NON-RANDOM DISJUNCTION IN DROSOPHILA [1]

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THE stability of the ring chromosomes in *Drosophila melanogaster* raises certain questions about the nature of chromosome structure and reduplication, since it seems possible that such chromosomes would be subject to loss by interlocking of daughter chromatids after reduplication. In a set of experiments designed to determine the extent to which newly derived ring chromosomes might be lost at the meiotic divisions, it has been found that the generalization that disjunction of chromatids at the second meiotic division is random does not appear to be completely valid when the chromatids are structurally different. The evidence for such non-randomness is presented below.

EXPERIMENTS INVOLVING ATTACHED X CHROMOSOMES

The type of genetic constitution which regularly manufactures ring chromosomes is that used by SIDOROV, SOKOLOV and TROFIMOV (1935, 1936) and by STURTEVANT and BEADLE (1936) in their demonstration of single crossing over within heterozygous inversions. The essential features of their analyses of the results of crossing over in this type of tetrad are incorporated into figure 1. At the reduction division the X-chromatids separate from the Y-chromatids; the figure shows only the second division segregation for the X-chromatids. The tetrads with no, one, or two exchanges are represented by the symbols E_0, E_1 or E_2, respectively. The two exchange tetrads (E_2) may involve 2, 3, or 4 strands and are designated as E_2-2s, E_2-3s and E_2-4s. It is to be noted that there are two different genetic consequences from the single exchange tetrads (E_1), and two from each of the three types of two exchange tetrads (E_2-2s, E_2-3s and E_2-4s). These different possibilities are distinguished by the letters a and b following the tetrad type. Three exchange tetrads are not considered here since, as will be shown below, they are relatively rare and can contribute little to the analysis.

From figure 1 it is clear that 50 percent is the maximum frequency with which rings may be expected, regardless of the distribution of tetrads of the different ranks since the no-exchange tetrads give rise only to attached X's and the two-exchange tetrads to 50 percent more attached X's than rings in the viable X-chromosome-bearing gametes.

Neither the work of SIDOROV, SOKOLOV and TROFIMOV nor that of STURTEVANT and BEADLE suggests any deficiency of the ring class. In the first case,

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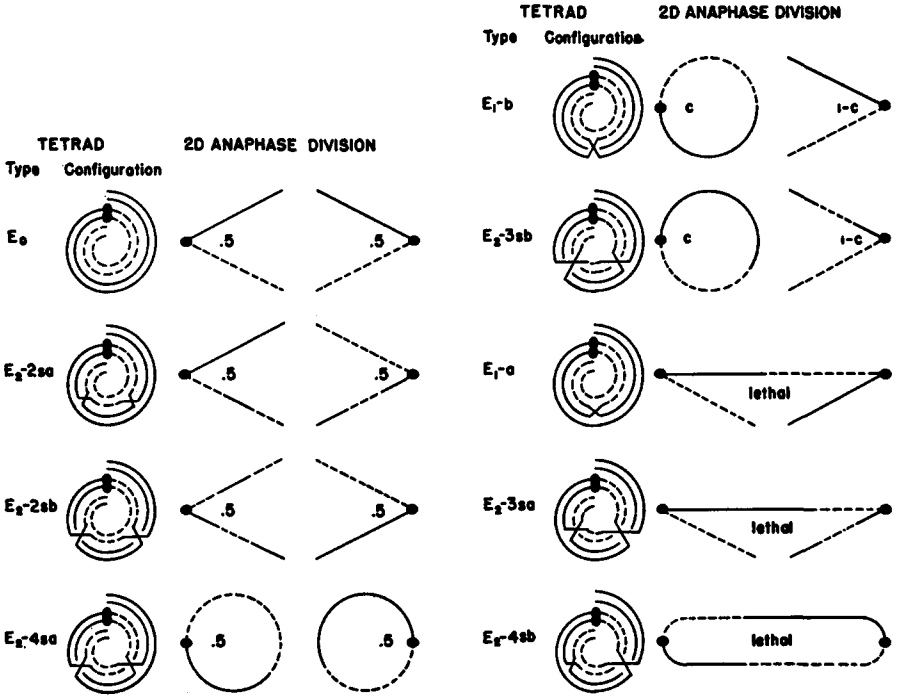

FIGURE 1.—Genetic consequences of no, one and two exchanges in attached X chromosomes heterozygous for an inversion. Fragments resulting after exchange are not diagrammed. Symbols are explained in the text.

the ratio reported is 824 attached X chromosomes to 1084 ring X chromosomes, and in the second 316 attached X chromosomes to 337 rings. It is a striking peculiarity of both sets of data that the frequency of rings exceeds 50 percent. SIDOROV, SOKOLOV and TROFIMOV, recognizing this discrepancy, make a viability correction which brings the percentage of rings down to 54 and suggest further that some undetected superfemales, phenotypically like the ring-bearing class, may have been confused with that class. The data of STURTEVANT and BEADLE, while less extensive, appear equally inconsistent with the tetrad analysis since (a) they conclude, as a best estimate of tetrad frequency, that $E_0 = .048$, $E_1 = .908$ and $E_2 = .044$, which should have given 294 rings to 359 attached X's and (b) the nature of their attached X chromosome, heterozygous for the y^4 inversion, was such as to produce a euchromatic duplication and deficiency ring chromosome which might have an adverse viability effect on the ring-bearing class.

The bearing of this type of experiment on the question discussed in the introduction and the incompatibility of the published data with the simplest type of tetrad analysis has led to a repetition of this experiment using an improved method. To circumvent viability complications, it was necessary to synthesize an attached X chromosome, heterozygous for an inversion, which could, by crossing over, give rise to ring chromosomes viable in the male, *i.e.*,

without any appreciable duplications or deficiencies. The method of doing this is described in detail elsewhere (NOVITSKI and LINDSLEY 1950); it consists essentially of combining, in a 3N female, a ring chromosome with good male viability (X^{c2}, *cv v f*), a chromosome with normal sequence (*y Hw*) and an inverted chromosome (*In* (1) sc^8, *f y*, *In* (1) EN), and extracting the double crossover in which one of the strands of the ring has crossed over with a chromatid of each of the other chromosomes, thus producing an attached X chromosome effectively heterozygous for an inversion. The crossover product ring from this chromosome is structurally like the X^{c2} chromosome introduced initially into the 3N parent. If a period is used to designate the position of the centromere, the attached X chromosome synthesized in this way carries the mutant genes and their normal alleles in the following order, $y\ Hw\ cv^+\ v\ f \cdot y^+\ Hw^+\ cv\ v^+\ f$, and will be referred to as *Hw f* for the sake of brevity.

The F_1 of the cross *Hw f* ♀ ♀ × sc^{S1} *B In S* w^a sc^8 (Muller-5) ♂ ♂ are given in column A of table 1. Since the male parent did not carry any of the mutants found in the derived rings, the female ring-bearing F_1 are listed only in the total row. The ring chromosome carrying *f* but none of the other recessive mutants must have arisen in a three-exchange tetrad; the low number, 1,

TABLE 1

F_1 of y Hw v f. cv f♀♀ × sc^{S1} *B InS* w^a sc^8♂♂*(A) and* × *sc cv v f B*♂♂*(B)*
Region I, y-cv; Region II, cv-v; Region III v-centromere.

X chromosome constitution	Phenotype	A	B	A + B
Attached X, non crossover	*Hw f* ♀♀	350	624	974
Attached X, crossovers in regions II and III	*Hw v f* ♀♀	17	52	69
Attached X, crossovers in regions I and III	*Hw cv f* ♀♀	30	19	49
Total attached X		397	695	1092
Ring X chromosomes				
Crossovers in region I	*v f B* ♀♀	.....	178	
	v f ♂♂	99	215	314
Crossovers in region II	*cv v f B* ♀♀	....	477	
	cv v f ♂♂	242	542	784
Crossovers in region III	*cv f B* ♀♀	....	432	
	cv f ♂♂	240	472	712
Crossovers in regions I, II and III, from 3 exchange tetrads	*f B* ♀♀	....	3	
	f ♂♂	1	1	2
Ring bearing ♀♀	*B* ♀♀	730	....	1820
	(cv) (v) f B ♀♀	....	1090	
Ring bearing ♂♂	*(cv) (v) f* ♂♂	582	1230	1812
Total ring classes		1312	2320	3632
Patroclinous ♂♂	*B* w^a ♂♂	1883	....	5488
	sc cv v f B ♂♂	....	3605	

compared to the total is considered ample justification for disregarding tetrads of rank higher than two in the analysis.

Since half of the attached X gametes are lost by fertilization with an X-bearing sperm whereas all the ring chromosome gametes can be recovered, the attached X class must be multiplied by two for comparison with the total of the ring class or, alternatively, the attached X class may be compared directly with either the male or the female ring class. The data indicate quite clearly that there is an excess, well beyond 50 percent, of the ring class. An additional set was run in which the *Hw f* ♀ ♀ were mated to *sc cv v f B* ♂ ♂ ; the results are given in column B of table 1. In this case all the F_1 are homozygous or hemizygous for *f* and the female progeny carrying the ring, as well as the male, also carry other markers so that any viability complications arising from an inequitable distribution of mutant characters should decrease the frequency of the ring classes to a greater extent than the attached X classes. Here, as before, there is an excess of rings, the ratios calculated in the way suggested above being 1390 attached X to 2320 rings, 695 attached X to 1090 rings or 695 attached X to 1230 rings. It should also be noted here that the suggestion of SIDOROV, SOCOLOV and TROFIMOV that undetected superfemales contribute to the excess is invalid in this experiment since they would have a unique phenotype, *Hw f B*, even if none of the typical superfemale characteristics were obvious.

It seems unquestionable that the discrepancy is real and that, therefore, the tetrad analysis presented earlier is in some way defective. The effects of relaxing certain of the assumptions inherent in the analysis will be considered briefly. The assumption of sister strand crossing over in a tetrad analysis reduces the calculated number of no-exchange tetrads (WEINSTEIN 1936). The present problem centers around an observed deficiency of attached X chromosomes, which can be considered mathematically as arising from a negative number of no-exchange tetrads, and the further reduction of this number by the assumption of sister strand crossing over would enhance rather than alleviate the difficulty in interpretation.

Secondly, it has been assumed that the first division is reductional and the second equational. If the opposite were the case, even if only in a proportion of all tetrads, the chromatid bridges shown in figure 1 which are assumed to give rise to lethal zygotes, following STURTEVANT and BEADLE (1936), would instead be first anaphase bridges insuring the passage of a Y chromosome to the functional egg nucleus. As pointed out by the above workers, this would increase the number of patroclinous males recovered but would not alter the theoretically expected proportions of attached X or ring chromosomes. They conclude that, since the ratio of patroclinous males to total females approaches but does not exceed a 2:1 ratio (the value given by the data in table 1 is 1.88:1), second division bridges must be lethal. It may be added, as a corollary to this, that in no appreciable proportion of the tetrads can the first division have been equational rather than reductional.

Finally, it has been taken for granted that the two-, three- and four-strand

types of two exchange tetrads occur in the ratio of 1:2:1, *i.e.*, there is no chromatid interference. From figure 1 it is obvious that any excess of rings over attached X's would have to come from one of the four-strand double classes, E_2-4sa. It is possible to calculate the relative frequencies of the three types of two exchange tetrads (the nature and extent of chromatid interference) necessary to account for the observed excess of ring chromosomes from the data in table 1. Attached X chromosomes (XX) bearing gametes arise from no exchange, two-strand double exchange, and a quarter of the single- and three-strand double exchange tetrads, and are recovered as viable zygotes in that half of the cases where the egg is fertilized by a Y-bearing sperm:

$$2XX = E_0 + E_2\text{-}2s + \tfrac{1}{4}\, E_1 + \tfrac{1}{4}\, E_2\text{-}3s.$$

Ring chromosomes (X^c) are derived from a quarter of the single and three-strand double exchange tetrads, and from half of the four-strand double exchanges:

$$X^c = \tfrac{1}{4}\, E_1 + \tfrac{1}{4}\, E_2\text{-}3s + \tfrac{1}{2}\, E_2\text{-}4s.$$

Furthermore the total of the X-chromosome-bearing gametes should equal the number of Y-chromosome-bearing gametes which may be estimated by doubling the frequency of patroclinous males (Pat ♂ ♂):

$$2\ \text{Pat}\ ♂\ ♂ = E_0 + E_1 + E_2\text{-}2s + E_2\text{-}3s + E_2\text{-}4s.$$

If the values of XX, X^c and Pat ♂ ♂ (1092, 3632 and 5488 respectively) from table 1 were the consequences of regular segregation, the above equations would be satisfied by the following equalities:

$$\begin{aligned} E_2\text{-}4s &= 4208 \\ E_1 + E_2\text{-}3s &= 6112 \\ E_0 + E_2\text{-}2s &= 656. \end{aligned}$$

However, since in ordinary crossover experiments all the four-strand double exchange tetrads and half of the single and three-strand doubles give rise to gametes carrying single crossover chromatids, such an array of tetrads would give rise to $(4208 + \tfrac{1}{2} \cdot 6112)/(4208 + 6112 + 656)$ or 68 percent recombination between X chromosome genes at the extremes of the chromosome. This value is clearly contradicted by the failure of recombination to exceed 50 percent in such experiments.

From the above it seems reasonable to suppose that the appropriate solution is consistent with the usual assumptions of conventional tetrad analyses, lack of sister strand crossing over and chromatid interference, but that after the ring chromosomes are formed by crossing over, more of them get into the functional egg nucleus than do the attached X chromosomes. Such a non-randomness of segregation might be visualized as operating in the following way: During the first anaphase, as the two X-chromatids progress towards the presumptive region of the functional egg nucleus, an orientation of the dyad is set up in which the ring chromatid, perhaps because of its smaller size, tends to occupy a position away from the first metaphase plate. At the second division, which immediately follows the first, the ring may then be advan-

tageously located for inclusion in the female oötid. The orientation of the dyad assumed responsible for determining the chromatid to be included in the functional egg nucleus may be more specifically defined as an orientation of the centromere region. The questions arising from this consideration appear to be similar to those involved in the directed second division segregation of normal chromatids by first anaphase bridges produced by single crossing over within inversion heterozygotes (STURTEVANT and BEADLE 1936) where the chromatid bridge orients the centromere region of the dyad prior to the second division (CARSON 1946).

EXPERIMENTS WITH ROD CHROMOSOMES

Non-randomness resulting from competition of structurally different chromatids for inclusion in the functional egg nucleus might be limited to the sort of experiment described previously but could be a more general property of the meiotic process in Drosophila. There are several general types of chromosome combinations that give rise, by crossing over, to dissimilar chromatids: translocation heterozygotes, duplication and deficiency heterozygotes. These, as a rule, produce also inviable zygotes or involve chromosomes of low viability which may lead to certain ambiguities in interpretation. The experiments to be described here make use of a heterochromatic deficiency for *bb* and block A (*Bk A*) of the X chromosome. The deficiency for *Bk A* has been reported to have no effect on the viability or fertility of an otherwise normal male, or when homozygous in the female, but decreases the length of the metaphase X chromosome by about a third (MULLER and GERSHENSON 1935; MULLER, RAFFEL, GERSHENSON and PROKOFYEVA-BELGOVSKAYA 1937).

The chromosomes used are those synthesized by RAFFEL and MULLER (1942) by combining the right and left ends of certain scute inversions, *In* (1) sc^8, *In* (1) sc^4, and *In* (1) sc^{81}. These inversions have been analyzed in detail by the above workers and have been shown to have the following characteristics: The left breakpoint of the inversion is to the left of the scute gene in the case of *In* (1) sc^8 and to the right in the other two; the right breakpoint is to the left of *bb* and *Bk A* in *In* (1) sc^4 and to the right in the other two. The crossover product $sc^4\ sc^8$ carries a scute gene at the base as well as the tip but is deficient for the heterochromatic region including *bb* and *Bk A*. The $sc^{81}\ sc^8$ combination, like the sc^8 chromosome, has a scute gene at the base and the heterochromatic region including the *bb* locus and *Bk A* at the tip. The essential difference between the two types of chromosomes is that $sc^4\ sc^8$ lacks the heterochromatic region, whereas both sc^{81} and sc^8 carry it at the end and so are physically longer.

The initial cross consisted of females of the compositions *y* sc^4 *car m* w^a sc^8/ *In* (1) *dl-49, y w* lz^s and sc^{81} *car m* w^a/*In* (1) *dl-49, y w* lz^s mated to sc^8 *f v cv* males. F_1 ♀ ♀ of the genotype sc^8 *f v cv*/*In* (1) *dl-49, y w* lz^s were mated to y^2 *cv v f* ♂ ♂; their progeny consisted of 674 *cv v f* ♀ ♀, 781 *y* ♀ ♀, 609 *cv v f* ♂ ♂ and 414 *y w* lz^s ♂ ♂. Since all classes should have been equal in

frequency, the inequalities give a measure of the effect of the mutant genes in the two sexes in decreasing the viability. There were 86 percent as many *cv v f* ♀ ♀ as *y* ♀ ♀ and 78 percent as many *cv v f* ♂ ♂. In the crossover experiments below, the complementary crossover types counted as females carry less extreme combinations and therefore the figure of 86 percent should represent the maximum deviation that can be attributed to inviability.

The progeny of the cross *y sc*^{4} *car m w*^{a} *sc*^{8}/*sc*^{8} *f v cv* ♀ ♀ × *y*^{2} *cv v f* ♂ ♂ are given in table 2. The crossovers are classified separately for sex and for the presence of yellow, which marks the shorter deficient chromosome. The identifiable classes are more numerous in the male progeny than the female, being 1 (1′ + 1″), 2 (2′ + 2″) and 3 in the female and in some instances classification of *car* was not possible because of the presence of *w*^{a} and *v*. In those cases a vertical arrow through the classes points to the more general class for which the identification was unambiguous. A dash indicates that that class could not have been identified in any case. A question mark, as in the 3,4 yellow crossover type, indicates an ambiguous class; in this instance such crossovers would probably have been included in the 2″ class.

TABLE 2

Experiment C. F_1 *of* $\frac{y\ sc^4\ 1'\ car\ 1''\ 2'\ m\ 2''\ 3\ 4\ w^a\ sc^8}{Het \qquad f \qquad v\ cv \qquad sc^8}$ ♀♀ × *y*^{2} *cv v f* ♂♂. *The abbreviation "Het" refers to the heterochromatic block missing in the sc*^{4} *sc*^{8} *combination chromosome. Other symbols are explained in the text.*

Crossover regions	*y* ♂♂	Het ♂♂	*y* ♀♀	Het ♀♀	Crossover regions	*y* ♂♂	Het ♂♂	*y* ♀♀	Het ♀♀
0	648	344	771	435	1′, 2′	↓	3		
					1″, 2′		1		
1′	67				1 , 2′	9	4		
1″	89	↓	↓	↓	1″, 2″	4	2		
1	156	82	164	91	1′, 2″	2	0	↓	↓
2′	274	118			1 , 2	15	10	9	6
2″	29	13	↓	↓	2′, 4	18	8	....	....
2	303	131	305	167	2″, 4	0	2	....	....
3	206	90	194	97	2′ 3	14	10		
4	44	32	....	....	2″, 3	0	1	↓	↓
					2 , 3	14	11	22	17
1′, 4		2	....	....	3 , 4	?	3	....	....
1″, 4	↓	5	....	....	2′, 2″	2	2	....	....
1 , 4	16	7	....	....	1″, 2′, 4	1	0		
1′, 3		7			1′, 1″, 3	1	0		
1″, 3	↓	11	↓	↓	1′, 2′, 4	1	0		
1 , 3	21	19	35	24	1′, 2′, 3	1	0		
					1 , 2″, 3	0	1		

Simple inspection is sufficient to show that there is a pronounced excess of progeny carrying the smaller chromosome regardless of crossover class or sex, and that this excess is well beyond any expectation formulated in terms of viability effects. The female crossovers in region 1, for instance, give a ratio of 164/91, yet the larger class is homozygous for the mutants *y, cv, v* and *f*, the smaller wild-type.

A different picture is presented by the cross of $sc^{81}\ sc^{8}/sc^{8}$ ♀ ♀ × $y^{2}\ cv\ v\ f$ ♂ ♂ (table 3). Class for class, the ratios vary around 1 : 1. This is not unexpected since both chromosomes carry the heterochromatic region at the ends. This cross constitutes an additional control on the preceding one and shows that the deviations from a 1 : 1 ratio in that case must be a function of the left end of the $sc^{4}\ sc^{8}$ combination chromosome.

TABLE 3

Experiment D. F_1 of $\frac{\text{Het car } 1''\ 2'\ m\ 2''\ 3\ 4\ w^{a}\ sc^{8}}{\text{Het} \quad / \quad v\ cv \quad sc^{8}}$ ♀♀ × $y^{2}\ cv\ v\ /$ ♂♂.

Crossover regions	*car* ♂♂	non-*car* ♂♂	/ ♀♀	non-/ ♀♀	Crossover regions	*car* ♂♂	non-*car* ♂♂	/ ♀♀	non-/ ♀♀
0	296	323	391	378	1″, 4	?	4	....	....
					1″, 3	?	3	....	....
1″	30	24	....	....	2′, 4	11	6	....	....
2′	107	74	↓	↓	2″, 4	0	1	....	....
2″	11	22			2′, 3	16	10	↓	↓
2	118	96	150	157	2″, 3	0	1		
3	87	108	102	119	2 , 3	16	11	17	7
4	27	24	....	....	2′, 2″	?	1	....	....

INTERPRETATION OF THE ROD CHROMOSOME RESULTS

The tetrad analysis for the $sc^{4}\ sc^{8}/sc^{8}$ combination is diagrammed in figure 2. It is conventional in all respects except that it is assumed that when the two structurally different chromatids separate at the second meiotic division the probability that the shorter will be included in the functional egg nucleus is not .5 but c, with the probability for the longer being $(1-c)$. Reference to figure 2 reveals that non-randomness of this type would be effective only in the single exchange tetrads (E_1) and the three-strand doubles (E_2-3sa and E_2-3sb). The frequency of each type of recovered strand may be found by summing the proportions of each rank of tetrad giving rise to that type. The abbreviations non, sgl and db are used to indicate non-crossover, single and double crossover strands, respectively; the superscript $^{-}$ designates those recovered strands having the heterochromatic deficiency (*i.e.*, the shorter chromosome) and the superscript $^{+}$ the contrary class. Absence of a superscript represents the sum of the two classes. In the absence of chromatid interference $E_2\text{-}2s = E_2\text{-}3sa = E_2\text{-}3sb = E_2\text{-}4s = \frac{1}{4}\ E_2$, then

$$\begin{aligned}
non^{-} &= \tfrac{1}{2}\ E_0 + \tfrac{1}{2}\ cE_1 + \tfrac{1}{8}\ cE_2 + 1/16\ E_2 \\
non^{+} &= \tfrac{1}{2}\ E_0 + \tfrac{1}{2}\ (1-c)E_1 + \tfrac{1}{8}\ (1-c)E_2 + 1/16\ E_2 \\
non &= E_0 + \tfrac{1}{2}\ E_1 + \tfrac{1}{4}\ E_2 \\
sgl^{-} &= \tfrac{1}{2}\ cE_1 + \tfrac{1}{4}\ cE_2 + \tfrac{1}{8}\ E_2 \\
sgl^{+} &= \tfrac{1}{2}\ (1-c)E_1 + \tfrac{1}{4}\ (1-c)E_2 + \tfrac{1}{8}\ E_2 \\
sgl &= \tfrac{1}{2}\ E_1 + \tfrac{1}{2}\ E_2 \\
db^{-} &= 1/16\ E_2 + \tfrac{1}{8}\ cE_2 \\
db^{+} &= 1/16\ E_2 + \tfrac{1}{8}\ (1-c)E_2 \\
db &= \tfrac{1}{4}\ E_2
\end{aligned}$$

From the equations for non, sgl and db it is clear that the aberrant distribution of crossover types does not affect the usual tetrad analysis provided only that the complementary classes are added together. Table 4 sums the data for the scute crosses; the five triple crossovers have been entered as doubles since the overall frequency of three exchange tetrads must be negligible. The frequencies of tetrads of different ranks have been calculated individually for the male and female classes, using the equations for the sums of the complementary types. With the tetrad values given, it is possible to

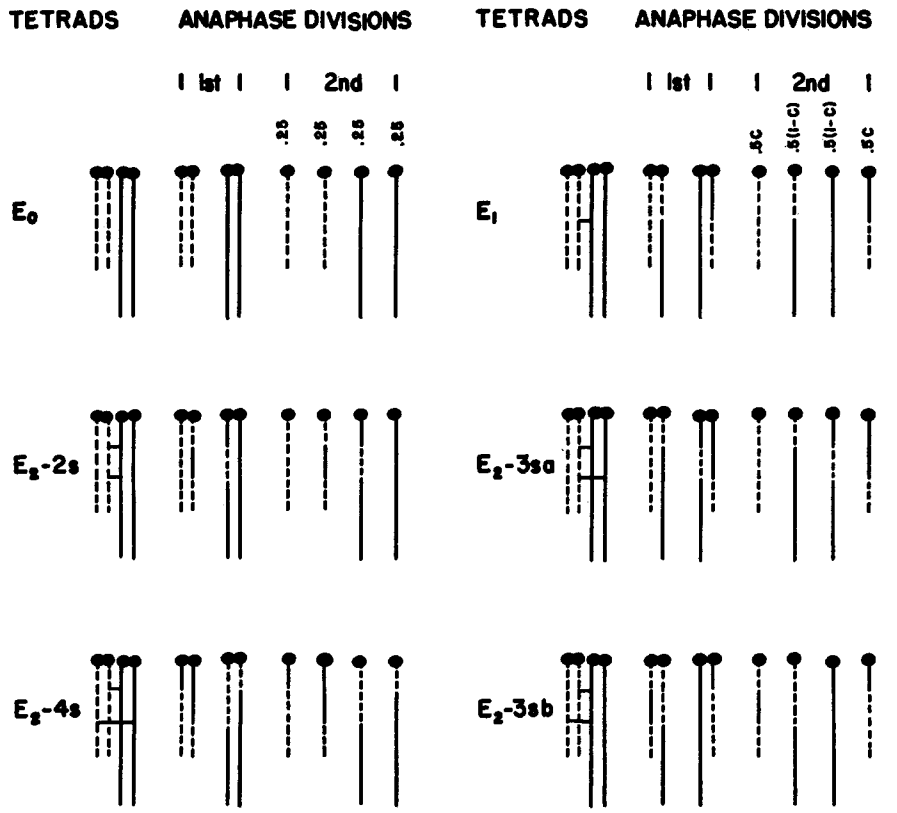

FIGURE 2.—Results of exchange in rod X chromosomes, one of which is deficient for part of the heterochromatic region. The symbols are the same as in figure 1.

calculate the value of c from six independent sets of values (complementary classes give, of course, identical values since the sum of those classes was used for calculating tetrad frequencies). These values listed in table 4 under column c range from .67 to .72, a rather remarkable and undoubtedly to some extent coincidental agreement. Nevertheless, they show quite clearly that the observed deviations, even in the non-crossover class, are accountable for on the assumption that the shorter chromosome is included in the functional egg nucleus about twice as frequently as the longer.

Similar calculations for the $sc^{81}\ sc^{8}/sc^{8}$ cross are presented in table 4. Certain of the double crossover classes are not determinable; a correction has been made by adding seven to this class from the single crossovers, since the ratio of the complementary classes from the other doubles is 1:1 (35/35) and there are seven flies in the complements of the ambiguous classes. The chromosomes carrying the sc^{81} end are entered in the $^{-}$ rows. The derived values of c are aberrant (.35 and .92) when calculated from the calculated double crossover classes of both sexes; the low number of individuals in these classes is undoubtedly responsible for these values. For the non-crossovers and singles, however, they range from .47 to .53, *i.e.*, segregation is random.

TABLE 4

Summation of the non-, single and double crossovers from tables 2 and 3, with the calculated frequencies of tetrads of different rank, and the values for c.

	$sc^{4}\ sc^{8}/sc^{8}$				$sc^{81}\ sc^{8}/sc^{8}$			
	♂		♀		♂		♀	
	N	c	N	c	N	c	N	c
non^{-}	648	.68	771	.70	296	.47	391	.51
non^{+}	344		435		323		378	
non	992		1206		619		769	
sgl^{-}	709	.71	663	.67	262	48	276	.53
sgl^{+}	335		355		245		252	
sgl	1044		1018		507		528	
db^{-}	90	.72	66	.67	25	.35	7	.92
db^{+}	58		47		34		17	
db	148		113		59		24	
Total	2184		2337		1185		1321	
E_2	592		452		236		96	
E_1	1496		1584		778		960	
E_0	96		301		171		265	

ANALYSIS OF THE ATTACHED X CHROMOSOME RESULTS

The above formulation may be applied to the tetrad analysis of the attached X chromosome heterozygous for an inversion. The tetrad frequencies will refer here only to that half of the gametes receiving an X centromere, although the estimate of the total number of such gametes comes from the number of patroclinous males, that is, those receiving a Y chromosome. There are four distinguishable classes, the total of females with attached X's, those with attached X's equational for one or more of the heterozygous mutants, ring-bearing males and females and patroclinous males, to be abbreviated as XX, c-o XX, X^{c}, and Pat ♂ ♂, respectively. From figure 1 it can be seen that

$$XX = E_0 + (1-c)E_1\text{-b} + E_2\text{-2s} + (1-c)E_2\text{-3sb}.$$
$$X^{c} = cE_1\text{-b} + cE_2\text{-3sb} + E_2\text{-4sa}$$

Equationals are detected only in half the cases of homozygosis, so

$$\text{c-o XX} = \tfrac{1}{2}\,E_2\text{-2sa} + \tfrac{1}{2}\,(1-c)E_2\text{-3sb}$$

and the number of Y-bearing gametes recovered as patroclinous males after fertilization by an X-bearing sperm should equal one-half those receiving X-chromatids, or

$$\text{Pat } ♂\,♂ = \tfrac{1}{2}(E_0 + E_1 + E_2)$$

Furthermore, $E_1a = E_1b = \tfrac{1}{2} E_1$, E_2-2sa = E_2-2sb, E_2-3sa = E_2-3sb, E_2-4sa = E_2-4sb and in the absence of chromatid interference, E_2-2s = E_2-3sa = E_2-3sb = E_2-4s = $\tfrac{1}{4} E_2$.

A very convenient expression for c is given by

$$c = \frac{X^c}{2\,\text{Pat } ♂\,♂ - XX - X^c} - \frac{E_2(1-c)}{4E_1 + 3E_2}$$

(The derivation of this solution is tedious; its validity may be demonstrated, however, by substituting for Pat ♂ ♂, XX, and X^c the terms given in the initial equations.)

E_2 is somewhat smaller than E_1 in the usual tetrad analysis (see, for instance, the values calculated for E_1 and E_2 from the scute inversion experiments). Disregarding the last term of the equation in computing c leads to an overestimate which equals zero when c is one, becoming progressively larger as c decreases and amounting perhaps to as much as five percent when $c = .5$. The extent of the error is insignificant compared to the inherent variability of the raw data. The advantage to the simplified equation is that it does not involve the number of the crossover attached-X's, a class which is small, difficult to determine accurately and, for the data collected by SIDOROV, SOKOLOV and TROFIMOV, not available.

In determining c, the observed number of XX progeny must be doubled since a half are lost by fertilization by an X-bearing sperm, and when the derived ring chromosome is lethal in the male, as it is in experiments of SIDOROV, SOKOLOV and TROFIMOV, and of STURTEVANT and BEADLE, that figure must also be doubled. The calculated values are given in table 5.

TABLE 5

Determination of c from the data on the attached-X experiments.

Experiment of	2 Pat ♂♂	2XX	X^c	c
STURTEVANT & BEADLE	2196	632	674	.76
SIDOROV, SOKOLOV & TROFIMOV (1936)	7062	1648	2168	.67
Experiment A	3766	794	1312	.79
Experiment B	7210	1390	2318	.66

DISCUSSION

The possibility that newly formed ring chromosomes may be lost in some fraction of all cases by an interlocking of daughter chromatids after the first reduplication cannot be denied nor confirmed from the experiments with an attached X heterozygous for an inversion. The degree of non-randomness of disjunction (c) necessary to account for the observed data has been calculated

by assuming no loss; it seems evident that a higher value for c with some loss of ring chromosomes would be equally compatible with the data. Although the information from the two kinds of experiments, with attached X's and with the scute inversions does not give striking differences in the value of c, it seems reasonable to suppose that the magnitude of the competition between structurally dissimilar chromatids must be some function of the degree and kind of dissimilarity.

As mentioned previously, a number of different kinds of genetic constitutions should give rise to asymmetrical dyads at the second meiotic division, with subsequent non-random disjunction. A number of such instances can be found in previously published work and may be considered briefly here. MATHER (1939) mentions that "the sc^8 deficiency chromosome requires further comment . . . (it) shows a very peculiar type of segregation from normal sc^8 chromosomes. In the daughters of a sc^8/sc^8 def. female the deficiency is recovered just twice as frequently as the corresponding portion of the other chromosomes. . . . The reasons for this behavior are not fully known, but it seems to be bound up with a maternal effect." Further, STURTEVANT and BEADLE (1936), from a similar experiment found a total of 186 sc^8 progeny and 133 sc^8 deficiency progeny. The sc^8 deficiency chromosome is lethal in the male, unlike the $sc^4\ sc^8$ combination chromosome used in the experiments described in this paper; the deviation from the 2:1 ratio expected, $(♀♀+♂♂):♀♀$, is significant ($P < .002$) but agrees with $sc^4\ sc^8/sc^8$ experiment ($P = .25$) despite a distribution of mutants in their experiments which should decrease the deficiency class disproportionately and the existence of one highly aberrant crossover class (the complementary types of crossovers in region 4 were in the ratio of 29 sc^8 chromosomes to 2 sc^8 def. chromosomes) tending to obscure the extent of the discrepancy.

The bearing of the suggestion of non-random separation on the interpretation of segregation in translocation heterozygotes is well illustrated by the data presented by GLASS (1934, 1935). Three different translocations, each involving a breakpoint at the right end of the second chromosome and one near the centromere of the third, were studied, in the heterozygous state, with respect to the frequencies of different combinations produced. GLASS points out that ". . . one of the complementary aneuploid classes is almost twice the size of the other" and further tests show that ". . . the phenomenon is not one of differential viability of the classes; nor can it be due either to a gametic lethal, or to lethality of gametes carrying a particular type of chromosomal abnormality." He concludes that possibly "the presence of ordinary recessive lethals, by crossing over, eliminates the non-disjunctional zygotes carrying them in homozygous condition," but points out that the assumption of maximum crossing over could be responsible for a loss only one-third as great as that observed. The fact that all three translocations show precisely the same effect when heterozygous (V_3/+, 279/151; V_4/+, 1242/709 and V_5/+, 246/125) can be considered fairly strong evidence that the accidental occurrence of a recessive lethal in the translocation stocks is not responsible for the approximate 2:1 ratio of the aneuploid types.

Interpretation on the basis of non-random disjunction is not only consistent with the experiments reported above, but, in fact, leads to a simplified concept of the nature of the segregation process in these translocation heterozygotes. Let 2L · 2R and 3L · 3R represent the normal second and third chromosomes, respectively, 2L · 2R + 3L and · 3R the translocated chromosomes, with the position of the centromere marked by the dot. When an exchange occurs in arm 2R, a dyad of composition 2L · 2R/2L · 2R + 3L is formed and the resulting competition for inclusion in the oötid leads to a more frequent recovery of 2L · 2R than 2L · 2R + 3L. The aneuploid gametes are of the following constitutions: 2L · 2R, · 3R (the more frequent) and 2L · 2R + 3L, 3L · 3R (the less frequent). Segregation at the first meiotic division is always of the centromere region of one chromosome arm from the centromere region of the homologous arm, giving rise to dyads of composition 2L · 2R/2L · 2R, 3L · 3R/3L · 3R and 2L · 2R + 3L/2L · 2R + 3L, · 3R/ · 3R from the no-exchange tetrads and to dyads of composition 2L · 2R/2L · 2R + 3L, 3L · 3R/3L · 3R and 2L · 2R + 3L/2L · 2R, · 3R/ · 3R when an exchange occurs between the centromere and the translocation. Thus, the observed frequency of aneuploid gametes may not necessarily result from a type of segregation at the first division different from that which produces orthoploid gametes, but may be interpreted as a simple consequence of exchange between the centromere and the translocation breakpoint. It should be pointed out that such an interpretation might be applicable for this type of unequal-armed translocation, but can hardly be considered an explanation for the production of aneuploid gametes by other types of translocations.

Although the production of structurally asymmetrical dyads in Drosophila is achieved only by the use of special genetic constitutions, there is, in some other animals, one chromosome pair for which this could be a regular feature, namely, the sex chromosomes. Where the female is the heterogametic sex, a difference in size of the sex chromosomes, with a homologous region between the centromere and the differential segment allowing exchange, could lead to an excess of gametes carrying one of the sex-chromosomes, with the extent of the excess depending upon the frequency of exchange and the degree of competition. However, where the male is heterogametic and all the products of meiosis are functional, non-randomness could have no role without the further assumptions that there is, in fact, an orientation of the second meiotic division spindle with respect to the first and that some physiological or genetic mechanism (as, for instance, chromatid bridges involving any one of the autosomes) operates to eliminate certain of the spermatids differentially. Evidence available at present does not support such a scheme (SCHULTZ and ST. LAWRENCE 1949) but without a more thorough knowledge of the chromosome behaviour in such cases it cannot be dismissed as a possibility to account for some measure of the observed discrepancies in the primary sex ratio.

SUMMARY

The frequency of recovery of ring chromosomes from attached X chromosomes heterozygous for an inversion is shown to be inconsistent with the

expectations based on a tetrad analysis; the cause appears to be non-random disjunction at the second meiotic division when two structurally dissimilar chromatids compete for inclusion in the functional egg nucleus. Experiments involving structurally dissimilar rod chromosomes confirm this effect and the bearing of this phenomenon on the interpretation of previously existing data, particularly that on segregation in translocation heterozygotes, is discussed. It is suggested that such non-random disjunction could be responsible to some extent for observed deviations from the expected 1 : 1 sex ratio in animals where sex is determined by a heteromorphic pair of chromosomes.

LITERATURE CITED

CARSON, H. L., 1946 The selective elimination of inversion dicentric chromatids during meiosis in the eggs of *Sciara impatiens*. Genetics **31:** 95–113.

GLASS, H. B., 1934 The effect of lethal genes on the non-disjunctional classes in mutual translocations of Drosophila. Z. i. A. V. **67:** 255–258.

1935 A study of factors influencing chromosomal segregation in translocations of *Drosophila melanogaster*. U. of Mo. Res. Bull. **231:** 1–28.

MATHER, K., 1939 Crossing over and heterochromatin in the X chromosome of *Drosophila melanogaster*. Genetics **24:** 413–435.

MULLER, H. J., and S. M. GERSHENSON, 1935 Inert regions of chromosomes as the temporary products of individual genes. Proc. Nat. Acad. Sci. **21:** 69–75.

MULLER, H. J., D. RAFFEL, S. M. GERSHENSON, and A. A. PROKOFYEVA-BELGOVSKAYA, 1937 A further analysis of loci in the so-called "inert region" of the X chromosome of Drosophila. Genetics **22:** 87–93.

NOVITSKI, E., and D. L. LINDSLEY, 1950 Construction of tandemly attached X chromosomes. D.I.S. **24.**

RAFFEL, D., and H. J. MULLER, 1940 Position effect and gene divisibility considered in connection with three strikingly similar scute mutations. Genetics **25:** 541–583.

SCHULTZ, J., and P. ST. LAWRENCE, 1949 A cytological basis for a map of the nucleolar chromosome in man. J. Hered. **40:** 31–38.

SIDOROV, B. N., N. N. SOKOLOV, and I. E. TROFIMOV, 1935 Forces of attraction of homologous loci and chromosome conjugation. Nature **136:** 108–109.

1936. Crossing over in heterozygoten Inversionen. Genetica **18:** 291–312.

STURTEVANT, A. H., and G. W. BEADLE, 1936 The relations of inversions in the X chromosome of *Drosophila melanogaster* to crossing over and disjunction. Genetics **21:** 554–604.

WEINSTEIN, A., 1936 The theory of multiple-strand crossing over. Genetics **21:** 155–199.