figure 4). (b) On the assumption that the multiple ex-

change chromosomes do indeed result from a One Step process, the analysis of the tetrad types which generate *y sp-w N* and + *w* + phenotypes predicts the recovery of 14 + *sp-w N* and + *w N* chromosomes from such tetrads. If the tetrad analysis is valid, the deficiency in this class of chromosomes would indicate that the multiple exchange phenotypes are not generated in a single meiotic event. While neither of these lines of evidence constitute unequivocal evidence for the Two Step Mechanism, it is felt that, taken together, they are sufficiently convincing to merit the conclusion that such a mechanism does generate a considerable portion of apparent multiple crossover chromosomes.

One point of concern is the failure to recover a greater excess of *y w* + and *y sp-w* + double crossover phenotypes relative to the *y sp-w N* and + *w* + triple exchange classes. An initial reciprocal mitotic crossover in regions 1 or 2 can generate both the double and triple crossover chromosomes, the former requiring a meiotic crossover in region 3, the latter requiring an exchange in regions 1 or 2 (see Figures 5 and 6). Since the probability of the meiotic exchange occurring in region 3 is at least 50 times as great as the probability of an exchange in regions 1 or 2, a corresponding excess of *y w* + and *y sp-w* + phenotypes would be expected relative to the apparent triples. It is probable that many chromosomes in females generating *y w* + and *y sp-w* + offspring were scored as transposes even though they were, in fact, genuine gonadal mosaics. It can be seen (Table 3) that there is a high rate of sterility in testcrosses (compare the number of noncrossovers in the left column with the number of fertile offspring in the right column). We have found that transpose-bearing females are much more fertile than noncrossover females. Thus, the test system selects against the detection of noncrossover chromosomes and enhances the chance of a female being scored as a transpose. In addition, all females having only one noncrossover offspring in a brood of transpose sisters, were scored as transposes even though they might be extreme gonadal mosaics.

It is important to compare the relative frequencies of double crossover chromatids detected in the rod X and attached-X chromosome tests since it could be suggested that attachment of the X's results in crossover events not normally occurring in rod X's. This might predict that the frequency of double exchange tetrads would be higher in the attached-X chromosome. In the rod X tests, only one fourth of all double exchange tetrads are detectable as double crossover chromatids. Thus, the one double crossover recovered in 37,489 gametes from the rod X tests (line B, Table 1) indicates the occurrence of four double exchange tetrads in this sample. In the attached-X chromosome studies, four genuine double crossover chromosomes in regions 1 and 2 were recovered from 94,500 gametes. However, each gamete in the attached-X series represents the recovery of two chromatids from a meiotic tetrad; that is, the number of chromatids sampled is twice the number of gametes. Thus, 4/189,000 or one double crossover chromatid was recovered per 47,250 sampled chromatids in the attached-X series and one in 37,489 in the rod X tests. This comparison indicates an absence of any gross change in the frequency of double exchange tetrads resulting from attachment of the X chromosomes.

The senior author is deeply grateful to PROFESSOR WILLIAM M. HEXTER who fostered my interest in genetics and directed the first tests of the Two Step Mechanism. We wish to acknowledge the helpful suggestions of PROFESSORS WILLIAM K. BAKER and HEXTER on the manuscript.

SUMMARY

Experiments were designed to determine whether rare multiple crossovers in short genetic regions are generated in multiple exchange tetrads in meiosis or by a single meiotic crossover in a cell carrying a previously occurring mitotic crossover. Crossing over was measured between the markers *l(1)J1 y*$^+$ *sp-w* + and *l(1)J1*$^+$ *y w N* in each arm of rod and attached-X chromosomes. Crossover values in the rod X chromosomes were 1.16 and 0.64 in the *y-w* and *w-N* regions respectively. In 37,489 offspring scored, one double crossover was recovered. The autosomal inversions, *Cy* and *Ubx*^{130} increased crossing over in both regions.—Attached-X data indicated that in a gametic sample of 94,928, crossover values were 1.38 and 0.81 for the *y-w* and *w-N* regions. Four females yielding offspring carrying apparent multiple exchange chromosomes were gonadal mosaics for crossover and noncrossover chromosomes. In addition, 13 multiple exchange chromosomes were recovered from nonmosaic mothers. These chromosomes fell into two groups: the first group could only be generated by a genuine double exchange tetrad, the second could be generated either by multiple exchange tetrads in meiosis or by successive single crossovers in mitosis and meiosis. An analysis of the tetrads generating the multiple exchange types indicated a deficiency in certain expected classes. The results of the tetrad analysis and the observation of gonadal mosaics in females favor the model of generation of apparent multiple exchange chromosomes by successive mitotic and meiotic crossovers.—It is concluded that interference is positive, but not complete in short genetic regions and that apparent multiple crossover chromosomes may actually result by a Two Step Mechanism.

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