THE MEIOTIC BEHAVIOR OF REVERSED COMPOUND RING X CHROMOSOMES IN DROSOPHILA MELANOGASTER[1]

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A reversed compound ring X chromosome is composed of two complete, or essentially complete, X chromosomes, each attached to the same centromere at one end (as in attached-X chromosomes) with the distal tips of the two chromosomes attached to each other by a heterochromatic segment, thus forming a continuous ring, the component chromosomes of which can synapse with each other simply by the collapsing of the ring. Such a compound ring was first synthesized by NOVITSKI (1954), but the absence of heterozygous markers in this ring greatly limited the analysis of this particular compound.

An analysis of reversed rings is of interest in a number of connections. First, even though only three of the six possible compound X chromosomes have been analyzed extensively, it is already evident that the results from all these compounds cannot be rationalized on the basis of what is known about crossing over in normal (unattached) X chromosomes. It is therefore of some importance that a study be made of the behavior of the various compound X chromosomes for the purpose of further testing the current notions about chromosome behavior. Second, in common with other ring chromosomes, reversed rings provide a test that can distinguish between crossing over taking place among all four nonsister chromatids of the tetrad with no sister-strand exchange as contrasted to crossing over occurring between only two of the four nonsister chromatids with sister-strand exchange then randomizing the strands (as suggested by LINDEGREN and LINDEGREN 1937; SCHWARTZ 1953). Third, reversed rings are structurally very similar to reversed acrocentric compound X chromosomes (a reversed acrocentric compound is structurally similar to an attached-X in which the centromere has been moved from a median position to a subterminal one), which behave extremely atypically in two respects during meiosis when contrasted to either free X or attached-X chromosomes (SANDLER 1954). Specifically, (1) in crosses of females that carry reversed acrocentric compounds, the level of exchange (as measured by the rate of homozygosis) is markedly affected by whether these compound-bearing females carry a homolog for the compound (the Y chromosome), the exchange frequency being much higher when they do. (2) The frequency of one-exchange tetrads (the term "tetrad" is used here to mean the complex of the four X's formed by replication of the two chromosome elements of the compound chromosome, and does not include the heterochromatic homolog, if any) is very nearly, or perhaps actually, zero, although there is a high frequency of

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both no-exchange and two-exchange tetrads. It is, therefore, of interest to compare the meiotic behavior of reversed acrocentrics and reversed rings. Finally, physical considerations suggest that ring chromosomes might, after crossing over or replication, be mutually interlocked so that in the progeny of ring-bearing parents there would be a reduced recovery of rings owing to their elimination as interlocked complexes. Reversed rings represent a situation in which genetic evidence for interlocking of rings might be obtained.

Results indicate the following: (1) The data from reversed rings are consistent with the usual assumptions about the nature of crossing over (i.e., that all four nonsister chromatids participate in exchange at random, with sister strands never crossing over) and are inconsistent with the assumption of sister-strand crossing over as outlined here. (2) Reversed rings and reversed acrocentrics are similar in that both have (a) a reduced frequency (or absence) of single exchanges as compared with exchanges of ranks zero and two, (b) about the same frequency of no-exchange and double-exchange tetrads, (c) about the same distribution of exchanges along the length of the chromosome, and (d) the same reduction in crossing over when the parental compound-bearing females do not carry a homolog for the compound. (3) There is a sizable reduction in the recovery of reversed rings in the progeny of compound-bearing females in excess of that which can be accounted for by either reduced viability of ring-bearing individuals or by a tetrad analysis, suggesting the possibility of unresolved interlocked complexes in reversed rings. However, reasons for believing that this reduction in the recovery of rings is caused by some factor other than interlocking of rings will be considered.

Synthesis of reversed rings

The reversed ring used in this analysis was synthesized in two steps: (1) constructing an attached-X chromosome homozygous for distally placed heterochromatin and (2) attaching these heterochromatic regions to form a ring (fig. 1).

The precise method employed involved constructing a special attached-X chromosome. One arm of this chromosome was *In(1)sc*^{8} (a long inversion with a large block of proximal heterochromatin placed distally and carrying a normal allele of *y* distal to this heterochromatin) carrying the markers *cv*, *v*, and *f*. The other arm was a crossover product of *In(1) sc*^{81} and *In(1)EN*, making the chromosome similar to the *sc*^{8} inversion distally but different proximally in that it carries an amount of heterochromatin different from *sc*^{8}, and carries a mutant allele of *y* (in addition to the normal allele of *y* carried at the tip). This arm was marked by the mutants *m* and *car*. Such attached-X females with no homolog were X-irradiated with approximately 2000r and crossed to attached-XY (YSX.YL), *y B* males carrying no homolog. The female progeny from this cross were examined for cases in which the attached-X chromosome had lost both distal *y*$^{+}$ loci simultaneously (i.e., *y* females). Such an attached-X would be recoverable, since it would carry a mutant allele of *y* proximally. Two ways in which such loss might be accompanied by the conversion of the attached-X chromosome to a reversed ring are shown schematically in figure 1.

Several possible reversed rings were obtained from this cross; one of the lines so obtained was used for analysis. A cytological examination of larval neuroblast

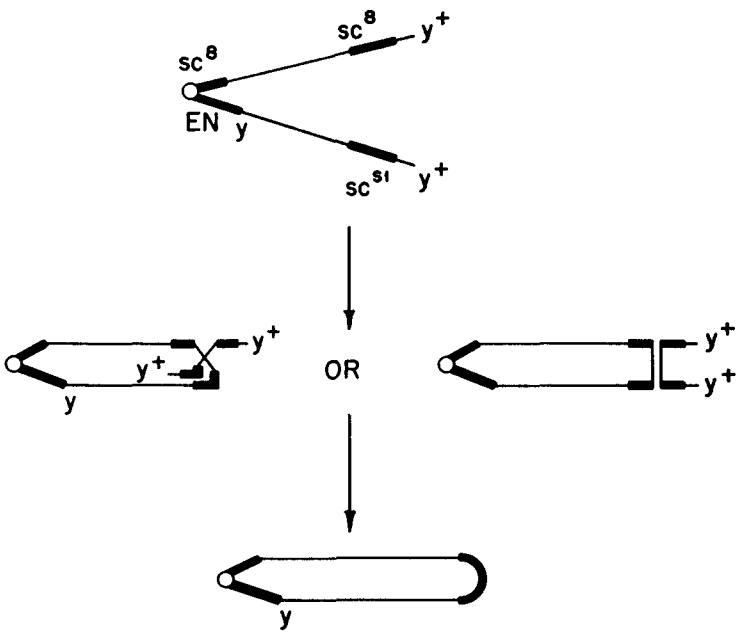

FIGURE 1.—The origin and structure of the reversed compound ring X chromosome. Line 2 shows two possible ways in which the attached-X, shown on line 1, might have been converted to the reversed compound ring, shown on line 3.

tissue from this line showed that it did carry a large ring X chromosome. Another cytological check at the end of the genetic analysis reconfirmed the presence of a ring.

METHODS AND RESULTS

The major genetic analysis performed on the reversed ring is as follows: Females carrying reversed rings, hemizygous for the mutant allele of *y* and heterozygous for the recessive markers *cv*, *v*, *m*, *f*, and *car*, which are 19.3, 3.1, 20.6, and 5.8 standard crossover units apart respectively, and carrying FR2 (a heterochromatic chromosome equivalent to the long arm of the Y chromosome and marked by the normal allele of *y*), which acts as a homolog for the compound, were crossed to *y B* males carrying the attached-XY chromosome and no homolog. It is not known for any marker whether it is in coupling or repulsion with any other marker because, although the markers originally went into the compound as *cv*, *v*, and *f* on one arm and *m* and *car* on the other, crossing over will change these relations. The progeny (F_1) from this cross were scored, and every *y* (but not *cv*, *v*, *m*, *f*, or *car*) female recovered was crossed to sibling males. In the F_2 (the progeny of these *y* females), females were scored. The coupling relations among the markers in the rings carried by the original parental females could be determined from the homozygous female progeny (of single females) in the F_1 and from the results of the progeny tests. This is so because if a female carries a reversed ring with any two markers in coupling, then a double exchange (one crossover to the right of the rightmost marker and the other to the

left of the leftmost marker) can result in a single female offspring homozygous for both markers. If, on the other hand, the original ring carried the two markers in repulsion, then, as in attached-X chromosomes, it requires an exchange of rank greater than two (such exchanges are assumed to occur sufficiently infrequently so that the error introduced by them here is small enough to be ignored) to produce an offspring simultaneously homozygous for both markers.

The progeny from the original cross are given, according to the determined genotype of the parental females, in table 1. The last five lines in the table are a summary. Since male progeny (*B* or *y B*) are recovered in this cross only when eggs are fertilized by sperm carrying the YSX.YL chromosome, and since female progeny are recovered only if eggs are fertilized by nullo-XY sperm, it is necessary, for male and female classes to be directly comparable, to correct the male classes for meiotic loss of the YSX.YL chromosome (SANDLER and BRAVER 1954). Experience has shown that the best correction is made by increasing the male classes by 16 percent. The corrected figures for the male classes appear in the first two lines of the summary. Although these corrected figures will be used throughout the analysis, it should be noted that none of the general conclusions reached depend on this correction. The numbers given in the lines of table 1 designated as "Matroclinous females" and "Total homozygous females" are obtained by deciding from the genotype of the parental female and from the observed homozygotes, how many of the *y* females scored were homozygous for wild type alleles, and assigning those to the homozygous class.

These data have been treated in a number of ways, which will be considered in the following sections.

ANALYSIS OF RESULTS

On the frequency of exchanges of rank one

A direct determination of the frequencies of the different rank exchanges in the reversed ring is not immediately possible because, for an estimate of these frequencies, the relative viability of ring-bearing females and males must be known. It is, however, still possible to determine whether single exchanges are absent, or at least very reduced in frequency, in the following way. A reversed ring compound, heterozygous for the markers *cv*, *v*, *m*,*f*, and *car*, the coupling relations among which may be symbolized *cv v f/m car*, for example, can be converted to one of the constitution *cv v car/ m f* by certain single exchanges or by certain double exchanges. A ring in which the coupling relations have been changed by crossing over will be referred to as a transpose. Now, since homozygous rings come only from double exchanges (exchanges of rank greater than two will not be considered; the conclusions to be drawn will not be affected by this exclusion), whereas a transpose may come from either a single or a double exchange, it is possible to determine, from the observed number of homozygotes, the number of transposed rings that are accounted for by double exchanges; the remainder presumably being caused by singles.

In practice, a transposed ring is not phenotypically different from the original ring, but, owing to the change in coupling relations, the difference can manifest itself in progeny tests. The data from the progeny tests have therefore been treated as follows. Parental females were chosen whose homozygous F_1 progeny indicated

TABLE 1

Results from matings of females carrying reversed compound ring X chromosomes and FR2 to males carrying the attached-XY chromosome marked with y B *and carrying no homolog. (Classes marked by an asterisk have been corrected for meiotic loss.)*

Phenotype of progeny	Genotype of parental ring-bearing females																
	cv v f / m car	*cv v car / m f*	*cv m car / v f*	*cv m f / v car*	*cv v m / f car + +*	*cv v f car / m*	*cv m f car / v*	*cv m / v f car*	*cv v / m f car*	*v m car / cv f*	*v m f / cv car*	*cv v m f / car*	*cv f car / v m*	*cv v m car / f*	*v m f car / cv*	*cv v m / f car*	Total
B♂♂	960	676	834	603	636	1,486	909	979	732	37	620	936	246	218	101	140	
y B ♂♂	95	66	56	76	60	147	91	86	74	5	56	88	23	29	9	13	
y ♀♀	294	211	245	228	214	536	325	314	267	10	232	342	89	73	29	49	
+ ♀♀	1	0	1	0	0	1	1	2	1	1	0	1	0	0	0	0	
y cv ♀♀	2	2	2		2	3	3	3			9	4	3	1	3	1	
y v ♀♀	18	16	15	22		22	46	23	23		3	2		1			
y m ♀♀	41	21	17	11		66	6	33	12			2	1				
y f ♀♀	8	3	1	1	1	6	2	1	1	1	2	10	1	7		4	
y car ♀♀	2	2	7	3	1	2	2	1			4	18		1			
y cv v ♀♀	19	8				5			8								
y v f ♀♀	17		19			12		13									
y m car ♀♀	7		7														
y v car ♀♀		6		9					16			3			1		
y m f ♀♀		19		11	2		8	17									
y cv m ♀♀			9	3			9				11	13	6	5		3	
y v m ♀♀					7			5	3				1			1	
y f car ♀♀					1	6	3			1			3				
y cv f ♀♀																	
y cv v f ♀♀	10					5											

y cv v car ♀♀		4															
y cv m car ♀♀			3														
y cv m f ♀♀				4			1										
y cv v m ♀♀					4							6		4		1	
y v m f ♀♀					1						17	11					
y m f car ♀♀							8		5								
y v f car ♀♀						15		14									
y v m car ♀♀														3			
y cv v m f ♀♀					3							9					
y v m f car ♀♀					2										1		
y cv v f car ♀♀						5											
y cv m f car ♀♀							1										
y cv v m car ♀♀														1			
y cv v m f car ♀♀					1												
B ♂♂*	1,142	804	992	718	757	1,768	1,082	1,165	871	44	738	1,114	293	259	120	167	12,034
y B ♂♂*	113	79	67	90	71	175	108	102	88	6	67	105	27	35	11	15	1,159
Matroclinous ♀♀	292	209	243	228	189	521	325	304	263	9	194	300	86	64	24	48	3,299
Total homozygous ♀♀	126	83	82	64	50	162	89	120	72	3	84	120	18	32	10	11	1,126
Exceptional ♀♀	1	0	1	0	0	1	1	2	1	1	0	1	0	0	0	0	9

that they were of the constitution *m f/v car* or *m car/v f* (*cv* was ignored because homozygosis for this marker is rare and its coupling relations with the other markers is consequently difficult to determine). It should be noted that the females used in this test comprise only a small fraction of the females whose progeny are given under these same genotypes in table 1. This is because the genotypic determinations in table 1 involve both the F_1 and F_2, whereas the determinations in this test are made only on the basis of the F_1. The nonhomozygous female progeny from parental females of these particular constitutions were classified, from the results of the progeny tests, according to whether they were transposed, nontransposed, or whether this was indeterminate. This last category comes from cases in which the critical homozygous types failed to appear among the F_2.

Suppose, for instance, that the female progeny of an original parental female included ten *y* females, one *m car* female, one *v f* female, two *v* females, and one *f* female. From the *m car* and *v f* offspring, the maternal genotype can be specified as *m car/v f*. The ten *y* females are then mated, and their female progeny scored. If, among the progeny of any such *y* female, a *m car* or a *v f* homozygote appears, the *y* female is classified as a nontranspose; if either a *m f* or a *v car* homozygote is recovered, the *y* female is classified as a transpose; if none of these types appear, a classification cannot be made. We may note two points about this analysis. First, the selection of the parental females is based solely on homozygotes for two markers simultaneously (*m car* and *v f* in the example); there is therefore no selection with respect to homozygotes for markers appearing singly, and these are the homozygotes that will be used in the comparison to follow. Second, the homozygous products of the very same exchanges determine whether a particular ring is a transpose; that is to say, the relative probabilities of classifying a ring as either a nontranspose or a transpose ought to reflect the frequencies of these types in the sample.

There is one additional complication. Occasional crossovers do occur between *v* and *m* and between *f* and *car*. Double exchanges, in which one of the crossovers is of one of these types can produce offspring homozygous for single markers and transposes that may be symbolized as *v m*/++ or *f car*/++. These transposes will be referred to as *v–m* transposes. The occurrence, among the progeny of a single female, of a homozygote for *v* and *m* simultaneously (or for *f* and *car* simultaneously) indicates that the parental female was of the constitution *v m*/++ (or *f car*/++). The proportion of females of this constitution, among all females, that can be so classified directly from the homozygotes among their progeny has been estimated in these experiments simply by classifying original parental females according to whether they were of this constitution from the F_2 data only (this can be done for every female without ambiguity) and then checking on the proportion of these that could have been so classified from the F_1 results only. This classification was possible in 74 percent (48/65) of the cases of *v m*/++, and in 51 percent (21/41) of the cases of *f car*/++.

In this experiment there were 27 females whose multiple homozygous F_1 progeny showed that they were of one of the desired constitutions (*v f/m car* or *v car/m f*). The F_1 progeny from these females included, in addition to those females homozygous for more than one marker at a time, 452 *y* females and 103 females homozygous for only one marker at a time. The 452 *y* females were found, upon testing, to include

174 nontransposes, 28 transposes, 15 *v–m* transposes (7 *v m*/++ and 8 *f car*/++), and 235 were indeterminate.

The 15 observed *v–m* transposes represent approximately 63 percent of the actual number of such transposes in the entire sample from the argument presented previously, which means that there must have actually been 24 such. This leaves 226 indeterminate nonhomozygous females (the 235 indeterminate *y* females less the 9 that were *v–m* transposes) to be divided into the nontranspose and transpose classes as 174 is to 28. These classes then become 195 and 31, respectively. In total, then there were observed 103 singly homozygous females and 83 (24 *v–m* transposes and 28 + 31 transposes) transposed and *v–m* transposed females.

Figure 2 shows the regions of exchange pertinent to this analysis, and table 2 gives the results from double exchanges according to region. From table 2, it can be seen (making the conventional assumptions about crossing over) that, whereas a precise determination of the relation between females homozygous for one marker at a time and transposes is not possible, from the double exchanges, there should be about two singly homozygous females recovered for each transpose, and approximately one singly homozygous female recovered for each *v–m* transpose. Taking into account the relative frequencies of transposes and *v–m* transposes, the expected recovery of singly homozygous females to transposed plus *v–m* transposed females from double exchanges (from the total of 186) becomes 115:71 as compared with the observed 103:83.

This agreement between the observed and the expected ratios, then, indicates that most, if not all, transposes are accounted for by double exchanges, and hence, single exchanges (half of which, in the *v* to *f* region, would produce transposed rings and none of which produce homozygosis), if they occur at all, must be rare.

On sister-strand crossing over

It has been shown (WEINSTEIN 1936; LINDEGRÈN and LINDEGREN 1937; SCHWARTZ 1953) that for chromosomes, other than rings, the expectations are the same whether it is assumed that crossing over takes place randomly between any two nonsister chromatids of a tetrad and that there is no sister-strand crossing over (the 'conventional' assumptions) or that crossing over occurs only between the two newly replicated strands (strands attached to the same centromere in the compound X chromosomes), with the probability of an effective sister-strand exchange in any region (i.e., an odd number of sister exchanges in the given region) being 1/2. For the

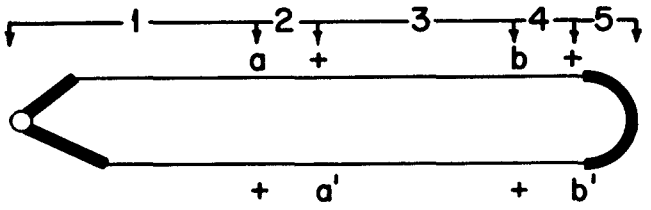

FIGURE 2.—The regions of exchange defined for those reversed compound rings that have been used to detect transposes of marker genes. The consequences of exchange in these regions are given in table 2.

TABLE 2

The expected relation between females homozygous for only one marker at a time, transposes, and v–m *transposes from exchanges of rank two*

Region involved	Products from	
	2-st-doubles	4-st-doubles
1, 2	1H: 1$\hat{T}$: 2N	1H: 1$\hat{T}$: 2N
1, 3	2H: 1T: 1N	2H: 1T: 1N
1, 4	1H: 1$\hat{T}$: 2N	1H: 1$\hat{T}$: 2N
2, 3	1H: 1$\hat{T}$: 2N	1H: 2T: 1$\hat{T}$
2, 5	2H: 1$\hat{T}$: 1N	1H: 1$\hat{T}$: 2N
3, 4	1H: 1$\hat{T}$: 2N	1H: 2T: 1$\hat{T}$
3, 5	2H: 1T: 1N	2H: 1T: 1N
4, 5	1H: 1$\hat{T}$: 2N	1H: 1$\hat{T}$: 2N

The "markers" a, a', b, and b' represent, generally, the coupling relations in the rings carried by the parental females used for the transpose analysis. Double exchanges involving regions 1, 5 (since they result in nothing of interest here) and 2, 4 (since they must be very rare) are omitted; all 3-strand doubles result in anaphase II bridges and are, for this reason, omitted. H = detectable homozygote for one marker; T = detectable transpose; $\hat{T}$ = detectable v–m transpose; N = neither H, nor T, nor $\hat{T}$. (It should be noted that this class contains some multiple homozygous types from 1, 4 and 2, 5 exchanges, but it is not used in subsequent analyses.) The "regions involved" are shown in figure 2.

ring chromosomes, however, the expectations from the two sets of assumptions are different. The data from single ring X chromosomes in Drosophila (MORGAN 1933) are not consistent with the assumptions in a sister-strand analysis as outlined, but are consistent with the conventional nonsister-strand assumptions. The data from ring chromosomes in maize (MCCLINTOCK 1938, 1941; SCHWARTZ 1953), on the other hand, are consistent with the sister-strand analysis, but not with the other. It will be shown here that, as with single rings in Drosophila, the data from the reversed ring are not consistent with the assumption of sister-strand crossing over as just considered, but is consistent with the usual, nonsister strand assumptions. It should be emphasized that this applies only to sister-strand crossing over that follows the rules given here, and does not apply to the question of sister-strand exchange in general; this point will be developed in a later section.

The genetically distinct crossovers (sister and nonsister) in the reversed ring are shown in figure 3. The consequences of all combinations of these exchanges are given in table 3. A similar analysis, making the conventional assumptions about crossing over, is given in figure 4 and table 4. The first comparison of importance here depends on the fate of the equal (E) anaphase II bridges produced by crossing over. A bridge, in figures 3 and 4, has been designated equal if two strands that tie the sister centromeres separating at anaphase II together are of exactly the same length. This equality results if the heterochromatic regions labeled 1 and 2 in figures 3 and 4 are each present once in each of the two strands connecting the centromeres of a bridge. Bridges of this type have been shown to be excluded from the nuclei of the second meiotic division (NOVITSKI 1955) resulting, after fertilization by an X-bearing sperm (or an XY-bearing sperm in these experiments) in a patroclinous male. This is in

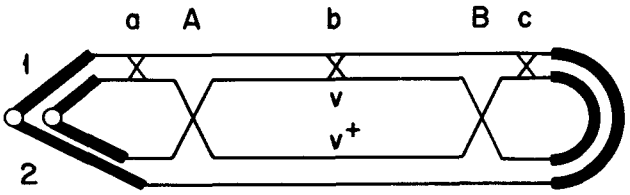

FIGURE 3.—The genetically distinct sister-strand (lower case) and nonsister-strand (upper case) exchanges in the reversed compound ring X chromosome. The numbers, 1 and 2, designate the two different heterochromatic regions. The genetic consequences of all combinations of these exchanges are given in table 3.

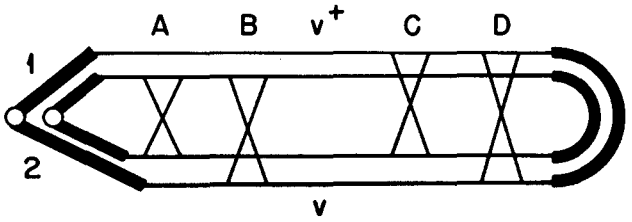

FIGURE 4.—The genetically distinct nonsister-strand exchanges in the reversed compound ring X chromosome. The consequences of all combinations of these exchanges are given in table 4.

contrast to anaphase II bridges generally (that is, either anaphase II bridges composed of only one strand, or unequal double-strand anaphase II bridges) that are lethal. Patroclinous males from this source are distinguishable from the regular males, which are the complementary products of the ring, because the parental females carry a marked homolog (FR2 carrying a normal allele of *y*) that the regular male class receives and are therefore non-*y*,*B*, but that the males from equal bridges do not receive and are therefore *y B*. Primary nondisjunction is one other predictable source of *y B* males. The complementary product to the *y B* males from primary nondisjunction is, however, the exceptional (i.e., y^+) female class. Although meiotic loss would be expected to result in some small excess of *y B* males over wild type females (SANDLER and BRAVER 1954), the observed excess (1,159 to 9; see table 1) is clearly much too large for this. It seems, therefore, most reasonable to suppose that all but a very small fraction of the exceptional males arise from equal bridges. The expected ratio of *y B* males to total homozygous females from double exchanges, making the conventional assumptions about crossing over, (figure 4 and table 4) is 1:1, with which the observed numbers 1,159 to 1,126 agree. This same expectation, assuming sister-strand crossing over (see figure 3 and table 3) is 3:2, with which the observations do not agree. Moreover, from the sister-strand analysis, it can be seen that one half the no-exchange tetrads should also produce patroclinous males (see table 3), which clearly are not recovered. This could also mean, of course, that sister-strand crossovers occur only in tetrads that also have nonsister exchanges.

Finally, it is possible to perform an analysis similar to that in figure 2 and table 2, to arrive at the expected ratio, from double exchanges, of singly homozygous females to females carrying transposed reversed rings, making the sister-strand assumptions. These expectations are approximately four singly homozygous females to one trans-

TABLE 3

The consequences of exchange in the reversed compound ring X chromosome of ranks 0, 1, and 2, supposing nonsister exchanges to occur between only the two "newly replicated" chromatids, with an effective sister-strand crossover occurring in any region with a probability of $\frac{1}{2}$

Rank and frequency	Crossovers involved		Products after anaphase II separation
	Nonsister	Sister	
$\frac{1}{2}$ E_0	None	None	Reversed ring (*v*/+) + reversed ring (*v*/+)
$\frac{1}{2}$ E_0	None	a, b or c	Bridge (E)
$\frac{1}{2}$ E_1	A or B	None	Reversed ring (*v*/+) + reversed ring (*v*/+)
$\frac{1}{2}$ E_1	A or B	a, b or c	Bridge ($\frac{1}{2}$ E + $\frac{1}{2}$ E′)
$\frac{1}{8}$ E_2	A and B	None	Reversed ring (*v*/+) + reversed ring (*v*/+)
$\frac{2}{8}$ E_2	A and B	a and c or a, b, and c	Reversed ring (*v*/*v*) + reversed ring (+/+)
$\frac{2}{8}$ E_2	A and B	b or a and b	Bridge (E′)
$\frac{3}{8}$ E_2	A and B	a or c or b and c	Bridge (E)

Exchanges have been omitted when such omission does not distort the relative frequencies of the distinct products given in the table. E_0 = no exchange, E_1 = single exchange, and E_2 = double exchange. E = equal and E′ = unequal. The "crossovers involved" are shown in figure 3.

TABLE 4

The consequences of exchange of ranks 0, 1, and 2, supposing no sister-strand crossing over in the reversed compound ring X chromosome. The "crossovers involved" are shown in figure 4

Rank and frequency	Crossovers involved	Products after anaphase II separation
E_0	None	Reversed ring (*v*/+) + reversed ring (*v*/+)
$\frac{1}{2}$ E_1	A or D	Reversed ring (*v*/+) + reversed ring (*v*/+)
$\frac{1}{2}$ E_1	B or C	Bridge (E′)
$\frac{1}{4}$ E_2	A and C	Bridge (E)
$\frac{1}{4}$ E_2	A and D	Reversed ring (*v*/+) + reversed ring (*v*/+)
$\frac{1}{4}$ E_2	B and C	Reversed ring (*v*/*v*) + reversed ring (+/+)
$\frac{1}{4}$ E_2	B and D	Bridge (E′)

pose and about two singly homozygous females to one *v–m* transpose (as contrasted to 2:1 and 1:1 making the conventional assumptions). Although the data (103 homozygotes to 83 transposed plus *v–m* transposed rings) do not agree with these expectations, this argument alone would not be convincing since the nonagreement could be the result of some frequency of single exchanges. Taken, however, in conjunction with the argument from the equality of total homozygotes and patroclinous males, it may be considered further evidence for the inconsistency of the data with the expectations assuming sister-strand crossing over.

On the frequency of exchanges of ranks zero and two

From the foregoing, it would appear (1) that the frequency of single exchanges in reversed rings is either zero or very close to it, and (2) that the data from reversed rings are best dealt with assuming a conventional tetrad analysis. The frequencies of no-exchange and two-exchange tetrads may now be estimated. The appropriate analysis is given in figure 4 and table 4. The data have been presented in table 1. It can be seen that the total number of ring chromosomes and ring chromosome derivatives (i.e., homozygous rings and anaphase II bridges) from double exchanges is simply 4 times "total homozygous females," since, from table 4, one fourth of all the doubles yield homozygotes. This is 4,504 (4 × 1,126). For each homozygote from double exchanges, one nonhomozygous female is produced. There are, therefore, 1,126 of these, leaving 2,173 (3,299 − 1,126) nonhomozygous females that come from the no-exchange tetrad class (since singles are assumed not to occur). Now, if there were no other complications, the total number of ring chromosomes and ring chromosome derivatives accounted for (4,504 + 2,173 = 6,677) should equal the observed number of regular (*B*) males, since one half of all the eggs receive the homolog for the compound. However, from table 1, it is seen that 12,034 males were recovered as contrasted to only 6,677 ring products accounted for. That this is not a relative inviability of the ring classes is shown by the observed equality between total homozygotes (1,126) and patroclinous males (1,159). This is especially convincing since homozygous females are certainly somewhat less viable than heterozygous females and the *y B* males are probably more viable than the *B* males as the YSX.YL chromosome may be duplicated for the *y* region. It is clear, then, that there is some source of ring loss that is not accounted for by either inviability of the ring-bearing classes or the tetrad analysis. It will be shown in the next section of this report that this ring loss comes only from the no-exchange class. This being so, the frequencies of no-exchange and two-exchange tetrads may be estimated, the regular male class being used as the total. This is equivalent to increasing the no-exchange female class by the difference between the observed and the expected number of regular males. The frequency of tetrads of rank two then becomes 0.37 (4,504/12,034), whereupon no-exchange tetrads must have a frequency of 0.63. It is also possible to estimate the recovery of rings from no-exchange tetrads; this is 2,173/7,530 = 0.29; that is, only 29 percent of the no-exchange tetrads are recovered.

The frequencies of exchange arrived at here are rather interesting since they are very similar to the exchange frequencies in reversed acrocentric compound X chromosomes (SANDLER 1954). The best comparison would come from computing exchange values for the reversed acrocentric by the same method that is used here for the reversed ring. In the report on the reversed acrocentric, the single exchange frequency was estimated from the difference between males and females recovered, assuming no viability differences between compound-bearing females and regular males. It might also be mentioned that the calculations of exchange values in that work contain a minor error in that one lethal class was not considered; the corrected values are, however, very close to those given and do show a value for the frequency of single exchanges of very close to zero (actually about 12 percent). A typical set of data

from a cross of females carrying reversed acrocentric compound X chromosomes (line ND 34 in table 10; SANDLER 1954) and FR2 by YSX.YL, *y B* males are:

Regular ♂♂ = 2,925
Nonhomozygous ♀♀ = 1,836
Homozygous ♀♀ = 652.

The male class has been corrected for meiotic loss, the homozygous female class has been corrected for those homozygous for wild type alleles, and the nonhomozygous female class has been corrected accordingly. On the assumption that single exchanges occur with a frequency of zero, double-exchanges become 0.45 (2 × homozygous females/regular males = 1,304/2,925), whereupon no-exchange tetrads must have a frequency of 0.55. The tetrad analysis is somewhat different from that for the reversed ring, and the appropriate analysis is given in SANDLER (1954).

This comparison of $E_0 = 0.55$ and $E_2 = 0.45$ for the reversed acrocentric and $E_0 = 0.63$ and $E_2 = 0.37$ for the reversed ring suggests that the rate of exchange in the two compounds is the same.

On the effect of FR2 on exchange

It has been shown (SANDLER 1954) that the rate of homozygosis in reversed acrocentric compound X chromosomes is markedly decreased if the parental compound-bearing females do not carry a Y chromosome (or FR2) as a homolog for the compound. To test for this phenomenon in reversed rings, females carrying reversed rings heterozygous for *m* and *v*, with no homolog for the compound were crossed to YSX.YL, *y B* males with no homolog. The progeny included:

y B ♂♂ = 684 (814)
Total ♀♀ = 243
y v ♀♀ = 15
y m ♀♀ = 13.

These results may be compared with those given in table 1. The appropriate figures here are:

Regular (*B*) ♂♂ = 10,113 (12,034)
Exceptional (*y B*) ♂♂ = 974 (1,159)
Total ♀♀ = 4,425
y v ♀♀ = 469
y m ♀♀ = 453.

The numbers in parentheses are the observed numbers corrected for meiotic loss.

To compare the rates of homozygosis in these two experiments by computing the "percentage homozygosis" as "number of homozygous females" divided by "total females," as may be done for the reversed acrocentric, would lead to spurious results if there were a difference in exchange frequency between the two experiments, since only 29 percent of the no-exchanges result in recovered females (see preceding section) whereas, from the tetrad analysis, 50 percent of the double exchanges are recoverable.

To circumvent this difficulty, the observed number of males (regular plus exceptional) will be used as the total. This number will be somewhat affected by exchange frequency since the exceptional male class is a double-exchange product, but since such males represent only a small proportion of the total number of males, this should have little effect on the results. In any case, this error would tend to obscure a difference between the two experiments. The exceptional males must be included in the total because, in the experiment in which the parental females did not carry FR2, these males are not distinguishable from the regular males. The comparison, then, for the case of homozygosis for *v*, becomes 4.2 (469/10,113 + 974) for the case in which the females carried FR2 and 2.2 (15/684) for the case in which they did not. It is clear that this same difference would manifest itself if homozygosis for *m* were measured. It appears, then, that the effect of FR2 on exchange (the rate of homozygosis being used as a direct measure of exchange) does extend to reversed rings.

Since this is so, very strong evidence may be presented indicating that the deficiency in recovery of ring-bearing females observed in the preceding section comes exclusively or almost exclusively from the no-exchange tetrad class. This may be done most simply by predicting the sex ratio in the experiment in which the compound-bearing females did not carry a homolog from the results of the experiment in which they did, assuming that only 29 percent of the no-exchange tetrads are recovered and that double exchanges are one half as frequent when the compound-bearing females do not carry FR2 as when they do. Arithmetically, this may be done as follows: from each 100 eggs, at the first meiotic division, 50 will receive a compound ring and 50 will not. All 50 of those that do not receive the ring result in males (since meiotic loss of the YSX.YL chromosome has been corrected for, the fact that only nullo-X eggs being fertilized by YSX.YL-bearing sperm are recovered and only ring or ring-derivative eggs being fertilized by nullo-XY sperm are recovered may be ignored); of the 50 that do receive the compound ring, 9.3 will have two exchanges (since E_2 here is 0.185 or $1/2 \times 0.37$) and the rest, 40.7, will have no exchanges; from the 9.3 with two exchanges there will be recovered 4.6 females and 2.3 males (see table 4); from the 40.7 with no exchanges, 29 percent, or 11.8, are recovered as females. In total, then, there should be 52.3 males and 16.4 females recovered or 3.2 males per female. By the figures corrected for meiotic loss given, the ratio of males to females is 3.3 (814/243). The data will not agree with expectations arrived at if the loss of rings is assumed to come at random from both the zero-exchange and the two-exchange tetrads.

On the distribution of exchanges along the length of the chromosome

It has been shown here that reversed acrocentric and reversed ring compound chromosomes behave very much alike. One further comparison between these two compounds is possible—a comparison of the distribution of exchanges along the length of the chromosome. This is most readily done by comparing the rates of homozygosis for individual markers among all homozygotes in the reversed acrocentric and reversed ring compounds. This comparison is given in table 5. It can be seen that the distribution of exchanges is very similar for the two compounds.

TABLE 5

A comparison of the relative rates of homozygosis in the reversed ring as compared with the reversed acrocentric compound X chromosome. Percentage homozygosis is computed as the number of homozygotes for a given allele over the total number of homozygous alleles

Allele	Reversed acrocentric homozygotes		Reversed ring homozygotes	
	Number	Percent	Number	Percent
cv	70	12	182	17
v	215	38	469	42
f	177	31	301	27
car	110	19	155	14

Data for the reversed acrocentric are from SANDLER (1954). The data, in both cases, were collected from females carrying a homolog for the compound chromosome.

DISCUSSION

A comparison of reversed acrocentric and reversed ring compounds

The reversed ring and the reversed acrocentric have been shown to behave alike according to all the criteria applied. Both compounds show the same effect on crossing over of a heterochromatic homolog for the compound, both show the same distribution of exchanges along the length of the chromosome, both show a reduction (or an absence) of single exchanges, and both show about the same frequencies of zero-exchange and two-exchange tetrads. They differ, in fact, only in that the reversed ring is recovered less often from no-exchange tetrads than expected, whereas no such deficiency is manifest in reversed acrocentric data. It now seems very likely that both the lack of single exchanges and the effect on crossing over of the Y chromosome, or FR2, (and, possibly also, as will be developed presently, the deficiency of rings from reversed ring-bearing parents) are different manifestations of the same cause. The absence of single exchanges is, of course, the most striking feature to be considered.

It would seem that, with respect to the lack of singles (and perhaps the other effects), there are only three possibilities: (1) that single exchanges are occurring, but that the crossover products are not recovered for some reason, giving the impression of an absence of singles; (2) that single exchanges are occurring, but that one or more of the assumptions that goes into the tetrad analysis is incorrect, with the result that single exchanges appear, from the analysis, not to be occurring; and (3) that there are in fact no singles occurring, which means that there exists some property or properties of chromosomes, that under ordinary conditions does not manifest itself, but that, under the structural conditions imposed on the chromosomes in reversed acrocentric and reversed ring compounds results in the aberrant behaviors noted. A comparison of reversed acrocentric, reversed ring, and attached-X compounds suggests that the structural condition in question is the interstitial heterochromatic segment. The first two of these possibilities were considered in some detail previously (SANDLER 1954), and were shown to be inadequate to explain the data from reversed acrocentrics. These same arguments, in general, apply also to reversed rings, by extrapolation if nothing else. There is, in addition, one rather general consideration that also tends to eliminate these possibilities. The fact that

the effect of a Y chromosome (or FR2) on crossing over manifests itself in both the reversed acrocentric and reversed ring compounds suggests that this effect and the absence of single exchanges are two indications of the same phenomenon. If, moreover, the deficiency of rings recovered from reversed ring females is not a consequence of interlocking, as it may very well not be, it seems reasonable that this too is a result of the same cause. If this is so, then it is certainly very difficult to see how either a lack of recovery of certain products or an error in the assumptions made in the tetrad analysis would be sufficient to account for all these phenomena.

One final point in this connection is of some consequence. From the results of the reversed acrocentric compound alone, it would be possible to conclude that some of the tetrads do not pair (the difficulty in synapsis being presumably caused by the reversed acrocentrics having to fold back on themselves in order to pair) and that this proportion has, of course, no exchanges. When, however, the compound does pair, then there are only double exchanges. The results from the reversed ring would seem to deny this possibility since the reversed ring should pair with the same ease as an attached-X. It seems necessary to conclude, therefore, that even though the component chromosomes of the compounds are paired with each other, they may either not cross over at all or they may cross over twice (or possibly more than twice).

Sister-strand crossing over

One of the specific results of this analysis of the reversed compound ring X chromosome is the inconsistency of the data with the supposition that crossing over occurs initially between only two of the four nonsister chromatids with sister-strand exchange then randomizing the exchange regions. This conclusion from the reversed ring agrees with that based on the data from single ring X chromosomes in Drosophila (MORGAN 1933). It has, on occasion, been argued that the nonagreement of the results from single ring X chromosomes with the expectations based on a sister-strand analysis may be interpreted as coming about because the ring is small and, if this results in a certain rigidity, each twist in the ring might be automatically accompanied by a second twist that, if a sister-strand exchange occurs at each twist, will result in an even number of sister exchanges in every tetrad. Such a situation would, of course, give results indistinguishable from those if no sister-strand crossing over takes place. Such an argument, however, based on the relatively small size of single rings, does not apply to the reversed ring since it appears to be a rather large ring in larval neuroblasts and in genetic length.

There is, perhaps, one qualification that ought to be considered. If, for any reason, only even numbers of sister-strand exchanges per tetrad could occur in the reversed ring, then the results from the reversed ring would be in agreement with the expectations (since, indeed, the experimental distinction between the conventional, as opposed to the sister-strand, hypothesis depends wholly on the occurrence of tetrads with an odd number of sister exchanges). The reason for suggesting this particular qualification is that one way of describing the observed absence of single exchanges in the reversed ring is to say that there can occur only an even number of nonsister exchanges. If this is so, then the possibility of only an even number of sister exchanges might be acceptable. Moreover, if this is so, then the difference between maize and

Drosophila, with respect to crossing over, is simply that in Drosophila sister exchanges always occur in pairs; a distinction much less profound than one that supposes that sister-strand crossovers never occur in Drosophila but are, in maize, a regular and essential part of the process of crossing over.

Possible interlocking of rings

It has been shown that although the data from reversed rings and from reversed acrocentrics suggest that the two compounds behave very much alike, they do differ markedly in one particular: whereas all the expected reversed acrocentric compounds are recovered from reversed acrocentric compound-bearing females, only 29 percent of the expected compound rings from no-exchange reversed ring tetrads are recovered, although all the expected products from double exchanges are observed. It has been shown, moreover, that this deficiency in rings cannot be attributed to inviability of the ring-bearing classes. Certainly, one possible explanation of this discrepancy is that some fraction of ring tetrads give rise to mutually interlocked ring complexes that result in lethal zygotes. If this is the proper explanation of the deficiency in the recovery of rings, then, surprisingly enough, it means that the rings from no-exchange tetrads are being lost by interlocking, which strongly suggests that crossing over is a process whereby the interlocked complexes may be resolved (a conclusion similar to that of MATSUURA 1940). The question that immediately suggests itself is how tetrads in which no exchange has occurred may become interlocked. There seem to be but two possibilities. Either (1) the process of replicating a new strand does not occur on only one surface of the original strand so that the new chromatid, when completely formed, is wound around the original chromatid, or (2) that sister-strand exchanges are occurring. The data collected to date are not sufficient to distinguish between the two possible origins of the interlocked complexes.

There is, however, one argument that ought to be mentioned suggesting that this deficiency of rings has an explanation other than that of elimination by interlocking. If there is some lethality from interlocking, then this lethality could be visualized as resulting from either the breakage and subsequent lethality of interlocked rings, or a lethality caused by the anaphase bridge-like structure of the unresolved interlocked complexes. If the former concept were the proper one, then it would seem that the breakage of one of the two interlocked rings would be sufficient to resolve the complex, which would mean that the observed lethality (70 percent of the no-exchange tetrads) actually represents only half the instances of interlocking. This supposition will clearly not fit the data. The alternative idea—that the interlocked complexes remain intact with the result that all instances of interlocking are lethal—will fit the data numerically; but this idea leads to a mechanical difficulty. It is difficult to see why such a complex would be lethal since it is structurally the same length as the equal anaphase II bridges that result, not in lethality, but in detectable nullo-X eggs.

Although these arguments are most certainly not conclusive, they do suggest that the observed deficiency of rings may not be a reflection of interlocking. If this is the case, then (1) these data represent evidence that chromosomes have some mechanism that enables them either to keep from becoming interlocked when they are struc-

turally rings, or to extricate themselves from the complex after becoming interlocked, and (2) it then seems reasonable to suppose that this observed discrepancy is somehow related to the other atypical meiotic behaviors of the reversed ring. One such relation, for example, is as follows. It may be imagined that in the reversed ring all the products of single exchanges are lethal. In the present analysis, this would appear as if the lethality occurred in the no-exchange class. This interpretation, although acceptable for the case of the reversed ring, would not be applicable to the reversed acrocentric, since there is no lethality unaccounted for in that compound.

SUMMARY

A genetic analysis has been made of a reversed compound ring X chromosome. This compound is structurally like an attached-X with its free ends connected by a heterochromatic segment or, which is the same thing, it is similar to a reversed acrocentric compound X chromosome with its free end attached to the centromere by a heterochromatic segment.

The analysis revealed the following points:

1. The data are found to be inconsistent with the assumption that crossing over takes place between only two nonsister chromatids (the "newly replicated" chromatids), with sister-strand crossing over then randomizing the exchange regions, and are found to be consistent with the conventional assumptions made about the nature of crossing over.

2. The compound ring behaves very much like the reversed acrocentric compound X chromosome in that (1) the presence of a Y chromosome, as a homolog for the compound, in the parental females approximately doubles the rate of exchange (as measured by the rate of homozygosis) and (2) the frequency of single exchanges is very nearly, or perhaps actually, zero although there is a high frequency of both no-exchange and two-exchange tetrads. These questions are discussed in text.

3. Unlike reversed acrocentric compounds, the reversed ring is not recovered as often as expected. It is shown that whereas all the products of the two-exchange class are observed, only 29 percent of the no-exchange tetrad class is recovered. The possibility of this depression in the recovery of reversed rings being caused by ring loss by virtue of the formation of mutually interlocked ring complexes is considered.

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