AUTOSOMAL HALF-TETRAD ANALYSIS IN *DROSOPHILA MELANOGASTER*[1]

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Received September 9, 1966

RECOMBINATION studies using tetrad or half-tetrad analyses are particularly valuable in investigating the reciprocality of crossover events. Until recent time, such investigations in Drosophila have been limited to half-tetrad analyses with reversed metacentric compound-X ("attached-X") chromosomes (see LINDSLEY and SANDLER 1963 for terminology). Thus, it is possible to construct compound-X chromosomes, heterozygous for a series of markers, and to study recombination by examination of the half-tetrad products of meiosis recovered in the daughters of females carrying such chromosomes. Classic notions concerning crossing over are based upon investigations of this sort (ANDERSON 1925; EMERSON and BEADLE 1935; BEADLE and EMERSON 1935; WELSHONS 1955). In recent years, recombination studies in a number of organisms have elaborated instances of nonreciprocality (see reviews by WESTERGAARD 1964; WHITEHOUSE and HASTINGS 1965). These deviations from the dogma of reciprocality in crossing over share one common feature, namely, that they involve studies of recombination between chromosomes labeled with very tightly linked genetic markers, i.e., separable sites within a cistron. Such cases, termed "conversions," may reflect important features of the process of crossing over, and consequently are the subject of numerous current investigations.

Half-tetrad analysis using compound-X chromosomes suffers from one major defect which makes systematic analysis of conversion in *Drosophila melanogaster* difficult, and perhaps impossible. Since conversions are expected to arise with low frequency in experiments involving very tight linkage, large scale sampling of meiotic products is essential. Following the synthesis in a single female of a compound-X chromosome heterozygous for a series of markers with a specific chromosomal distribution, meiosis with recombination will lead to the production of a genotypically heterogeneous population of compound-X females identical in phenotype. Subsequent experiments with such females requires single female matings and complete scoring of offspring phenotype in order to determine the chromosomal distribution of markers for each female parent. Such laborious practice seriously limits the usefulness of the compound-X system.

Recently, procedures have been developed for the construction of reversed metacentric compound chromosomes for all autosome arms in *Drosophila melanogaster* (RASMUSSEN 1960; LEWIS, personal communication). These stocks extend

[1] This investigation was supported by a research grant, GM 09886, from the Public Health Service.

half-tetrad analysis to all of the chromosomes of this species, and do not suffer the disadvantage described above for the compound-X system. The major feature of the compound-autosome stocks is illustrated in Figure 1 with compound-third chromosome arms. The figure shows parent females with compound-3L arms and compound-3R arms which are symbolized as C(3L); C(3R). Similarly, the male parent has C(3L′); C(3R′). The major classes of male and female progeny resulting from union of gametes produced by segregational meioses in both parents will be of two types: C(3L); C(3R′) and C(3L′); C(3R). The question of nonsegregation is discussed in a later section. If a specific C(3R′) chromosome is constructed carrying a series of heterozygous markers of known chromosomal distribution, then maintenance of the known chromosomal distribution of markers may be accomplished in mass cultures by passing this chromosome from generation to generation only through males. Large numbers of females may be produced, all carrying C(3R′), and these may serve as experimental material for half-tetrad analysis on a mass mating scale. Moreover, if one is able to impose upon the compound-autosome system, a system which selects for survival only progeny carrying interesting half-tetrads, then a useful tool for the systematic investigation of conversion is at hand. Just such an investigation is in progress in this laboratory, and involves half-tetrad analysis of compound-3R chromosomes. This study assumes that classic rules governing recombination, based upon half-tetrad analysis in single compound-X females, do apply to compound-3R half-tetrad analysis in mass matings. Consequently, an important preliminary to such an investigation would examine this assumption.

MATERIALS AND METHODS

The markers used in the half-tetrad analysis and their standard map positions (BRIDGES and BREHME 1944) are curled (*cu*:3–50.0), karmoisin (*kar*:3–52.0), ebony (*e*:3–70.7), and red (*red*:

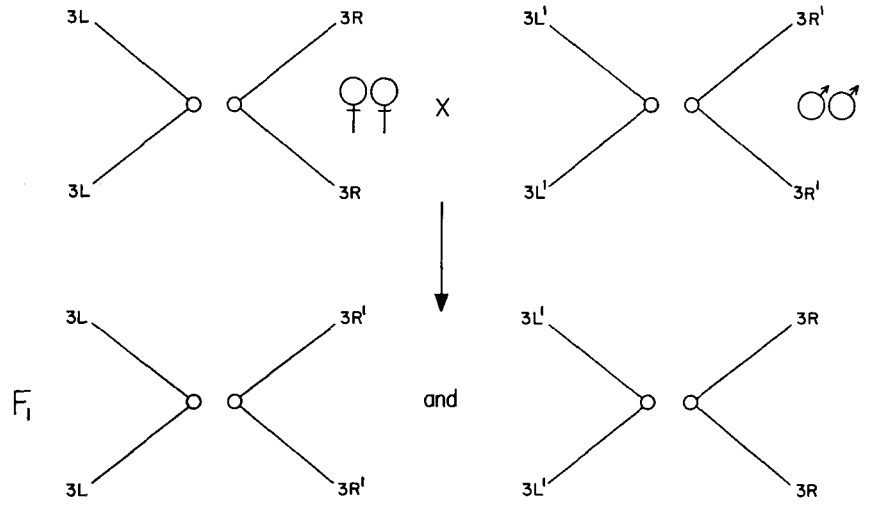

FIGURE 1.—Transmission in Compound-3 stock.

3–55.5) (OSTER 1954). Inversion (3L)Payne is an inversion in the left arm of chromosome 3 (BRIDGES and BREHME 1944), and *Cy, SM1* is a multiple break rearrangement involving most of chromosome 2 (LEWIS and MISLOVE 1953). Descriptions of other mutants used in this study may be found in BRIDGES and BREHME 1944. These mutants are: radius incompletus (*ri*), peach allele of pink (p^p), rosy (*ry*), glass (gl^3). The symbolism used to describe compound-autosomes conforms to that adopted for compound chromosomes in general (LINDSLEY, personal communication). Thus, C(3L)RM-P2, *ri* refers to a specific reversed metacentric compound-3L chromosome, designated Pasadena 2, and homozygous for the recessive mutant, radius incompletus.

The compound-3R chromosome used in this investigation was constructed by subjecting virgin females of the genotype *cu kar red e*/++++ to 4000r of X-irradiation, and then mating them to males which were C(3L)RM-P2,*ri*; C(3R)RM-SC1, *kar ry*. Surviving male progeny of the phenotype, *ri kar*+, *ry*+ carried newly synthesized C(3R) chromosomes. A separate stock was established from each male, but the present study was carried out with the derivatives of one such male.

All matings were carried out in half-pint milk bottles containing the standard cornmeal, molasses, yeast, and agar medium seeded with live yeast. Thirty virgin females, 12 to 24 hours old, were mated to 40 males of the tester stock, C(3L)RM-P2,*ri*; C(3R)RM-SB1, $p^p gl^3$, and permitted to lay eggs for three days. Parents were then transferred to fresh food bottles, and subsequent transfers were carried out every two days to provide a total of five broods. Cultures were incubated at 25 ± 1°C.

The use of males from the tester stock, C(3L)RM-P2,*ri*; C(3R)RM-SB1,$p^p gl^3$, made it possible to distinguish and discard those flies which received a compound-3R from their fathers. All other flies were then scored. All wild-type flies and certain classes of doubles with a recessive phenotype were scored and progeny tested. All other flies with a recessive phenotype were scored and then discarded. Each female to be progeny tested was mated with five males of the tester stock, and her offspring were scored. Each male to be progeny tested was mated to three tester stock virgin females. Two or three of his daughters, carrying the appropriate C(3R) chromosome, were mated in each of two vials with approximately five tester stock males. Because it was possible to test from four to six daughters of each progeny test male, the data gathered for males is generally more complete and thus more accurate than that for females.

Half-tetrad analysis of recombination in the compound-3R chromosome, *cu kar red e*/+++++, was carried out in the presence and absence of heterozygous, heterologous chromosome rearrangements. Experiments were conducted with four different female genotypes described below. The abbreviated notation in brackets will be used throughout this report.

1. +/+; C(3L)RM-P2, *ri*. [+;+] No rearrangements.
2. +/+; C(3L)RM-P5, + · *In(3L)Payne, ve h th* · +++. [+;*In(3L)Payne*] Inversion (3L)Payne present.
3. *Cy, SM1*/+; C(3L)RM-P2, *ri* [*Cy*;+] *Cy, SM1* present.
4. *Cy, SM1*/+; C(3L)RM-P5, + · *In(3L)Payne, ve h th* · +++. [*Cy,In(3L)Payne*] Both rearrangements present.

In the sections that follow the reversed metacentric compound autosomes will be referred to as attached-autosomes.

RESULTS AND DISCUSSION

The general features of recombination elaborated from classical studies with single attached-X females may be summarized as follows: 1. Crossing over occurs at the tetrad stage, and each exchange involves only two of the four chromatids. 2. Single exchanges occur at random between nonsister chromatids. 3. The frequency of homozygosis increases as a function of distance from the centromere. 4. There is no chromatid interference.

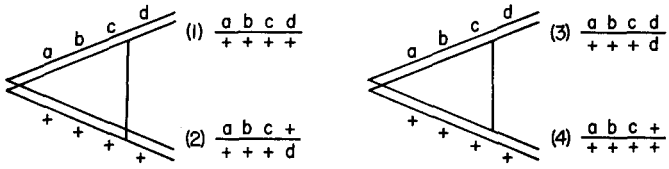

FIGURE 2.—Single exchanges in C(3R).

Following this model, we may consider the recombination results of mass matings of heterozygous, C(3R) females.

Single exchanges: Exchanges which occur in tetrads of attached-autosomes may be categorized as either reciprocal or nonreciprocal (Figure 2). A reciprocal exchange is one which occurs between two nonsister chromatids which are destined to be attached to the same centromere during the second meiotic division, while nonreciprocal exchanges involve nonsister chromatids which will be attached to different centromeres during the second meiotic division.

Both exchange types result in specific half-tetrad products, as illustrated in Figure 2. A reciprocal exchange yields two heterozygous attached-autosomes, one being indistinguishable from a nonexchange attached-autosome (Type-1), and one which is clearly an exchange attached-autosome (Type-2). In Type-2 there has been an exchange of markers between the two arms to the right of the crossover. On the other hand, a non-reciprocal exchange yields two distinguishable exchange attached-autosome classes, both of which are homozygous for the markers to the right of the crossover—in one case it is the recessive allele which is homozygous (Type-3) and in the other case the wild-type allele (Type-4).

If crossing over occurs in the tetrad stage, and exchanges occur at random

TABLE 1

Phenotypic frequencies of offspring of C(3R), cu kar red e/++++ *mothers with and without heterologous rearrangements*

Offspring phenotype	[+;+]	Heterologous rearrangements in mothers [+;*In(3L)Payne*]	[*Cy*;*In(3L)Payne*]	[*Cy*;+]
cu kar red e	50	49	51	32
kar red e	25	32	42	23
red e	51	51	65	28
e	266	244	290	131
cu kar red	23	15	38	23
cu kar	3	3	4	0
cu	1	0	0	1
kar red	8	7	17	4
red	4	2	11	10
+	2477	2551	2358	1113
Total	2908	2954	2876	1365

between nonsister chromatids, progeny tests of the wild-type offspring should reveal equal numbers of Type-2 and Type-4 flies. It is assumed that failure of a progeny test of wild-type offspring due to death without reproduction occurs at random. This assumption is implied in all ratios examined. Table 1 lists the phenotypic classes scored in each experiment. Table 2 presents the results of successful progeny tests carried out on male and female wild-type F_1. The number of Type-2 and Type-4 progeny for each region for all four experiments is taken from Table 2 and presented for comparison in Table 4. In all cases, the expected 1:1 ratio is found, supporting the conclusion that recombination occurs in the tetrad stage, and takes place at random between nonsister chromatids in the presence as well as the absence of heterologous rearrangements.

Double exchanges: Figure 3, which has been adapted from a diagram by WELSHONS (1955), illustrates the possible types of double-exchange products which occur when the first exchange is reciprocal and when it is nonreciprocal. The first exchange is taken as the exchange closest to the centromere. Once the first exchange has been determined, the second exchange can occur in such a way as to form a 2-strand, 3-strand, or 4-strand double. In the absence of chromatid interference, 2-, 3-, and 4-strand doubles should occur in a ratio of 1:2:1.

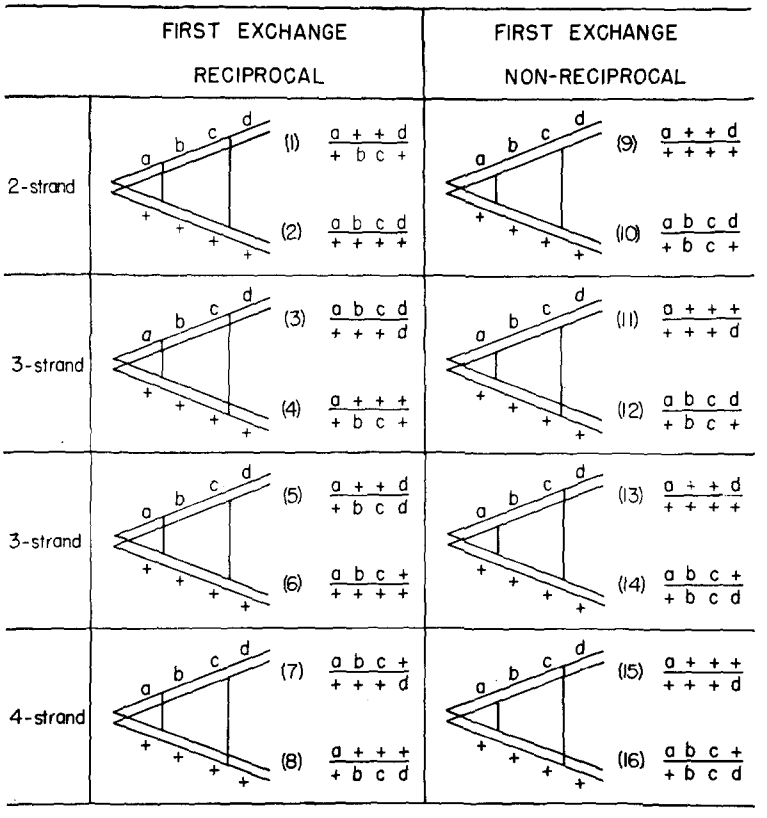

FIGURE 3.—Double exchanges in C(3R).

TABLE 2

Progeny test classification of wild-type offspring

Genotype	[+;+]	[+;*In(3L)Payne*]	[*Cy*;*In(3L)Payne*]	[*Cy*;+]
cu kar red e / + + + +	1065	1275	964	437
+ + + + / + + + +	21	17	18	10
cu + + + / + + + +	20	15	15	12
cu + + + / + *kar red e*	25	16	13	18
cu kar + + / + + + +	28	19	25	28
cu kar + + / + + *red e*	35	21	20	28
cu kar red + / + + + +	252	193	221	124
cu kar red + / + + + *e*	256	189	196	120
+ + + *e* / + + + +	15	12	20	17
+ + *red e* / + + + +	3	2	1	1
cu + + *e* / + + + +	3	3	4	5
cu + + + / + + + *e*	2	2	4	6
cu + + *e* / + *kar red* +	3	1	1	3
cu + + + / + *kar red* +	3	0	2	3
cu kar + *e* / + + + +	4	4	1	1
cu kar + + / + + + *e*	4	5	4	2
cu kar + *e* / + + *red* +	2	0	2	3
cu kar + + / + + *red* +	3	1	3	2
+ + *red* + / + + + +	0	1	0	0
Total	1744	1776	1514	820

TABLE 3

Progeny test classification of wild-type male offspring

Genotype	[+;+]	[+;*In(3L)Payne*]	[*Cy*;*In(3L)Payne*]	[*Cy*;+]
$\frac{cu\ kar\ red\ e}{+\ +\ +\ +}$	717	837	588	283
$\frac{+\ +\ +\ +}{+\ +\ +\ +}$	21	17	18	10
$\frac{cu\ +\ +\ +}{+\ +\ +\ +}$	15	11	8	5
$\frac{cu\ +\ +\ +}{+\ kar\ red\ e}$	20	9	6	13
$\frac{cu\ kar\ +\ +}{+\ +\ +\ +}$	18	8	14	21
$\frac{cu\ kar\ +\ +}{+\ +\ red\ e}$	28	14	12	19
$\frac{cu\ kar\ red\ +}{+\ +\ +\ +}$	137	110	103	71
$\frac{cu\ kar\ red\ +}{+\ +\ +\ e}$	149	98	95	86
$\frac{+\ +\ +\ e}{+\ +\ +\ +}$	15	12	20	17
$\frac{+\ +\ red\ e}{+\ +\ +\ +}$	3	2	1	1
$\frac{cu\ +\ +\ e}{+\ +\ +\ +}$	1	1	2	3
$\frac{cu\ +\ +\ +}{+\ +\ +\ e}$	0	1	4	5
$\frac{cu\ +\ +\ e}{+\ kar\ red\ +}$	1	1	1	1
$\frac{cu\ +\ +\ +}{+\ kar\ red\ +}$	2	0	2	1
$\frac{cu\ kar\ +\ e}{+\ +\ +\ +}$	4	2	1	1
$\frac{cu\ kar\ +\ +}{+\ +\ +\ e}$	0	2	4	2
$\frac{cu\ kar\ +\ e}{+\ +\ red\ +}$	1	0	2	2
$\frac{cu\ kar\ +\ +}{+\ +\ red\ +}$	0	1	1	0
$\frac{+\ +\ red\ +}{+\ +\ +\ +}$	0	1	0	0
Total	1132	1127	882	541

TABLE 4

Regional frequencies of Type 2 and Type 4 single-exchange progeny among both male and female offspring (Table 2)

Heterologous rearrangements		centromere-*cu*	*cu-kar*	*kar-red*	*red-e*	Total
[+;+]	Type-2		25	35	256	316
	Type-4	21	20	28	252	300
	Total		45	63	508	616
[+;*In(3L)Payne*]	Type-2		16	21	189	226
	Type-4	17	15	19	193	227
	Total		31	40	382	453
[*Cy*;*In(3L)Payne*]	Type-2		13	20	196	229
	Type-4	18	15	25	221	261
	Total		28	45	417	490
[*Cy*;+]	Type-2		18	28	120	166
	Type-4	10	12	28	124	164
	Total		30	56	244	330

It should be understood that Figure 3 illustrates only one combination of double exchanges between the four gene markers. In the following discussion of double exchanges, all combinations of double exchanges between the gene markers are included in the calculations.

Considering doubles in which the first exchange is reciprocal, there are only two groups of wild-type progeny which are unique in classification. These are *a*++*d*/+*bc*+ and *a*+++/+*bc*+ (Types 1 and 4 in Figure 3). The remaining products show a recessive phenotype or would be scored as single exchanges or non-exchanges. If the frequency of 2-strand doubles is equal to one-half the frequency of 3-strand doubles, then Type-1 and Type-4 should occur in a ratio of 1:1. The first two columns of Table 5 contain the pertinent data, indicating good agreement with the expected 1:1 ratio in all experiments.

Now consider those doubles in which the first exchange is nonreciprocal. Types (9) and (13) are identical genotypes, *a*++*d*/++++, as are Types (11) and (15), *a*+++/+++*d*. Types (9) and (13) will subsequently be referred to as Class A doubles and Types (11) and (15) as Class B doubles. If the frequency

TABLE 5

Summary of doubles found by progeny testing wild-type offspring (Table 2)

	First exchange reciprocal			First exchange nonreciprocal		
Heterologous rearrangements	Type-1	Type-4	Total	Class-A	Class-B	Total
[+;+]	5	6	11	7	6	13
[+; *In(3L)Payne*]	1	1	2	7	7	14
[*Cy*; *In(3L)Payne*]	3	5	8	5	8	13
[*Cy*;+]	6	5	11	6	8	14
Total	15	17	32	25	29	54

of 2-strand doubles is equal to the frequency of 4-strand doubles, then Class A and Class B doubles should occur in a ratio of 1:1. The data (Table 5, Columns 4 and 5) show that 7 Class A and 6 Class B doubles were found in progeny tests of the nonrearrangement females. The presence of the heterologous rearrangements did not alter this ratio. One further ratio should be considered. Doubles in which the first exchange is reciprocal (Type-1 + Type-4) and doubles in which the first exchange is nonreciprocal (Class-A + Class-B) should occur in a ratio of 1:2. Examination of these data in Table 5 indicates that there is no significant difference from the expected ratio in any of the experiments, either singly or pooled.

Consider next the visibly mutant F_1 (Table 1) which arose from doubles in which the first exchange is nonreciprocal. Inspection of Figure 3 will show that Types (10) and (12) are identical genotypes (*abcd*/+*bc*+) as are Types (14) and (16) (*abc*+/+*bcd*). Moreover, all of these show the recessive phenotype *bc*. Types (10) and (12) will be called Class C doubles and Types (14) and (16) are called Class D doubles. Class C and Class D doubles should be found with equal frequency, if the frequency of 2-strand doubles is equal to the frequency of 4-strand doubles. Expand this argument to consider doubles in which the first exchange is nonreciprocal for all combinations of double exchanges in the regions between curled (*cu*) and ebony (*e*). Three mutant phenotypic classes are expected (*kar*, *kar red*, and *red*). Of these, *kar* offspring were not found (absence of doubles between *cu* and *red*). The remaining two phenotypic classes, kar red and red flies were progeny tested and classified as either Class-C or Class-D doubles. The results of the successful progeny tests of these mutant classes of doubles, summarized in Table 6, indicate that the two classes occur in a 1:1 ratio.

Pooling the wild-type (Table 5) and visibly mutant (Table 6) flies which arose from doubles in which the first exchange is nonreciprocal, permits still another ratio to be examined. Class A + C and Class B + D doubles should occur with equal frequency, if the frequency of 2-strand doubles is equal to the frequency of 4-strand doubles. The pooled data summarized in Table 7 indicates that a 1:1 ratio of these pooled classes was found among the progeny of rearrangement and nonrearrangement mothers.

TABLE 6

Results of progeny testing doubles with a recessive phenotype

Heterologous rearrangements	Class C		Class D	
	cu kar red e / + *kar red* +	*cu kar red e* / + + *red* +	*cu kar red* + / + *kar red e*	*cu kar red* + / + + *red e*
[+;+]	2	2	3	2
[+;*In(3L)Payne*]	3	1	4	1
[*Cy*;*In(3L)Payne*]	8	6	7	5
[*Cy*;+]	1	5	3	4
Total	14	14	17	12

TABLE 7

Pooled data of wild-type and mutant doubles with a nonreciprocal first exchange

Heterologous rearrangements	Classes A+C	Classes B+D
[+;+]	11	11
[+;*In(3L)Payne*]	11	12
[*Cy*;*In(3L)Payne*]	19	20
[*Cy*;+]	12	15
Total	53	58

In summary, the analysis of double exchange progeny presented in Tables 5, 6, and 7 shows no indication of chromatid interference.

Calculation of crossover frequencies: The information in Tables 1 and 2 may be used to estimate crossover frequencies, and these calculations are presented in Table 8. For each region between genetic markers the frequencies were estimated in the following manner. The number of chromatids with an exchange in the region of interest is determined from both mutant (Table 1) and wild-type offspring (Table 2). The number of exchanges obtained from the wild-type offspring was adjusted for those progeny tests that failed by multiplying the observed exchanges by the total of wild-type progeny (Table 1) and dividing by the number of successful progeny tests. Thus, for the *cu–kar* interval in non-rearrangement mothers, the number of exchange chromatids scored in wild-type progeny is 87 (Table 2). Adjusting for the unsuccessful progeny tests leads to an estimate of (87)(2477/1744). To this is added 34 exchange chromatids observed in mutant progeny (Table 1) to yield a total of 157.5 exchange chromatids observed in the interval *cu–kar* in a total of 2908 progeny. Since each offspring represents two chromatids assayed, the frequency of recombination in the interval is obtained by 157.5/[(2908)(2)]. The crossover frequency for the region from the centromere to curled represents a special case, and is obtained in the following manner. The number of crossover progeny among the mutants may be read directly from Table 1. However, due to a bias in the data from wild-type female progeny (discussed in a later section), estimation of the number of wild-type offspring representing crossovers in this region is obtained by considering

TABLE 8

Percent crossing over in C(3R) chromosomes

Heterologous rearrangements	centromere-*cu*	*cu-kar*	*kar-red*	*red-e*
[+;+]	5.6	2.7	3.9	24.9
[+;*In(3L)Payne*]	4.7	2.0	2.8	19.1
[*Cy*;*In(3L)Payne*]	6.9	2.5	3.6	24.0
[*Cy*;+]	8.3	4.6	6.3	26.6
Standard	2.5*	2.0	3.5	15.2

*Estimated at one-half the distance between adjacent markers, *ri-p*^{p}.

the wild-type male progeny tests (Table 3) as the only successful tests. The number of crossovers observed in the male tests is then adjusted for the total of wild-type offspring tested. The sum of mutant crossovers (Table 1) and the adjusted number of wild-type crossovers (Table 3) is divided by the total progeny to yield the crossover frequency. Thus for nonrearrangement mothers, the crossover frequency for the region from centromere–*cu* is estimated as [77 + (39) (2477/1132)]/2908 or 5.6%.

A graphic summary of the recombination data of Table 8 is presented in Figure 4. The percent increase or decrease in recombination (relative to the standard map distances) is plotted for all regions for each of the four genotypes studied. Several points of interest are to be noted in the recombination data. (1) Recombination in the C(3R) chromosome, in the absence of heterologous rearrangements, is greater in all regions studied than the standard map distances obtained from free-third studies. (2) The presence of the second-chromosome multiple break rearrangement, *Cy*, *SM1*, leads to a considerable increase in recombination in the C(3R) chromosome in all regions studied. (3) In contrast, the presence *In(3L)Payne* in C(3L) is associated with a decrease in recombination in C(3R) in all regions. This decrease may be seen in two sets of comparisons. (A) Using the recombination data without rearrangements as a standard

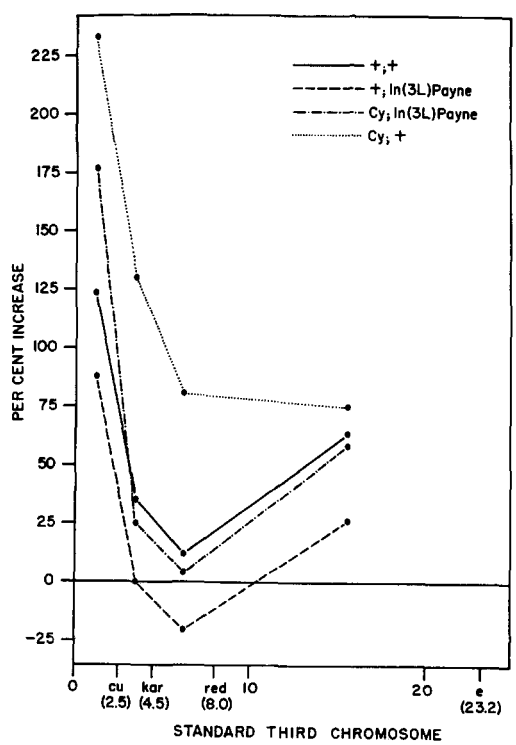

FIGURE 4.—Pattern of recombination in C(3R) with and without heterologous rearrangements, compared to standard map distances.

TABLE 9

Summary of crossover and noncrossover chromatids

Parents	Noncrossover		Single crossover		Double crossover		Total
	Number	Percent	Number	Percent	Number	Percent	
[+;+]	2497	71.59	968	27.75	23	0.67	3488
[+;*In(3L)Payne*]	2833	79.76	708	19.93	11	0.31	3552
[*Cy*;*In(3L)Payne*]	2251	74.34	761	25.13	16	0.53	3028
[*Cy*;+]	1082	65.97	535	32.62	23	1.41	1640

compared to the data obtained from mothers carrying *In(3L)Payne*. (B) Using the recombination data in the presence of *Cy, SM1* as a standard compared to the data obtained with both *Cy, SM1* and *In(3L)Payne*.

In order to determine how significantly the three rearrangement experiments differed from the nonrearrangement experiment, the data in Table 2 were regrouped as noncrossover, single crossover, or double crossover chromatids. Each chromatid was considered individually and independently of the others. A summary of these data will be found in Table 9. Inversion (3L)Payne shows a decrease in the classes of single and double crossovers, while *Cy, SM1* increases these two classes. A homogeneity test was performed between each rearrangement experiment and the normal attached-3R, and the results are given in Table 10. The decrease found with *In(3L)Payne* and the increase with *Cy, SM1* are both significant while the distribution of crossover and noncrossover chromatids in the *Cy, SM1*; *In(3L)Payne* experiment does not differ from the nonrearrangement attached-3R. Since the female data may introduce a bias into the calculation of homozygosis of wild-type alleles (see discussion below), the data

TABLE 10

Summary of homogeneity tests performed on crossover and noncrossover chromatids

Parents	Noncrossover	Single crossover	Double crossover	Total	P
[+;+]	2497	968	23	3488	
[+;*In(3L)Payne*]	2833	708	11	3552	
Total	5330	1676	34	7040	
χ^2	15.52	45.19	4.51	65.22	< 0.01
[+;+]	2497	968	23	3488	
[*Cy*;*In(3L)Payne*]	2251	761	16	3028	
Total	4748	1729	39	6516	
χ^2	1.68	4.20	0.46	6.34	0.05
[+;+]	2497	968	23	3488	
[*Cy*;+]	1082	535	23	1640	
Total	3579	1503	46	5128	
χ^2	5.03	9.02	6.89	20.94	< 0.01

TABLE 11

Comparison of the regional distribution of exchange classes

Parents	centromere-*cu*	*cu-kar*	*kar-red*	*red-e*	Total	P
[+;+]	116	90	137	848	1191	
[+;*In(3L)Payne*]	99	76	109	679	963	
Total	215	166	246	1527	2154	
χ^2	0.16	0.08	0.02	0.04	0.30	0.95
[+;+]	116	90	137	848	1191	
[*Cy;In(3L)Payne*]	132	98	136	814	1180	
Total	248	188	273	1662	2371	
χ^2	1.31	0.41	0.00	0.44	2.16	0.50
[+;+]	116	90	137	848	1191	
[*Cy*;+]	84	75	103	454	716	
Total	200	165	240	1302	1907	
χ^2	1.69	4.37	2.96	3.97	12.99	0.005

from males alone were classified into noncrossover and crossover chromosomes and tested for homogeneity with the combined data. No difference was found in any of the four experiments.

Another question which may be raised concerns the distribution of exchanges along the marked portion of the attached-3R. Table 11 summarizes homogeneity test comparisons of the crossover class distributions of mutant (Table 1) and wild-type (Table 2) progeny. Taking the progeny of nonrearrangement mothers as a standard, only the *Cy, SM1* experiment yielded a significant change in the distribution of crossovers.

Homozygosis of recessive and wild-type alleles: A major expectation of tetrad and half-tetrad analysis is that the percent homozygosis of each marker should increase with distance from the centromere. That this does indeed occur with attached-thirds can be seen in Table 12. The percent recessive homozygosis was calculated by totaling the number of times a recessive marker appeared, and dividing by the total number of flies scored. Thus, in the absence of rearrangements in female parents, there are 77 cases listed in Table 1 in which the cu phenotype appears. Since there were 2908 offspring, the percent recessive homozygosis for *cu* is $(77/2908) \times 100$ or 2.65%.

TABLE 12

Percent homozygosis of recessive and wild-type alleles

Parents	*cu*	*cu*+	*kar*	*kar*+	*red*	*red*+	*e*	*e*+
[+;+]	2.6	1.9	3.7	3.1	5.5	4.7	13.5	16.0
[+;*In(3L)Payne*]	2.3	1.6	3.6	2.5	5.3	3.7	12.7	12.0
[*Cy;In(3L)Payne*]	3.2	2.1	5.3	3.4	7.8	4.9	15.6	15.4
[*Cy*;+]	4.1	2.8	6.0	5.1	8.8	8.1	15.7	17.8

Calculation of the percent homozygosis for the wild-type allele is more difficult. Considering the nonrearrangement females in Table 2 there are 39 cases of *cu*+ in 1744 successful progeny tests. Adjusting for those wild-type flies which were unsuccessful in progeny tests, the percent homozygosis for *cu*+ among all flies is estimated by $(39/1744)\times(2477/2908)\times 100$ or 1.9%. Table 12 presents the percent homozygosis for both wild-type and recessive alleles.

It is evident from Table 12 that the percent homozygosis for the wild-type alleles is lower than that for the recessive alleles of those markers close to the centromere. The only explanation which can be offered for this discrepancy is that a bias was introduced by using the data gathered from testing wild-type females. Since each female produced relatively few progeny, determination of the genotype of a phenotypically wild F_1 female was often impossible. This difficulty is greater for markers closer to the centromere. In contrast, the data from wild F_1 males is more reliable. Each such male was mated, and several of his daughters were progeny tested to determine his genotype. Thus, the size of sample of progeny tests of each wild-type male permitted easy classification. In order to determine whether the data from females introduced a bias into the results, the male data alone (Table 3) were used to calculate the percent homozygosis of wild-type alleles. These results, presented in Table 13, show close agreement for homozygosis of wild-type and recessive alleles, and support the contention that the female data did bias the results.

Meiotic segregation of C(3L) and C(3R) chromosomes: In the introduction, discussion of transmission of attached-autosomes was restricted to a consideration of the classes of offspring produced by gamete unions resulting from meiotic segregation of C(3L) and C(3R) chromosomes (Figure 1). In two of the present experiments the C(3L) and C(3R) chromosomes were differentially marked in the parents, thus providing an opportunity to observe segregation. These data are presented in Table 14.

In both cases the male parent was the tester stock in which C(3L) is marked by the mutant phenotype, radius incompletus (*ri*), and C(3R) is homozygous for p^p gl^3. The female parent in both experiments carried the C(3L) heterozygous for *In(3L)Payne* and was ri^+, while the C(3R) chromosome was the subject of the recombination analysis. The two experiments differed in that the females, in one experiment, were heterozygous for the second chromosome rearrangement, *Cy,SM1*. Several features of interest are to be noted in Table 14. In ad-

TABLE 13

Percent homozygosis of wild-type alleles using data from males only

Parents	*cu*+		*kar*+		*red*+		*e*+	
	Number	Percent	Number	Percent	Number	Percent	Number	Percent
[+;+]	39	2.9	55	4.1	74	5.6	193	14.5
[+;*In(3L)Payne*]	32	2.4	45	3.4	54	4.1	148	11.3
[*Cy*;*In(3L)Payne*]	39	3.6	53	4.9	71	6.6	146	13.6
[*Cy*;+]	28	4.2	41	6.2	64	9.7	108	16.3

TABLE 14

Segregation of C(3L) and C(3R) chromosomes in crosses of C(3L), + · In(3L)Payne; *C(3R),* cu kar red e/++++ *females to males of the tester stock, C(3L),*ri; *C(3R),*p^p gl^3

Chromosome 2 in mothers	Brood No.	Segregational offspring		Nonsegregational offspring		Total
		ri^+; p^p gl^3	ri; $(p^p$ $gl^3)^+$	ri^+; $(p^p$ $gl^3)^+$	ri; p^p gl^3	
+/+	1	672	702	12	4	1390
	2	526	553	3	4	1086
	3	428	496	6	1	931
	4	672	688	3	4	1367
	5	465	487	4	3	959
	Total	2763	2926	28	16	5733
Cy, SM1/+	1	338	447	83	22	890
	2	423	555	113	32	1123
	3	467	553	64	31	1115
	4	356	449	52	31	888
	5	378	514	45	16	953
	Total	1962	2518	357	132	4969

dition to the two classes of progeny reflecting meiotic segregation of the C(3L) and C(3R) chromosomes, both experiments yielded two classes of offspring which could only result from nonsegregational meioses in both parents. Thus, the $ri^+(p^p\ gl^3)^+$ class are matroclinous for both C(3L) and C(3R), and the *ri*, p^p, gl^3 class are patroclinous for these chromosomes. Of particular interest is the more than tenfold increase in the frequency of nonsegregational offspring occasioned by the introduction of *Cy, SM1* into the maternal genotype. Assuming a constant frequency of nonsegregational meioses in the male parent common to both experiments, and in view of the fact that the introduction of *Cy, SM1* to the maternal genotype led to no significant drop in fertility, it is suggested that there is a high frequency of nonsegregational meioses in the attached-3 males. Since nonsegregational progeny were not reported in other extensive experiments (McCLOSKEY 1966), it is inferred that nonsegregational meioses in females is rare in the absence of chromosome rearrangements.

Examination of the distribution of nonsegregational offspring in both experiments reveals two other points of some interest. In both experiments the matroclinous progeny occur with greater frequency, and in the *Cy, SM1* experiment the discrepancy is most significant. The second point concerns homogeneity of the data on frequency of nonsegregational offspring in successive broods. In both experiments the patroclinous progeny frequencies are quite homogeneous. In contrast, there is a deviation from homogeneity for the matroclinous progeny which assumes statistical significance in the *Cy, SM1* experiments. Examination of the brood distribution of matroclinous progeny reveals a much greater frequency in early broods than in later broods. Explanation of the discrepancy between the two classes of nonsegregational progeny on the basis of decreased viability associated with the C(3L) chromosome marked with *ri*, and the C(3R)

chromosome marked with p^p gl^3 will not satisfactorily explain the extreme discrepancy in the *Cy*, *SM1* experiments. Moreover, in other experiments involving other marker systems (HOLM and CHOVNICK, unpublished), the nonsegregational progeny exhibit a similar discrepancy in the same direction. The authors suggest that these features of the distribution of nonsegregational progeny may best be understood with the following suppositions: (1) Nullo-3 eggs are uniformly less viable or are formed with a lower frequency than C(3L); C(3R) eggs. Either of these suppositions will explain the discrepancy between the two classes of nonsegregational progeny, and is consistent with the uniform brood distribution of patroclinous offspring. (2) In addition, the sharp decrease in frequency of matroclinous progeny in successive broods may be understood on the additional assumption that nullo-3 sperm do not store well.

The suggestions of lowered viability of nullo-3 eggs, and lowered capacity for storage of nullo-3 sperm, imply gene function in these cell types. These suggestions are in conflict, at least for sperm, with conclusions drawn earlier (MULLER and SETTLES 1927; MCCLOSKEY 1966), and further investigations bearing on these questions are in progress.

Question may be raised concerning the relationship, if any, of the nonsegregational meioses in females with the change in crossover frequencies seen in C(3R) in the presence and absence of *Cy*, *SM1* and *In(3L)Payne*. Unfortunately, the present data are inadequate, since segregation data could not be collected for those experiments in which *In(3L)Payne* was absent. In those experiments, both parents possessed the same C(3L) chromosome marked with *ri*. Moreover, in those experiments for which segregation data was collected, no effort was made to collect the recombination data such that it might be related to the segregation data. Consequently, it is not possible to compare the frequency of crossover chromatids among matroclinous progeny with those from segregational progeny.

SUMMARY

The principles of recombination established by half-tetrad analysis of single attached-X females also hold for attached-3R chromosomes, and these principles may be elaborated from mass matings of attached-3R females. The following observations demonstrate this similarity in C(3R) (Compound 3R): (1) Reciprocal and nonreciprocal single exchanges occur with equal frequency. (2) In the case of double exchanges there is no evidence of chromatid interference. That is, the second exchange in a double exchange tetrad is independent of the first. (3) Percent homozygosis of both wild-type and mutant alleles increases with the distance from the centromere. (4) The presence of the heterologous rearrangements, *Cy*, *SM1* or Inversion(3L)Payne or both, does not alter either the ratio between reciprocal and nonreciprocal single exchanges or the ratio between 2-strand, 3-strand, and 4-strand doubles. (5) The presence of *Cy*, *SM1* was shown to increase recombination along the marked portion of the attached-3R. However, crossover values were decreased in the presence of Inversion(3L)Payne, while both together increased it only slightly.—Observations on meiotic

segregation of the C(3L) and C(3R) chromosomes indicate that: (1) Nonsegregational meioses take place regularly in males with a high frequency (not estimated in the present study). It is inferred from other studies (McCLOSKEY 1966) that in the absence of chromosome rearrangements, nonsegregational meioses are rare in females. (2) Observations on the relative frequency of matroclinous and patroclinous progeny suggest either that nullo-3 eggs are produced with a lower frequency than C(3L); C(3R) eggs, or that nullo-3 eggs are uniformly less viable than C(3L); C(3R) eggs. (3) Observations on the brood distribution of matroclinous progeny suggest that upon storage, nullo-3 sperm are less likely to lead to successful fertilization.

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