A CLOSED X CHROMOSOME IN DROSOPHILA MELANOGASTER*

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From a pair of attached X chromosomes of *Drosophila melanogaster* there is derived occasionally a single detached X chromosome which shows all the characteristics of a normal X. In consequence a very small class of regular offspring is found in attached X lines. One yellow female having attached X chromosomes mated to scute broad apricot males produced a regular wild-type female, the phenotype to be expected if an egg bearing a detached X had been fertilized by X sperm; but among the offspring of the wild-type female there was an unexpected class of males. She had been mated to a forked bar male and produced, besides about 12 yellow and 35 scute broad apricot males, 7 forked bar males. The wild-type female had received one yellow-bearing chromosome from her mother and an X chromosome bearing genes for scute broad apricot from her father. If she had received in addition to a single yellow-bearing chromosome also a Y chromosome from her attached X mother, exceptional males would have been expected from Y eggs, but secondary exceptional wild-type females would also have been expected. In fact all of the daughters were heterozygous bar; moreover all of the forked bar males were found to be sterile, and were evidently primary non-disjunctional XO males. Some of the F_1 heterozygous bar females were mated to X-ple males ($sc \; ec \; cv \; ct^6 \; v \; g^2 \; f$) and again sterile non-disjunctional males were produced, and among the regular classes there were unusual ratios (table 1). Whereas about 50 percent of crossing over is expected between yellow and forked, only 6 among 225 regular males showed recombination of the characters.

TABLE 1

Offspring of $\dfrac{X^0 y}{fB}$ ♀ × X-ple ♂.

NO. OF CULTURE	♀ ♀		♂♂				
			NON-CROSSOVERS		CROSSOVERS		PATROCLINOUS
	+	fB	y	fB	yfB	+	X-ple
2.54a	70	82	56	87	0	3	2
2.54b		166	48	58	0	3	1

The yellow-bearing chromosome for two generations had shown certain peculiarities which were hereditary. Further tests showed that the chromosome is unique, in that among the offspring of females homozygous or heterozygous for the chromosome there are no single or triple crossovers, but there are many patroclinous males and gynandromorphs are unusually frequent (L. V. MORGAN 1929).

<div align="center">CYTOLOGY</div>

The unique chromosome in preparations of oogonial plates appears to be a thick almost circular ring, clearly hollow in the middle. The ring
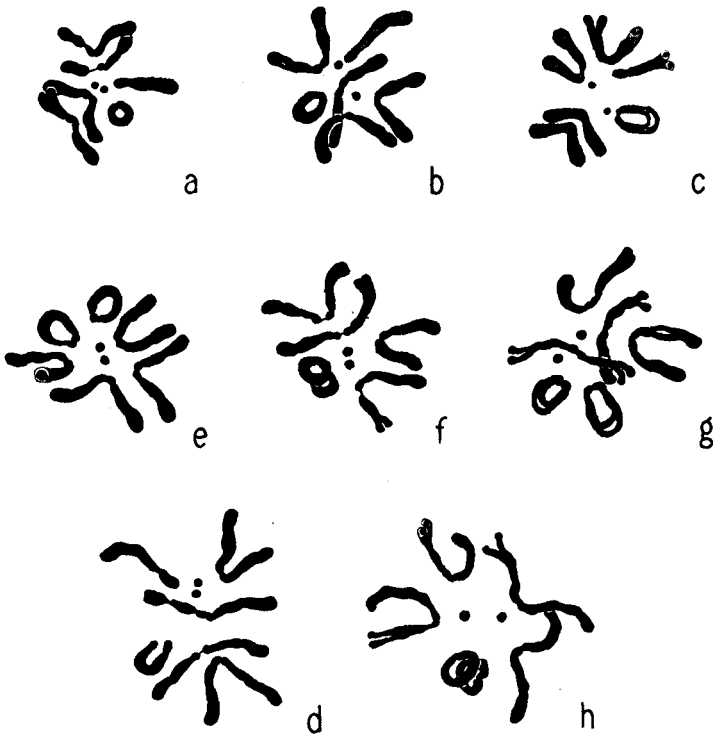

FIGURE 1.—The drawings represent metaphase-plates from the nerve cells of the ganglia of larvae of females carrying closed X^0 chromosomes. Figures a–d (from heterozygous females) contain one closed X^0 chromosome. Figures e–h (from homozygous females) contain two closed X^0 chromosomes. The drawings were made with the aid of camera lucida, at the level of the work table. The magnification is about 5300.

often appears to be closed, but at some optical levels it is somtimes slightly open on the side toward the centre of the plate, which led to its description "an almost or entirely closed and somewhat rounded letter U" (L. V. MORGAN 1926). Dr. DOBZHANSKY has very kindly made preparations and drawings of metaphase plates from the nerve-cells of the ganglia of female larvae (figure 1); in these the chromosomes are less contracted and

details of their structure are more clearly seen. In the larval nerve-cells it appears that the two ends of the curved chromosome are indeed in contact (as seen in all figures except one, figure 1d), and that they are directed toward the centre of the plate. It appears also that the ends of the chromosome are thinner than the middle, hence the apparent opening in the more contracted oogonial chromosomes, especially if the chromosome is inclined to the plane of the section, when at some optical levels only the thicker sides of the chromosome are seen.

When the chromosome lies flat in the plane of the section it appears like a ring sometimes elongated in the axis which passes between the ends. It can be interpreted as an X chromosome of which the spindle fibre end is as usual in a position near the centre of the metaphase plate and in which the usually free end of the chromosome has been brought into contact with the spindle fibre end. Thus the cytology presents an X chromosome whose ends are united to each other forming a ring or oval such that interchange between two chromatids can form a single chromatid only when exchange has taken place at two points simultaneously.

Dr. DOBZHANSKY's figures show further that, when the chromosome divides, the split seems not to begin at the end, as occurs when the end is free, but in the middle of the closed chromosome (figure 1c and 1g).

The genetic and cytological results can be explained on the hypothesis that the unique X chromosome is a closed chromosome in which the left end is attached to its own right end near the spindle fibre attachment.

ATTACHMENT OF THE ENDS OF THE CLOSED CHROMOSOME

There is evidence from genetic tests that the point at which the ends are united is close to genes at the extreme left end and at the right end of the chromosome.

First, there is abundant evidence that the left end of the closed chromosome which carries the gene for yellow (y) is never separated by crossing over from the right end carrying the normal allelomorph for bobbed ($+^{bb}$), a locus near the right end. More than 11,750 female offspring of females heterozygous for y-closed and for bb, mated to bb males, have been examined and none showed recombination of the extreme end characters. A stock, kept by mass cultures, of females heterozygous for y-closed and for bobbed mated to bobbed males was made from material from which the closed chromosome had always been selected by selection of females carrying the gene for yellow. For three years no recombination of the loci of y and $+^{bb}$ occurred. Other stocks not marked for bobbed show linkage of the characteristics of the closed chromosome to yellow, for by selection of yellow, the closed chromosome has been kept since it was first found in 1922.

In order to test the closeness of the point of union to the gene for yellow, flies were made heterozygous for yellow carried by the closed chromosome and for broad (locus 0.6), or for prune (locus 1.0) or for allelomorphs of white (locus 1.5). No crossing over occurred between yellow and the locus for broad (among 6568 flies) or between yellow and the locus for prune (among 2138 flies) but 87 flies have been obtained that were crossovers between the loci of yellow and white. Of these, 25 were bred and all of them showed that the closed characteristics remained with yellow. Therefore, the factor or condition responsible for the chromosome being closed is so far to the left in the chromosome that 100 percent of 25 random samples of crossovers between 0 and 1.5 showed the locus of the factor to be to the left of the break.

Similarly the linkage of the point of union to the locus for bobbed was tested; 113 flies were obtained that were crossovers between carnation (4.5 units to the left of bobbed) and bobbed (near the end of the chromosome). Of many that were mated, 15 bred and showed that the characteristics of the closed chromosome accompanied the not-bobbed gene of the closed chromosome in each case, that is, the chromosome is closed by the attachment of the distal end to a point near the right end of the chromosome.

The genetic results showing the union of the ends of the chromosome to each other (locus of yellow to locus of bobbed) and the linkage of the point of union to the left end and to the right end of the chromosome are evidence for the hypothesis that the ends are physically attached and that the attachment is the condition whose effects are the characteristics peculiar to the closed chromosome.

It has been stated that a stock heterozygous for y-closed and for bb was maintained for three years by selecting females heterozygous for yellow, all of which were also not-bobbed. The chromosome carrying bobbed was also marked by scute and no scute not-bobbed female had ever been observed. At the end of that time there appeared scute not-bobbed females and also the percentage of yellow males increased, a class which had been extremely small up to that time, owing partly to inviability when the X chromosome is closed. The chromosome carrying yellow was tested and now gave among 1721 flies close to the standard percentages of both single and double crossovers that are regularly found between normal Xs, and did not produce the high percentage of patroclinous males which is found when a closed chromosome is involved. The chromosome was no longer distinguishable from a normal X.

Since the closed X^0 was derived from attached XX, the reopening of the closed X^0 suggests that it might have originated by the separation of a single X from attached XX, but this is not a satisfactory explanation, for,

if opening of the closed X^0 could occur in that way, it would have taken place frequently instead of only once.

Flies carrying a closed X^0 chromosome are poorly viable, and homozygous females and males show low fertility and sometimes abnormalities of the eyes or legs. In the reopened X^0 these peculiarities have disappeared, so they may be due to irregularities caused by the closed condition. Jagged wings often found in the presence of duplication of sections of the X chromosome have not been observed.

It has been suggested that the closed X^0 may have originated as a translocation of the yellow end of one attached X to a point near the spindle fibre attachment of the other X, or as a complete inversion in one attached X followed by single crossing over with the other. But both hypotheses imply the presence of a slight duplication at the spindle fibre end and leave open the possibility of release of the yellow end as when a detached X is derived from attached XX.

DOUBLE CROSSOVERS

The crossovers which are found among the offspring of a female carrying one or two closed chromosomes prove, when the chromosomes are completely marked, to be exclusively doubles or quadruples. It has been shown that among at least 11,750 flies from heterozygous mothers and in various heterozygous stocks those chromosomes that retained the left end of the closed chromosome carrying yellow also retained the right end carrying not-bobbed, and this linkage can be assumed to hold for flies that cannot be rated for bobbed. (In the first count of experiment A_1 two flies are recorded as recombinations of yellow and bobbed. Subsequently, whenever there was doubt as to the classification of bobbed, the fly was tested and among those that bred none showed recombination. The two exceptions had almost certainly been wrongly classified.) Using this assumption in classifying it was found that among 16,914 flies (table 10), all the crossovers, of which there were 2,211, were doubles or quadruples and none were singles or triples.

The genetic results show that if interchange takes place between a closed X^0 chromatid and a normal X chromatid simultaneously at two points, viable zygotes of both complementary classes may reach the adult stage.

FREQUENCY OF DOUBLE CROSSOVERS

The attachment of the ends of an X chromosome affords a new situation for crossing over since single crossovers are not represented by the usual classes. It is of interest to know whether there is an effect on the frequency of double crossing over for the whole chromosome and for different regions of the chromosome. Frequencies of double recombinations have been determined by the method of alternated backcross devised by

BRIDGES and OLBRYCHT (1926) in mapping the X chromosome. Their results will be referred to as the alternated X-ple or a.X. values. The chromosomes used by them were so marked that crossing over could be followed in six regions, from scute at 0 to forked at 62 with little or no chance of double crossing over between the marked loci. The character bobbed near the extreme right end of the chromosome was not included but when the experiments with the closed chromosome were made, bobbed was introduced, thereby bringing under observation a seventh region of the chromosome to the right of forked.

Material descended from the a.X. stocks of BRIDGES and OLBRYCHT was used to make stocks heterozygous for $X^0\ y\ cv\ v\ f$ and for $X^0\ y\ ec\ ct^6\ g^2$ and also to make the apposers and the testers that were used. The symbol X^0 is used to designate the closed chromosome.

The combined experiments with marked flies heterozygous for closed X^0 involved 16,914 flies not including those of non-disjunctional classes. As already stated, yellow in the closed chromosome is always linked to not-bobbed so that among males in which bobbed does not show, because of the normal allelomorph of bobbed carried in the Y chromosome, and among flies of experiment B_2, where the tester was not bobbed, all yellow chromosomes were rated as not-bobbed, and not-yellow as bobbed. The apparent single crossovers were in this way found to be doubles that involved the seventh region.

It is apparent from the counts of observed offspring of heterozygotes (appendix, table 10) that factors for differential viability are present. Flies containing a closed X^0 (yellow males and females heterozygous for yellow) occur less frequently than the complementary types, class for class, and furthermore the difference is proportionately greater among crossovers than among non-crossovers. The ratio of the frequency of non-crossovers of X^0 classes to non-crossovers of X classes in the combined experiments is 73 to 100, and for crossovers the ratio is 29 to 100 in bottles containing a single female parent.

Viability probably varies with the conditions of the environment. When only the eggs laid by one heterozygous female in one day developed in a bottle, the ratio of X^0 to X non-crossovers in one experiment was 89 to 100. In mass cultures of heterozygous females back-crossed to normal X males, there are few if any yellow males whereas with uniform viability they are expected to equal nearly 1/4 of the population. Homozygous cultures hardly survive even under favorable conditions.

The mortality occurs partly at least in the pupal stage. Of 62 dead pupae found in pair cultures of heterozygous females, 87 percent belonged to the classes carrying a closed X^0; they were either X^0 males or females heterozygous for X^0.

In the first two experiments (A_1 and A_2) $y\ cv\ v\ f$ females heterozygous for X^0y and sc were mated to $ec\ ct^6\ g^2\ bb$ males, with or without scute, and F_1 females were mated singly to X_8 males which carried all of the recessive genes except scute. Cultures that gave yellow males in F_2 are included in table 10 and those that gave not-yellow (or scute) sons were used as controls (appendix, table 11). The control involved a total of only 2,623 flies, but recombination percents (table 2) corresponded well enough with the a.X. values to show that the stock had not changed since it had been used by BRIDGES and OLBRYCHT, and more significant results of the larger a.X. experiment could be used. The map distances derived from the a.X. values are not the same as the present standard values for the X chromosome which have been more recently obtained from a wide range of material, but since the stocks used in the experiments were made from alternated X-ple material, the a.X. values are suitable for comparison.

TABLE 2

Recombination values of the eight chief characters used in the experiments with the closed chromosome obtained from two control experiments and from "a.X." data of BRIDGES *and* OLBRYCHT.

	REGION							TOTAL NUMBER OF FLIES
	sc-ec 1	*ec-cv* 2	*cv-ct⁶* 3	*ct⁶-v* 4	*v-g* 5	*g-f* 6	*f-bb* 7	
From a.X.	6.8	9.6	8.2	14.8	11	11.4		20,786
From two		10.6	8.3	16.2	11.1	12.7	10.5	2,623
controls	7.2							585

In order to compare the rates of crossing over in different regions of the chromosome the total percent of recombination observed in each region (appendix, tables 10 and 12) has been divided by the number of the a.X. map units that represent the length of the region. The horizontal axis of diagram 1 represents the chromosome map, the vertical axis the frequency of crossing over expressed as percent per map unit of the total number of observed recombinations for each region. In order to eliminate the factors for differential viability among offspring of heterozygotes, the percents of recombination have been calculated separately for offspring of the two classes, with and without a closed X^0.

The frequency of crossing over in the first and seventh regions of flies *homozygous* for closed X^0 cannot be observed directly because no new characters can be introduced at the ends of the chromosome; double crossovers involving either of the two end regions therefore appeared to be singles and the doubles involving both end regions appeared to be non-crossovers. The percent of apparent singles from females homozygous for closed X^0 is 3.42. The percent of crossovers involving either the first or the seventh region among flies that received the closed X^0 from *hetero-*

zygous mothers is 3.44, a close agreement for both regions together. In default of direct values for doubles involving these regions, the singles have been allocated as doubles in the same proportions as were found in experiments A_2 and Aa, in which the closed X^0 came from heterozygotes. Similarly a certain percentage of phenotypical non-crossovers from homozygotes, corresponding to the percentage of crossovers from heterozygotes involving both the first and seventh regions, have been rated as crossovers of the 1, 7 class. The percentages obtained are nearly equal to the percentages that closed X^0 crossovers are of the closed X^0 class from heterozygotes.
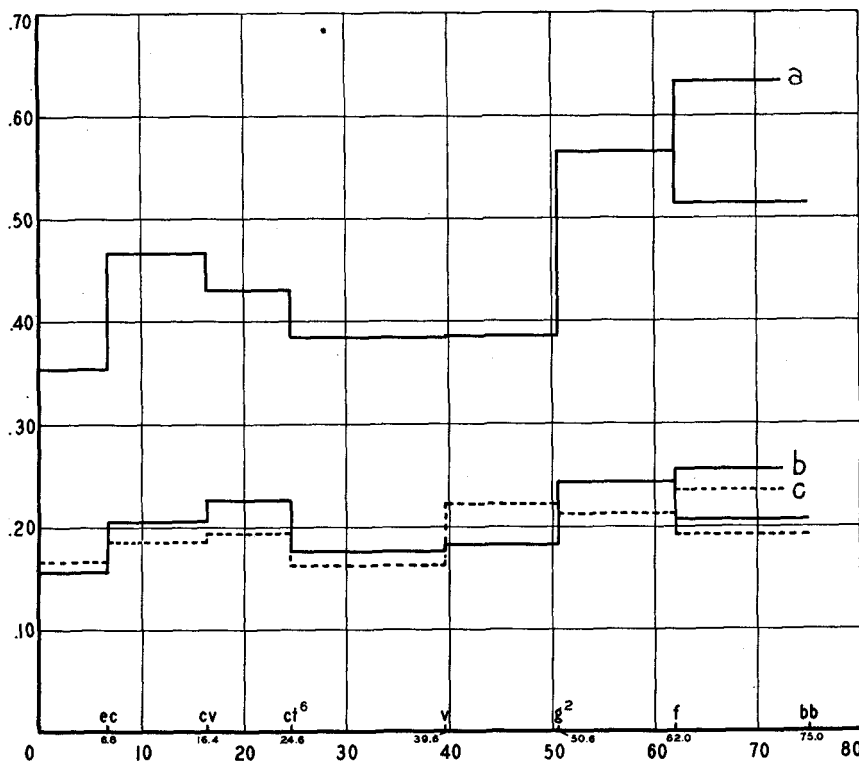

DIAGRAM 1.—Frequency of double crossing over found in flies marked for seven regions of the X chromosome. (a) Percents per map unit of double crossovers from females heterozygous for closed X^0 as shown by offspring not carrying a closed X^0. (b) Same from offspring carrying a closed X^0. (c) Percent per map unit among offspring of females homozygous for closed X^0.

The ratio of the percent of crossing over among flies of the closed X^0 class from heterozygotes to the percent of crossing over among the normal X class is a little less than 1:2 for every region of the chromosome. It will be shown later that this ratio is due in all probability to a lethal class among closed X^0 crossovers, and in the comparisons with normal crossing over that will follow, values for the normal X class will be used.

Crossing over in heterozygous and in homozygous X^0 females (diagram 1) is lowest in the fourth region and increases on both sides; after a maximum in region 3 or 2, there is a decrease to the left. A low rate of crossing over at the left end of the X^0 chromosome has been found, as already stated, in experiments with chromosomes marked close to yellow, for no recombination occurred of yellow and broad or yellow and prune.

It is difficult to determine the percent of crossing over per unit of map distance at the right end because the distance from forked to the end of the chromosome was not known for the a.X. chromosome, and that distance has been found from unrelated experiments to be very variable. If the value of about 13 units found by STERN (1926) is used, the percent per unit drops for the seventh region, but, if 1ð.55 units found in the small control experiment is used, the curves rise. In experiment B_1 and B_2 when a new dominant character Beadex-2, 3.3 units to the right of forked, was introduced into the apposing chromosome, the drop found for 13 units from forked to bobbed was progressive from forked to beadex to bobbed. The most recent values for forked to beadex and beadex to bobbed obtained from many different stocks are 2.6 and 6.6 respectively. Using these values the percent of crossing over per unit increases progressively from the fourth region to the right end of the chromosome.

COMPARISON WITH A NORMAL X CHROMOSOME

In order to make percentages derived from alternated X-ple values comparable with those from experiments with closed X^0, some corrections are necessary. Assuming that single crossovers with the closed chromosome are inviable, only double crossover frequencies for two normal Xs can be compared with the crossover frequencies in females heterozygous for normal X and closed X^0; accordingly, percentages of doubles from a.X. have been computed as of the total number of flies minus the singles and triples, except that the undetectable singles of the seventh region are not subtracted, which makes the percentages for a.X. too low. Furthermore, since in the a.X. experiments the seventh region was not marked, crossovers that were in fact doubles involving the seventh region appeared to be singles and have been subtracted as such from the grand total when singles have been subtracted. It is therefore necessary for comparison to subtract from the closed X^0 totals all the doubles involving the seventh region. Exceptional classes have also been subtracted.

The results obtained from the corrected data are given in table 3. They show that the frequency of total double crossing over in heterozygotes, as indicated by the normal chromosomes that are recovered, is less than the frequency of double crossing over between normal Xs.

A more detailed comparison has been made for different regions of the chromosome. The curves of diagram 2 have been obtained from the corrected data showing the percents of total crossing over per map unit for six regions based on doubles and quadruples when the seventh region of the chromosome is not under observation; the percents for a.X. being as explained a little too low. Disregarding for the present the left end of
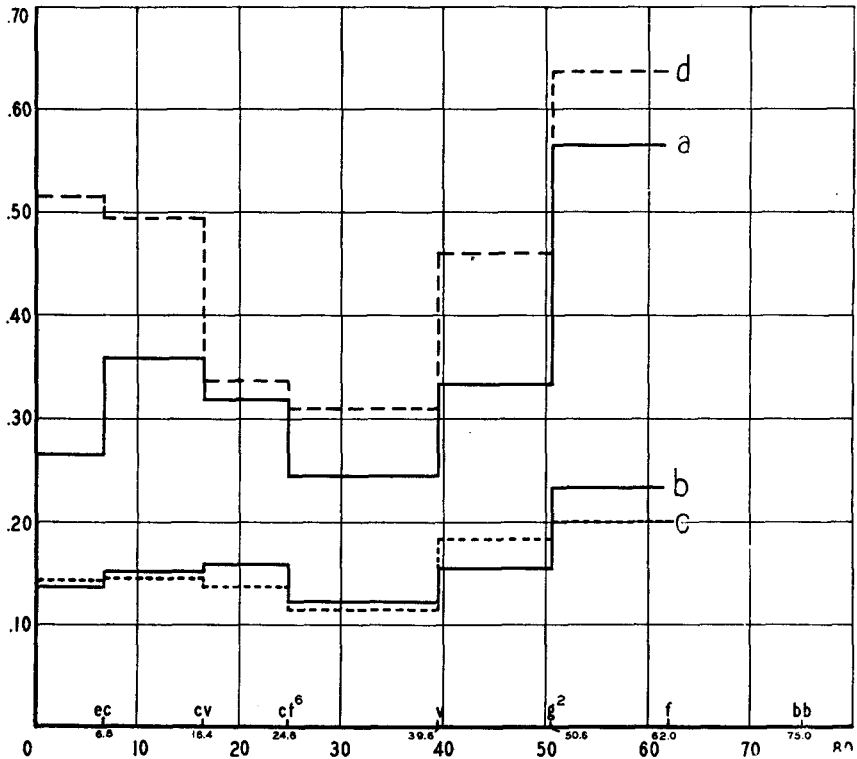

DIAGRAM 2.—Frequency of double crossing over with closed X⁰ from data corrected for comparison with alternated X-ple (a.X., BRIDGES and OLBRYCHT) in six regions of the X chromosome (a, b, c as in diagram 2, d percents per map unit of double crossovers from corrected data of a.X.)

the chromosome it is apparent that the percent of crossing over in the normal class from heterozygotes is for every region to the right of *cv* somewhat, though not constantly, lower than the percent of crossing over between normal Xs. From *cv* to *ct* the percents are most nearly equal, and the relative percents for heterozygotes fall progressively from *ct* to *g*. The percents for homozygous closed X⁰ from *cv* to *g* follow more closely the changes in percents for normal Xs, maintaining a nearly 1:3 ratio; this continues in the *g* to *f* region, in which the percent from heterozygotes again approaches more nearly the normal X value. The result may be stated in another way; the ratios of the frequencies of double crossovers

from two normal chromosomes and from a closed and a normal chromosome vary in different parts of the chromosome from cv to f, while the relative frequencies of double crossovers from chromosomes that are alike, either both normal or both closed, are more constant from cv to f.

TABLE 3

Relative percents of crossovers from females heterozygous for closed X^0 and from females homozygous for normal X or for closed X^0.

	CORRECTED TOTAL ADULT FLIES — REGION NOT OBSERVED		PERCENT OF DOUBLE CROSSOVERS OF SIX REGIONS AS OF CORRECTED TOTAL	RATIO OF PERCENT OF DOUBLE CROSSOVERS FROM X^0/X TO PERCENT FROM X/X	TOTAL OBSERVED REGULAR ADULT CLASSES	PERCENT OF DOUBLE CROSSOVERS OF SEVEN REGIONS AS OF TOTAL OBSERVED REGULAR ADULT CLASSES	TOTAL POPULATION	PERCENT OF SINGLE CROSSOVERS AS OF TOTAL POPULATION	RATIO OF PERCENT OF SINGLE CROSSOVER CHROMATIDS FROM X^0/X TO PERCENT FROM X/X
	SEVENTH	FIRST							
a.X. $\dfrac{X}{X}$		11111*	14.0*				20786	46.7	
X^0/X $\dfrac{X}{X^0}$		9488	10.8	0.77					
		6564	4.95	0.35					
Homozygous X^0		2580	4.76						
Control $\dfrac{X}{X}$	1420*		20.7*						
X^0/X $\dfrac{X}{X^0}$		9924	14.72	0.71	10168	16.7			
		6674	6.54	0.32	6746	7.54			
Homozygous X^0		2616	6.46		2646	7.1			
Exp. J₂ X^0/X $\dfrac{X}{X^0}$					1928	20.4	3135+	30.8+	0.66
					1438	9.4			

* Corrected totals for a.X. and control are too high because they include undetectable singles of the seventh and first regions respectively; the percents are correspondingly too low (see text).

+ Computed for first 6 days of laying (see table 7).

For the seventh region the percents of crossing over for the closed chromosome were directly compared with those from the small control experiment, which had been made to check the material for a.X. values (see table 3). In the control, the first region was not marked and corrections were made similar to those made for comparison with the a.X chromosome, unmarked in the seventh region. Again the percents from the control are a little too low owing to undetectable singles of the first region being included in the total. This does not affect the conclusion that there is no relative decrease in crossing over at the right end of the closed X^0 (table 4). Ratios for the normal X class from heterozygotes show a decrease for the v-g region consistent with that found in the comparison with a.X. (diagram 2) and an increase toward the right end. The results for closed X^0 classes are not entirely consistent.

TABLE 4

Ratios of the percents of crossing over in regions 2 to 7 in flies heterozygous and homozygous for closed X^0 to the percents of crossing over obtained from a small control experiment. The percents are obtained from corrected data and are based on the frequency of observed double and quadruple crossovers.

		y-ec	ec-cv	cv-ct	ct-v	v-g	g-f	f-bb
X/X⁰	X		0.60	0.72	0.72	0.66	0.71	0.80
	X⁰		0.27	0.38	0.31	0.38	0.29	0.34
Homozygous X⁰			0.23	0.32	0.28	0.32	0.26	0.21

A reduction in crossing over at the left end of the chromosome beginning in the second or first region was found to occur with a closed X^0 when all regions were included (diagram 1), and appears also when the crossovers involving the seventh region are not included (diagram 2). Crossing over between two normal Xs on the other hand increases progressively from the region that is the minimum for all the chromosomes, continuously, to the extreme left end of the chromosome.

The attachment of the left end of the closed chromosome to the right end is in a sense a translocation, and, as in translocations generally, there is a decrease in crossing over near the region of attachment; but this is not a reduction due to the conflict of attractions of genes of the sort described by DOBZHANSKY (1931) in the true translocations. The X chromosomes are intact and neither chromosome is attracted to parts of another that are controlled by two different spindle fibres as in cases cited by him. The results are in accord with the view of BEADLE (1932) and of OFFERMAN and MULLER (1932) (see also GRAUBARD 1931) that proximity to the spindle fibre attachment is a factor that reduces crossing over, for in the closed chromosome the attachment of the yellow to the bobbed locus has brought the left end of the chromosome near to the spindle fibre attachment.

INVIABLE CROSSOVERS

Crossing over, or exchange of segments between chromatids, takes place in Drosophila in the four-strand stage (BRIDGES 1916, ANDERSON 1925, L. V. MORGAN 1925, STURTEVANT 1931). In females heterozygous for closed X^0 and normal X two chromatids have no free end which imposes a new condition on the recovery of crossovers. For, if a closed and a normal chromatid exchanged segments at one level only (diagram 3, S), the result would be two tandem chromatids, attached together, because the "yellow" or distal end of the original closed chromatid is inseparable from its spindle fibre end; the chromatid complex would have a spindle fibre attachment at one end (derived from the normal X) and a second attachment (derived from the originally closed X^0) in the middle of the new double chromatid.

If the double complex passes entirely into one nucleus, non-disjunctional females with united Xs would be expected. Non-disjunctional females are not unusually frequent and a few that were tested contained separate X chromosomes, not a double complex. It is therefore assumed that a complex carrying two spindle fibre attachments may be lethal and some evidence for the assumption will be given later. DOBZHANSKY (1931) has pointed out that among translocations some would be expected leading to the formation of chromosomes having more than one spindle fibre attachment and there are no descriptions of such chromosomes in the literature.
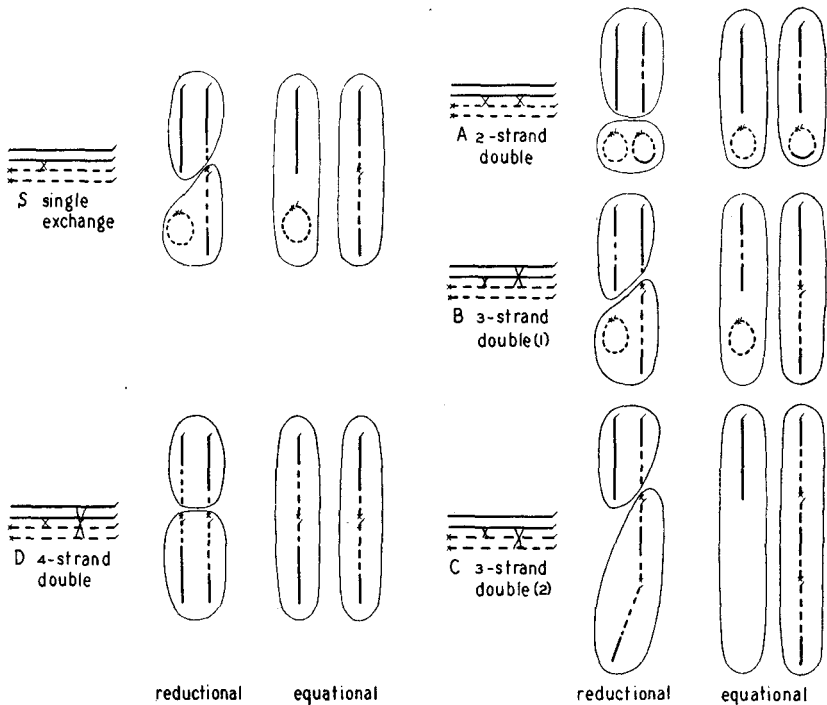

DIAGRAM 3.—Analysis of chromatid formation and distribution at the first meiotic division, when exchanges between homologues have taken place.

Double exchange (that is, two exchanges at the same time, not at the same level [ANDERSON, 1929]) may involve two or three or all four of the chromatids at once. Diagram 3 represents the four possible second exchanges between homologous strands that may occur simultaneously with a first exchange between homologues. A 2-strand double exchange leaves all the chromatids separate from one another and may result in a non-crossover and a double crossover of each kind, closed and normal (diagram 3A). The term normal designates a chromatid which contains the spindle fibre end derived from a normal chromosome.

A 4-strand double exchange is equivalent to two simultaneous single exchanges (diagram 3D) and is by hypothesis therefore lethal for all four chromatids.

A 3-strand double exchange may be one of two kinds. If two of the three strands are normal chromatids (each with a free end, diagram 3B) there may be recovered a closed X^0 non-crossover and a normal X double crossover since the exchanges lead from one normal spindle fibre end to the free end of the other normal chromatid. From the other normal spindle fibre end, however, exchange leads to the end of the closed chromatid attached to its own spindle fibre end, and the result is a lethal double complex.

If two of the three exchanging strands are closed chromatids one non-crossover normal chromatid may be recovered, but the other three chromatids will all be united (diagram 3C) and will *a fortiori* be lethal.

Closed X^0 double crossovers can therefore be derived from 2-strand double exchange only, but normal double crossovers are derived from both 2-strand and 3-strand exchange. It has been noted that the ratio of the percent of double crossovers among closed X^0 F_1s to the percent of double crossovers among normal X F_1s is very nearly the 1:2 ratio to be expected if exchange between homologous strands is at random.

The 1:2 ratio for closed X^0 to normal X double crossovers shows that homologous strands cross at random when two or three strands are involved in double exchange. The closed X^0 tells nothing about the frequency of 4-strand double exchange but EMERSON and BEADLE (in press) have found from data for attached X that 2-strand and 4-strand double exchanges are equally frequent. This result and the equality between 2-strand and 3-strand exchanges shown by closed X^0 are to be expected if when there is crossing over at one level a second crossing over at another level takes place by random exchange of homologous chromatids. The expectation is the same if triple exchange is random (see diagram 4 and table 5). It may be concluded therefore that exchange between homologous chromatids is at random.

PATROCLINOUS MALES

Patroclinous males occur in the proportion of 1 to 3 normal X double crossovers. This is chosen as a critical ratio because of the good viability of the normal class and because almost all double crossovers are derived from double exchange which perhaps is the source also of almost all patroclinous males. It has been found that closed X^0 double crossovers are half as frequent as normal X doubles and it has been shown that half of the closed X^0 doubles expected by random exchange between homologous chromatids are probably inviable. An analysis of the distribution of chromatids shows that patroclinous males may arise at the expense of

closed X^0 doubles. A closed X^0 double crossover from 3-strand double exchange involving two closed X^0 chromatids (diagram 3C) would be inviable because it would be united with two other chromatids, in a lethal triple complex. If at the first meiotic division two of the chromatids that are going together to one pole (either equationally or reductionally) drag the third chromatid away from the other pole, one daughter nucleus might
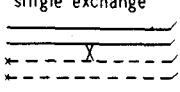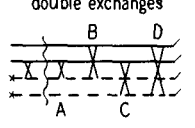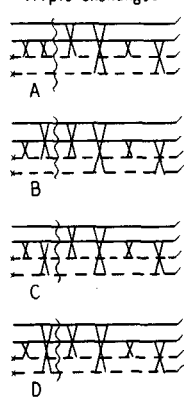
	non-c.o.		c.o.		no-X	invi-
	X^0	X	X^0	X	½	able
single exchange						
	1	1				2
ratio	1:1					
double exchanges						
A	1	1	1	1		2
B	1			1		2½
C		1			½	4
D						8½
ratios	2	2	1	2	½	8½
	1:1		1:2	4:1		
triple exchanges						
A	2	2	2	2		8
B	2		1	4	½	8½
C		2	1	2	½	10½
D			2	4	1	9
ratios	4	4	6	12	2	36
	1:1		1:2	6:1		

DIAGRAM 4.—Chromatids and ratios to be expected from random exchanges between homologous chromatids in heterozygotes for closed X^0 and normal X, on the assumptions that double and triple complexes are lethal and that patroclinous males are mainly derived from double exchange C. A closed chromatid is represented by a broken line and the end marked "x" is attached near the spindle fibre to the same chromatid. On the right of the wavy lines are represented the different exchanges that might occur simultaneously with the exchange or exchanges on the left of the line, by double and triple exchange.

contain only one X chromatid and at the second division one no X nucleus might be formed. If such a nucleus remained in the egg, it would, if fertilized by Y sperm, be lethal in the egg stage according to Li (1927), and if fertilized by X sperm it would contain the chromosome complement

of a patroclinous male. If patroclinous males have this origin the expected frequency from random double exchange is 1 to 4 normal X doubles, which is lower than the observed frequency of 1 to 3. (From infrequent triple exchange the expectation, if patroclinous males are due to formation of triple complexes, is still lower; see diagram 4 and table 5).

TABLE 5

Classes and Ratios; (1) To be expected (a) If homologous strands of closed X^0 and normal X cross at random and if patroclinous males are derived from one kind of 3-strand double exchange, and united chromatids produce inviable eggs.

TYPE OF EXCHANGE	NON-CROSSOVERS X^0 : X		DOUBLE CROSSOVERS X^0 : X		NO X EGGS BY X SPERM (PATROCLINOUS MALES)	INVIABLE EGGS	RATIO OF X NON-CROSSOVERS TO INVIABLE EGGS
none	1	1	0	0		0	
ratio	1:1						1:0
single	1	1	0	0		2	
ratio	1:1						1:2
double	2	2	1	2	0.5	8.5	
ratio	1:1		1:2		4:1		1:4
triple	4	4	6	12	2	36	
ratio	1:1		1:2		6:1		1:9

(b) If all strands cross at random

TYPE OF EXCHANGE	NON-CROSSOVERS X^0 : X		DOUBLE CROSSOVERS X^0 : X		NO X EGGS BY X SPERM (PATROCLINOUS MALES)	INVIABLE EGGS	RATIO OF X NON-CROSSOVERS TO INVIABLE EGGS
none	1	1				0	
ratio	1:1						1:0
single	6	8	0	0		10	
ratio	75:100						0.8:1
double	10	16	1	2	2.5	28.5	
ratio	62:100		1:2		4:5		1:2
triple	19	39	19	29	11	139	
ratio	50:100		2:3		3:1		1:3

(2) Observed

	NON-CROSSOVERS		DOUBLE CROSSOVERS		NO X EGGS BY X SPERM	INVIABLE EGGS	RATIO OF X NON-CROSSOVERS TO INVIABLE EGGS
ratio	1:1		1:2		3:1		1:1.2

The source of some of the patroclinous males may therefore be looked for in other kinds of exchanges and although little is known about the separation of chromatids at meiosis in Drosophila a formal anslysis can be made, presenting at least some of the problems involved.

Two-strand double exchange (diagram 3A) results in four single chromatids which may be distributed regularly among the four maturation nuclei. Four-strand double exchange (diagram 3D) results in two double

complexes. The remaining two kinds of exchange, namely single exchange and 3-strand double exchange involving two normal X chromatids (diagram 3S and B) are alike in that each results in a single normal chromatid (in the second case a double crossover), a single closed chromatid, and a double complex with two spindle fibre attachments. The non-crossover
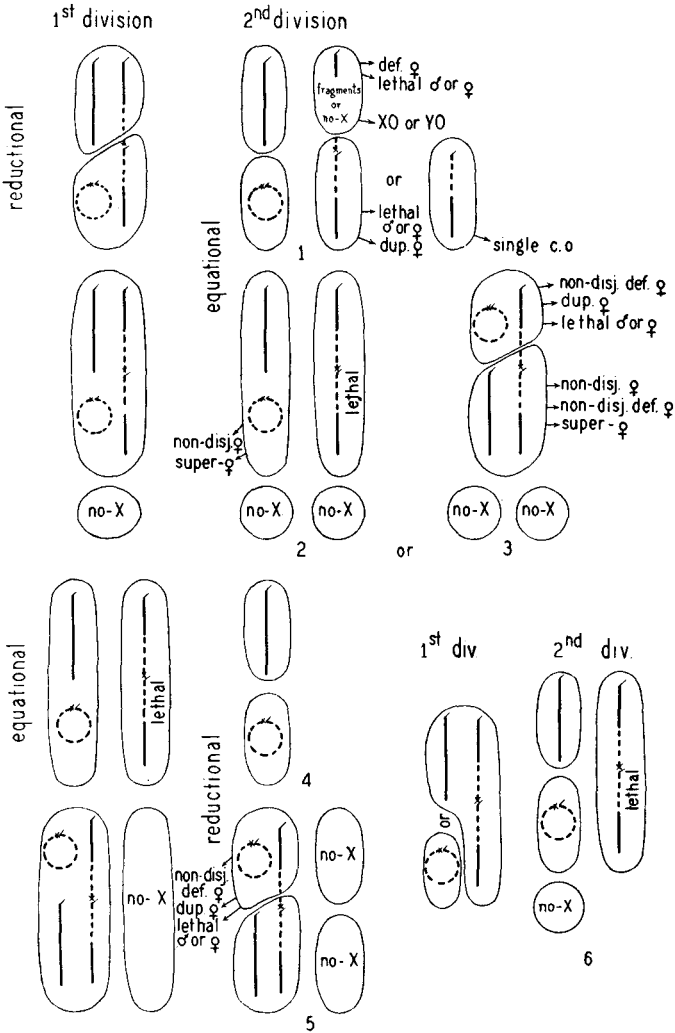

DIAGRAM 5.—Schemes of meiotic divisions in heterozygotes after single exchange (S of diagram 3) or 3-strand double exchange that involves two normal X chromatids (B of diagram 3).

chromatids from single exchange (S) should be recovered to account for the frequency of observed non-crossovers and the 1:1 ratio between the normal X class and the closed X^0 class. The normal X double crossover from exchange B should be recovered to account for the 1:2 ratio of closed X^0 doubles to normal X doubles.

If the reductional division takes place first, each daughter nucleus will contain one of the single chromatids resulting from exchanges S or B, and if these single chromatids are to be recovered the nuclei must be viable. The first division can be truly reductional (that is, the sister chromatids can remain together at the fibre end) only in case the normal component of the double complex is torn apart, and the complex is not lethal in the sense that has been assumed (see diagram 3 and 5). If chromatids are severed in this way, one of the daughter nuclei at the second (equational) division might receive the normal X chromatid and the other might receive a fragment or fragments of a chromatid; a nucleus with a fragment and its spindle fibre attachment might produce an inviable zygote or, if fertilized by X sperm, might produce a female with a deficiency in one of her X chromosomes. No such flies have been observed. If a fragment with a spindle fibre attachment were small, and sometimes went with the whole chromatid at the second division, duplications would be expected among the offspring, but these were not found. If all fragments were lost a nucleus containing no X chromatid might contribute to the class of patroclinous males.

The other daughter nucleus of the first reductional division would contain the spindle fibre ends of the closed chromatids (diagram 3S); one chromatid would be a closed non-crossover and the other chromatid might carry an extra piece torn from the normal chromatid that had become united to it by crossing over. The second division would give a nucleus with a closed chromatid, and one of a kind that would produce inviable zygotes or females with duplications, which are not found. If the united chromatids separated at the first division at their point of attachment single crossovers with the fibre attachment of the original closed X^0 would be expected but are not found (diagram 5, scheme 1). Similarly duplications and single crossovers might sometimes be expected from 4-strand double exchange (diagram 3D).

Three strand exchange that involves two closed X^0 chromatids (diagram 3C) and that results in a normal X chromatid and three chromatids united together might be expected to produce similar exceptional classes by rupture of chromatids if the first division is reductional. Or the three united chromatids might all go into one nucleus, giving the results already formulated.

If however the first meiotic division were equational, the recovery of single chromatids after exchanges S and B would involve no united chromatids whenever the double complex segregated from the two single chromatids (diagram 5, scheme 4). Assuming that double complexes are lethal no viable chromatids would be expected after 4-strand double exchange with this kind of equational division.

But on chance association of spindle fibre ends, the ends of a free and of an attached chromatid would go together at a first equational division as frequently as the ends of the free chromatids would separate from the ends of the attached chromatids. The resulting nuclei after the second division would be of the same kinds as after a first reductional division (see diagram 5, scheme 1) and classes would be expected that have not been observed.

If all four X chromatids went into one first daughter nucleus and no X into the other, the first division would be neither equational nor reductional. Following out such a possibility to the second division the no X nucleus might divide into two no X nuclei and the other nucleus divide also. But whether such a nucleus underwent the second division reductionally (diagram 5, scheme 5) or in either of two ways equationally (diagram 5, scheme 2 or 3) non-disjunctional and other classes that have not been observed should result.

It appears that the kind of assortment of chromatids that best fits the observed classes, except for the excess of patroclinous males, is one which segregates the free chromatids from a double complex at the first division (diagram 5, scheme 4).

If exceptionally, at the first division, two united chromatids and only one of the single chromatids went together into one nucleus and the single chromatid separated from the double complex at the second division (diagram 5, scheme 6), the 1:1 ratio between non-crossovers and the 1:2 ratio between double crossovers would be maintained and there would be an increase in the frequency of no X eggs, and therefore of patroclinous males, derived from one half of the nuclei that after the second division contained no X.

The observed percent of double crossovers from homozygous closed X^0 females is in agreement with expectation on the assumptions that have been made from a study of heterozygotes. Two-strand exchange would give viable double crossovers as in heterozygotes and 3-strand exchanges would produce patroclinous males. The double crossovers recovered should therefore be the same percent of the total regular classes as are closed X^0 doubles of the total closed X^0 class from heterozygotes which was realized (table 3 and diagrams 1 and 2). The percent of patroclinous males from homozygotes, if they are derived mostly from 3-strand exchange, would be expected to equal the percent of patroclinous males as of a total consisting of the closed X^0 class plus the patroclinous males from heterozygotes. Homozygotes gave 12 percent among a total of 2121 flies (table 12). Heterozygotes of experiment Aa gave 10 percent among 2087 flies of the corresponding classes and 9 percent occurred among another total of 2588. This is a close agreement but viability of the closed

X^0 class as compared with that of the normal X class was exceptionally low in both the experiments with heterozygotes. In another experiment (B_2, table 10) in which viability of closed X^0 flies as compared with viability of normal X flies was almost twice as good, patroclinous males were only 5.6 percent of a corresponding total of 1534. There was no measured control of the viability of F_1s from homozygotes but among 3 cultures there was an average of only 62 flies of regular classes from a culture; larvae were numerous, but not pupae and many of the pupae were dead; also many flies that hatched did not survive. It may be concluded that, although differential viability obscures the result, the unexpectedly high percentage (12 percent) of patroclinous males from homozygotes may not be at variance with the assumption that they originate mainly from eggs in which triple chromatid complexes are formed by crossing over.

INVIABLE EGGS

In Drosophila, the first meiotic division is not completed until after the sperm has entered the egg (HUETTNER 1924, GUYÉNOT and NAVILLE 1929) and since crossing over takes place at the four strand stage, which occurs at meiosis, irregularities due to crossing over should affect the egg stage (see PLOUGH 1921, GOWEN 1929a and b). It follows that a certain proportion of the eggs laid by flies heterozygous for closed X^0 and normal X would be expected to be inviable if double or triple crossover complexes are lethal as has been assumed, but that if crossing over were reduced, or prevented, a corresponding reduction in inviable eggs would be expected.

Dr. JACK SCHULTZ suggested a comparison of the viability of eggs from females heterozygous for closed X^0 and normal X with viability of eggs from females in which the normal X has been replaced by an inverted X called Δ-49 (MULLER and STONE 1930, OFFERMAN and MULLER 1932) preventing nearly all crossing over.

The flies used were $X^0\ y/g^2$ females mated to $y\ g^2\ bb$ males, $X^0\ y/\Delta$-49 $cm \pm bb$ females mated to $y\ \Delta$-49 $cm\ bb$ males, and for control Δ-49/g^2 females by g^2 males. A single female was put into a vial with many males, and the next day transferred to a bottle in which was placed food darkened by lampblack and contained in the depressions of handles of two paper spoons. On the first day following and on five successive days the spoons were removed and new spoons put into the bottle with the flies; the eggs on the spoons that had remained a day with the flies were counted and the spoons put into new bottles supplied in the regular way with fly food. On the fourth day eggs still unhatched were counted. Eight females of each kind were mated and the eggs that were laid on the first six days were followed in this way.

The result is consistent with the hypothesis for the percent of inviable eggs was reduced from 31.3 percent to 6.3 percent when crossing over with

a closed X^0 was reduced by the inversion in the Δ-49 chromosome (see table 6).

TABLE 6

Percent of classes representing crossovers from females carrying inverted and closed X^0 chromosomes (deduced values in italics).

EXPERIMENT	HETEROZYGOTE	PATROCLINOUS ♂♂	SINGLE CROSSOVER	DOUBLE CROSSOVER	INVIABLE EGGS	TOTAL
A.H.S. (unp.)	ClB/X	0.5				1743
	ClB/X		0.5			
C	ClB/X⁰	0.52		0.1		6122
L₁	Δ-49/X	0.27				1798
L₂	Δ-49/X⁰	0.33				4150
K₁	Δ-49/X⁰	0.5				2099
M	Δ-49/X	0.3				1329
J.S. (unp.)	Δ-49/X		13.0	0.4		1309
J₃	Δ-49/X				3.2	3498
J₁	Δ-49/X⁰			0.0	6.3	3383
J₂	X⁰/X		0.0		31.3	3326
		2.36		*14.3*	*33.2*	*3135*

Furthermore the presence of some inviable eggs from heterozygotes for closed X^0 and Δ-49 is consistent with the conclusion reached from analysis of maturation divisions that usually two single chromatids segregate from a double lethal complex. Dr. SCHULTZ found that heterozygotes for Δ–49 and normal X gave 13 percent of single crossovers due to single exchange in the region not included in the inversion of the Δ-49 chromosome, and only 0.4 percent of doubles (which involved the left end of the chromosome). The kind of segregation cited has been shown to be the only scheme on which inviable eggs would regularly be produced by single exchanges (see diagram 5). Since Δ-49 gave 3.2 percent of inviable eggs (due to unknown causes) in the control with normal X (experiment J₃, table 6), it may seem that 6.3 percent of inviable eggs from heterozygotes for Δ-49 and closed X^0 is not high enough to correspond with 13 percent of single crossovers from heterozygotes for Δ-49 and normal X; that they do correspond is however the most consistent conclusion especially since single crossover chromatids from closed X^0 and normal X seem to be only 0.66 times as frequent as single crossovers from normal Xs.

A second conclusion from analysis of meiotic divisions, namely, that patriclinous males mostly result from double exchange, is further shown by the percents of patroclinous males found from flies heterozygous for closed X^0 and for Δ-49 or ClB, another longer inversion.

The frequency of non-disjunctional classes, especially of males is known to be high among offspring of flies heterozygous for inversion and normal X (see table 6). For example in experiment L, Δ-49/X females gave 0.27

percent of patroclinous males, and ClB/X females gave 0.5 percent as computed from unpublished data of Dr. STURTEVANT. Flies heterozygous for closed X^0 and the inversion Δ-49 gave 0.33 percent in the controlled experiment L, and flies heterozygous for X^0 and ClB gave 0.52 percent; that is when inversions are present closed X^0 gives the percents of patroclinous males that are normal for the inversions and not the high percents that are characteristic for heterozygotes of closed X^0 and normal X. The percent of all crossovers from ClB/X females is said to be not more than 0.5 percent and ClB/X^0 females gave only 0.09 percent of double crossovers. The reduction of double crossovers by the inversion of a chromosome is accompanied by decrease of patroclinous males among offspring of flies carrying closed X^0. That patroclinous males are seldom derived from single exchange is indicated because there is no increase in their percentage from closed X^0/Δ-49 females as compared with that from normal X/Δ–49 females although some single exchange occurs with the inversion Δ–49.

The results just cited in regard to patroclinous males and inviable eggs obtained from flies heterozygous for closed X^0 and inversions, and the results from flies heterozygous for closed X^0 and normal X, namely the absence of increase in the frequency of non-disjunctional females, the absence of a class with united Xs, and of duplications and deficiencies, the near agreement between the frequency of patroclinous males and 1/4 of the frequency of normal X double crossovers, suggest that in eggs containing closed X^0 there is usually segregation of single chromatids from united chromatids at the first maturation division when two chromatids become united by single exchange. Such a segregation is equivalent to one kind of equational division.

FREQUENCIES OF ALL CLASSES

Whatever the mechanism by which exchange between chromatids produces lethal effects in flies heterozygous for closed X^0 the inviable eggs and patroclinous males together should, on the hypothesis that inviability of eggs is due to lethal effects of crossing over, correspond to single crossovers, triple crossovers, and about ¼ of the double crossovers from normal Xs. Patroclinous males were 2.36 percent of the deduced total population in the egg count experiment, and inviable eggs were 33.2 percent of that total (see table 7); the classes enumerated are therefore 35.56 percent of the total, a value which is low compared with normal X; but it is of the order to be expected, and is in agreement with other results with closed X^0 that show a lower rate of crossing over than that which occurs between normal Xs. From corrected data for alternated X-ple, the percent of doubles among the normal X offspring of closed X^0 heterozygotes is

0.77 times the percent of doubles from the a.X. control, and the percent of computed singles as of the whole population in the egg count experiment is 0.66 times the percent of singles in a.X. (see table 3).

Values from preliminary unpublished data recently obtained by Dr. BRIDGES in an experiment (425 C) with completely marked Xs are compared in table 8 with deduced values from the egg count experiment J_2.

TABLE 7

Observed and deduced frequencies of classes of experiment J_2. Offspring of $X^0 y/g^2$ females by yg^2bb males. Deduced values are in italics.

		X	non-crossovers	849	27.1 percent
			doubles	224	7.15 percent
	adult flies	X^0	non-crossovers	754 = 89 percent of 849	24.0 percent
			doubles	*73* (89 percent of 112 = 99)	2.33 percent
		patro-clinous males	XO assumed to be mostly from 3-strand double exchange (2)	74 (74:224 = 33:100) (56:224 = 25:100) 56 may be expected from 3-strand double exchange (2)	2.36 percent
Total no. of eggs 3326	inviable in late stages	X^0	X^0 non-crossovers	*95* (849–754)	3.03 percent
			X^0 2-strand crossovers	*26* (on basis of viability of non-crossovers, 99–73) 0.83 percent	
	inviable eggs	1040	inviable complexes from exchanges (single crossovers)	*966*	30.81 percent
			YO assumed to be of same origin and frequency as observed XO	*74*	2.36 percent
				3135	
	not accounted for from all classes			191	
				3326	

Non-crossovers from closed X^0 heterozygotes are 1.38 times as frequent as from normal flies. The deduced value for single crossovers in J_2 is 0.65 times the percent of singles from normal Xs. The percent of double crossovers in experiment J_2 is based on observed crossovers among flies carrying normal X; crossovers in experiment J_2 occurred with the maximum frequency that was observed among various experiments (see table 10,

appendix, and table 3); corrected for average frequency the value is 11.2. The consistently lower percentage of normal X doubles in every region of the chromosome in the more extensive experiments with heterozygotes as compared with alternated X-ple (diag. 3) seems to show that the true value for frequency of double crossovers from heterozygous X^0 is lower than for flies carrying only normal Xs.

TABLE 8

Percents of classes derived from females heterozygous for closed X^0 and from normal females in two 6-day experiments. Percents of experiment 425 C are computed from preliminary unpublished data of C. B. Bridges on crossing over of completely marked X chromosomes. Deduced percents from experiment J$_2$ are from table 7.

EXPERIMENT	P$_1$	NON-CROSSOVER	SINGLE AND TRIPLE CROSSOVE RS	DOUBLE AND QUADRUPLE CROSSOVERS	TOTAL
J$_2$	X^0/X	54.2	30.8	14.3 (11.2)*	3135
425 C	X/X	39.3	47.6	12.2	1243

* Percent deduced from other experiments.

FREQUENCY OF MEIOSIS WITHOUT EXCHANGE

If the conclusion that homologous strands cross at random, reached by analysis of crossing over in attached XX and in closed X^0 is applied to BRIDGES's data for the normal X chromosome (table 9), it can be shown that some crossing over between normal chromatids takes place at meiosis in all eggs in Drosophila.

TABLE 9

Percent of eggs of Drosophila melanogaster in which no exchange between chromatids of the X chromosome occurs at maturation, computed from observed crossovers from completely marked X chromosomes. (Observed values are from preliminary unpublished data of C. B. Bridges's experiment 425 C).

	NON-CROSSOVER	SINGLE	DOUBLE	TRIPLE	QUADRUPLE	TOTAL
Number	1050	1332	330	17	2	2731
Percent	38.3	48.7	12.1	0.6		
	0.6	1.8	1.8			
	—	—	—			
	37.7	46.9	10.3			
	10.3	20.6				
	—	—				
	27.4	26.3				
	26.3					
	—					

1.1 percent of chromatids resulting from no exchange
0.25 percent of eggs in which no exchange takes place

If exchange takes place at random among normal homologous chromatids, 8 non-crossover to 24 single crossover to 24 double crossover to 8 triple crossover chromatids is the expectation from triple exchange. The observed percent of triple crossovers from completely marked chromosomes was 0.6 percent; if three times 0.6 is subtracted from 12.1 percent of observed double crossovers there remain 10.3 percent of doubles from double exchange. The expectation from double exchange is 4 non-crossover to 8 single crossover to 4 double crossover chromatids; if twice 10.3 (which is the expected percent of single crossover chromatids due to double exchange) and three times 0.6 (the percent of single crossover chromatids expected from triple exchange) are subtracted from the observed 48.7 percent of single crossovers, there remain 26.3 percent due to single exchange. An equal percent of non-crossover chromatids is to be expected due to single exchange. When all percents of non-crossovers due to triple, double, and single exchange have been subtracted from the observed percent of non-crossovers there remain only 1.1 percent of chromatids to be expected from 0.25 percent of eggs or no eggs in which maturation has taken place without any exchange between chromatids.

Since crossing over is less frequent in heterozygotes for closed X^0 there may be some eggs in which no crossing over takes place.

The ratio of normal X non-crossovers to inviable eggs is expected by random exchange between homologous chromatids to be 1:2 as far as it is affected by single exchange, and 1:4 as far as affected by double exchange. The ratio will be increased in the proportion of 1:0 as far as no exchange occurs (tables 5 and 7). The observed ratio is 27:33, which is not inconsistent with expectation.

SISTER STRAND CROSSING OVER

Up to this point, ratios that have been examined have been based on exchange (found to be random) between homologous chromatids and it has been tacitly assumed that crossing over does not take place between sister strands. There is in fact evidence for the assumption.

STURTEVANT (from results not yet published but soon to appear) has found that crossing over does not take place between sister strands when crossing over is unequal.

The percentages of non-crossovers, crossovers, patroclinous males and inviable eggs in the population from females heterozygous for closed X^0 (table 7) are found to be consistent with expectation if the ends of the closed chromosome are inseparable, and if double or triple complexes resulting from crossing over of chromosomes are lethal, and if crossing over between non-sister chromatids is at random.

If, however, crossing over is random for all four strands the ratios depart from expectation (see table 5). Single, double, and triple exchanges between homologous chromatids would result in equal numbers of closed X^0 and normal X non-crossovers, which is almost certainly the true ratio, there being a relatively greater inviability in the closed X^0 class in stages after egg laying. If sister strands cross over, single exchange between normal sister strands would give rise to viable apparent non-crossovers, but between closed sister chromatids would give rise to an inviable double complex, reducing the percent of closed X^0 non-crossovers and also increasing the percent of inviable eggs or of patroclinous males. Double exchange if it included exchanges between sister strands would also change the ratios in the same direction. The expected ratios due to different kinds of exchange are given in table 5.

The observed ratios then show that sister strand crossing over if it occurs at all is not as frequent as crossing over between homologues. WEINSTEIN (1932) has reached the same conclusion mathematically.

Evidence that sister strand crossing over does not occur when conjugation of homologous chromosomes is prevented is derived from the experiment with heterozygous closed X^0 and inverted X (appendix, experiment J_1, table 13). Conjugation between homologous strands was almost prevented by the inversion Δ-49 and inviable complexes from single and odd numbered exchanges would have lowered the percent of the closed X^0 non-crossover class if exchange had occurred between sister closed X^0 chromatids. But no reduction was observed; the ratio of closed to normal non-crossovers was no more different from 1:1 than the deviation shown by other experiments to be probably due to differential inviability.

SUMMARY

A female Drosophila carrying attached X chromosomes and homozygous for yellow produced a daughter with a single maternal X chromosome that was "closed" (X^0).

The closed X^0 may conjugate with another X chromosome since double crossovers are recovered from females heterozygous or homozygous for closed X^0.

There is genetic evidence that the chromosome is closed by union of its ends since the loci for yellow and bobbed have never been found to be separated by crossing over, nor have the characteristics of closed X^0 ever been separated by crossing over from either of the two loci, which are near the ends of the chromosome.

No single crossovers are recovered from females carrying closed X^0 whether homozygous or heterozygous.

The percents of double crossovers among the normal offspring of females heterozygous for closed X^0 are somewhat lower than among normal controls for every region of the chromosome. The most marked difference in frequency of doubles is at the left end of the chromosome. This region in the closed chromosome is near the spindle fibre attachment, a condition which is known to decrease crossing over.

In cytological preparations the closed chromosome appears as a slightly elongated closed ring.

Low viability of closed X^0 is shown by the great difficulty in maintaining homozygous stock under the most favorable conditions, by low frequency of closed X^0 males in backcrosses, and by a high percentage of closed X^0 classes among dead pupae from backcrosses. The survival of some adults and the observations on dead pupae indicate a variable degree of inviability after the egg stage.

A single exchange of segments (crossing over at one level only) between a closed X^0 and a normal X chromatid would result in a double complex, consisting of two chromatids attached together, because the two ends of the closed chromatid are united. Among F_1s from flies heterozygous for closed X^0 there is no class of non-disjunctional females containing united X chromosomes as would be expected if double complexes survive.

A 2-strand double exchange (one in which only two homologous chromatids take part) would produce two separate double crossovers, one closed, one normal.

A 3-strand double exchange in which two normal strands and one closed strand take part would produce a normal double crossover.

The other kind of 3-strand double exchange, in which two closed chromatids and one normal chromatid take part, would unite three chromatids into one complex, and no closed X^0 double crossover would be recovered.

A nearly $1:2$ ratio of the percent of closed X^0 double crossovers (among the closed X^0 F_1s from heterozygotes) to the percent of normal X doubles (among normal X F_1s) has been observed. It is the ratio to be expected if homologous strands cross at random; that is if 2-strand and each of two kinds of 3-strand double exchange are equal. These results together with the equality of 2-strand and 4-strand double exchange found by EMERSON and BEADLE (in press) in attached X show that exchange between homologous strands is at random.

Patroclinous males are about $\frac{1}{3}$ as frequent as normal X double crossovers from heterozygotes; these might result from irregularities due to formation of united chromatids at maturation and together with inviable eggs represent classes due to exchange.

Patroclinous males would be expected from the second kind of 3-strand double exchange if two united chromatids going to one pole dragged the third one that is also united to them out of its nucleus leaving one nucleus with a single X chromatid after the first division. The expected frequency of patroclinous males from 3-strand exchange would be 1/4 of the frequency of normal double crossovers, a little less than the observed 1/3. Some patroclinous males may be derived rarely from other exchanges.

The percent of double crossovers from homozygotes is in agreement with the conclusions reached from heterozygotes in regard to double crossovers. The true percent of patroclinous males from homozygotes is obscured by low viability of regular classes but the observed percent appears to be consistent with expectation on the assumption that patroclinous males are derived mainly from 3-strand double exchange.

Lethal effects of exchange would affect the egg stage of Drosophila. Inviable eggs were found to be 33.2 percent of the deduced number representing the total population in F_1 from females heterozygous for closed X^0. This is in agreement with the hypothesis that patroclinous males are derived mostly from one kind of 3-strand double exchange and that double chromatid complexes resulting from one exchange between two chromatids are lethal. On the hypothesis, patroclinous males, which are about 2.3 percent of the population, and an equal number of YO gametes, which are lethal in the egg stage, represent one kind of double crossover, and the remainder of the lethal eggs, or about 31 percent of the population, represent single crossovers. The percents are in agreement with the hypothesis if crossing over takes place somewhat less frequently between closed X^0 and normal X than between normal Xs. Evidence for reduced frequency of crossing over is found in a relatively high percent of non-crossovers among F_1s from flies heterozygous for closed X^0, and a relatively low percent of double crossovers from heterozygotes as compared with values obtained from controls.

If the first meiotic division when closed X^0 is present is reductional, nuclear division after exchanges that produce double complexes would be expected to lead to severed chromatids, but no flies containing corresponding deficiencies or duplications have been observed. Patroclinous males would be expected if some nuclei were formed without any X chromatid.

If the first meiotic division is the kind of equational division that would segregate two single chromatids from a double lethal complex the observed ratios are most nearly satisfied.

If inviable eggs and patroclinous males result from irregularities of crossing over their frequency should be reduced if crossing over is reduced. Heterozygotes for closed X^0 and normal X gave 31 percent of inviable eggs; when crossing over was reduced by an inversion in an X chromosome,

heterozygotes for closed X^0 and the inversion (Δ-49) gave only 6.3 percent of inviable eggs.

Double crossing over is almost entirely prevented and single crossing over is reduced to 13 percent in heterozygotes for normal X and inversion Δ-49. Heterozygotes for closed X^0 and Δ-49 gave 6.3 percent of inviable eggs but no excess of patroclinous males over the percent obtained from heterozygotes for Δ-49 and normal X. This agrees with the hypothesis that with closed X^0, patroclinous males represent one kind of double crossover, and inviable eggs represent almost entirely single crossovers, crossing over being relatively infrequent when closed X^0 is present.

The observed ratios are those to be expected if exchange between homologous strands is random and if sister strands do not cross over.

When conjugation between homologous strands is almost prevented by an inversion in the apposing X chromosome of a female heterozygous for closed X^0, the ratio of the two classes of non-crossovers in F_1 is 1:1 which is not expected if sister strands cross over independently of crossing over between homologues.

The conclusion that homologous strands cross at random applied to unpublished data of BRIDGES from completely marked X chromosomes, indicates that exchange between normal X chromosomes probably takes place as a rule in all eggs of *Drosophila melanogaster*.

LITERATURE CITED

ANDERSON, E. G., 1925 Crossing over in a case of attached X chromosomes in *Drosophila melanogaster*. Genetics 10: 403–417.

———— 1929 Studies on a case of high non-disjunction in *Drosophila melanogaster*. Z.I.A.V. 51: 397–441.

BEADLE, G. W., 1932 A possible influence of the spindle fibre on crossing over in Drosophila. Proc. Nat. Acad. Washington 18: 160–165.

BRIDGES, C. B., 1916 Non-disjunction as proof of the chromosome theory of heredity. Genetics 1: 1–52, 107–163.

BRIDGES, C. B. and OLBRYCHT, T. M., 1926 The multiple stock "X-ple" and its use. Genetics 11: 41–56.

DOBZHANSKY, TH., 1931 The decrease of crossing over observed in translocations, and its probable explanation. Amer. Nat. **65**: 214–232.

1932 Studies on chromosome conjugation I. Translocations involving the second and the Y chromosomes in *Drosophila melanogaster*. Z.I.A.V. **60**: 235–286.

EMERSON, S. and BEADLE, G. W. (In press). Crossing over near the spindle fibre in attached X chromosomes of *Drosophila melanogaster*. Z.I.A.V.

GOWEN, J. A., 1929a The cell division at which crossing over takes place. Proc. Nat. Acad. Sci. Washington **15**: 266–268.

1929b The gene in cellular biology. Abst. Amer. Soc. Zool., Anat. Rec. **44**: 292.

GRAUBARD, MARC A., 1932 Inversion in *Drosophila melanogaster*. Genetics **10**: 81–105.

GUYÉNOT, E. ET NAVILLE, A., 1929 Les chromosomes et la reduction chromatique chez *Drosophila melanogaster*. La Cellule **39**: 24–82.

HUETTNER, ALFRED F., 1924 Maturation and fertilization in *Drosophila melanogaster*. J. Morph. and Physiol. **39**: 249–266.

LI, JU-CHI, 1927 The effect of chromosome aberrations on development in *Drosophila melanogaster*. Genetics **12**: 1–58.

MORGAN, L. V., 1925 Polyploidy in *Drosophila melanogaster* with two attached X chromosomes. Genetics **10**: 148–178.

1926 Correlation between shape and behavior of a chromosome. Proc. Nat. Acad. Washington **12**: 180–181.

1929 Composites of *Drosophila melanogaster*. Publ. Carnegie Instn. Washington **399**: 223–296.

MULLER, H. J. and STONE, W. S., 1930 Analysis of several induced gene rearrangements involving the X chromosome of Drosophila. Anat. Rec. **47**: 393–394.

OFFERMAN, C. A. and MULLER, H. J., 1932 Regional differences in crossing over as a function of the chromosome structure. Proc. Sixth Int. Congress Genetics **2**: 143–145.

PLOUGH, H. H., 1917 The effect of temperature on crossing over in Drosophila. J. Exp. Zool. **24**: 147–200.

1921 Further studies on effect of temperature on crossing over. J. Exp. Zool. **32**: 187–202.

SEREBROVSKY, A. S., 1926 Influence of the "purple" gene on crossing over between "black" and "cinnabar" in *Drosophila melanogaster*. J. Exp. Biol. (U.S.S.R.) **2**: 55–100.

STERN, C., 1926 An effect of temperature and age on crossing over in the first chromosome of *Drosophila melanogaster*. Proc. Nat. Acad. Sci. Washington **12**: 530–532.

STURTEVANT, A. H., 1931 Two new attached X lines of *Drosophila melanogaster*, and further data on the behavior of heterozygous attached X's. Publ. Carnegie Instn. Washington **421**: 61–81.

WEINSTEIN, A., 1932 A theoretical and experimental analysis of crossing over. Proc. Sixth Int. Congress Genetics **2**: 206–208.

APPENDIX

TABLES 10–13

For every type of complementary offspring the class first recorded is that containing the left end of the maternal chromosome that is described above the line.

TABLE 10

Experiment A_1 and A_2;

$$\text{♀ offspring of } \frac{X^0y \quad cv \quad v \quad f}{ec \quad ct^6 \quad g^2 \quad bb} \text{ ♀} \times y\,ec\,cv\,ct^6\,v\,g^2 f\,bb(X_8)\,♂.$$
$$1 \quad 2 \quad 3 \quad 4 \quad 5 \quad 6 \quad 7$$

Experiment A_a;

$$\text{offspring of } \frac{X^0y \qquad ec \quad ct^6 \quad g^2}{sc \quad br \quad w^a \quad cv \quad v \quad f} \text{ ♀} \times sc\,w^a\,ec\,cv\,ct^6\,v\,g^2 f♂.$$
$$1' \quad 1'' \quad 1''' 2 \quad 4 \quad 5 \quad 6\,7$$

Experiment B_1;

$$\text{offspring of } \frac{X^0y \quad cv \quad v \quad f}{sc \quad ec \quad ct^6 \quad g^2 \quad Bx^2 \quad bb} \text{ ♀} \times X_8♂.$$
$$1 \quad 2 \quad 3 \quad 4 \quad 5 \quad 6 \quad 7' \quad 7''$$

Experiment B_2;

offspring of same type of ♀ $\times y\,ec\,cv\,ct^6 v\,g^2 f\,(X_7)\,♂.$

EXPERIMENT	TYPE	NUMBER OBSERVED		TYPE	NUMBER OBSERVED		TYPE	NUMBER OBSERVED	
A_1, A_2	0	1849	2070	1,6	5	19	2,6	11	44
A_a		1687	3298		12	35		12	64
B_1		1333	1600		5	14		6	32
B_2		1371	1495		6	10		5	25
Total	0	6240	8463	1,6	28	78	2,6	34	165
A_1, A_2	1,2	2	0	1,7	3	18	2,7	10	37
A_a		1	3		4	31		14	63
B_1		0	1		2	12		5	23
B_2		0	0		1	12		8	9
Total	1,2	3	4	1,7	10	73	2,7	37	132
A_1, A_2	1,3	1	1	2,3	0	0	3,4	6	7
A_a		0	0		0	1		5	4
B_1		0	0		0	1		1	8
B_2		0	0		1	0		0	8
Total	1,3	1	1	2,3	1	2	3,4	12	27
A_1, A_2	1,4	6	8	2,4	3	13	3,5	6	18
A_a		7	9		7	18		10	34
B_1		1	6		3	11		3	16
B_2		1	2		5	8		5	8
Total	1,4	15	25	2,4	18	50	3,5	24	76

TABLE 10 (*Continued*).

EXPERIMENT	TYPE	NUMBER OBSERVED		TYPE	NUMBER OBSERVED		TYPE	NUMBER OBSERVED	
A_1, A_2	1,5	5	20	2,5	15	36	3,6	15	48
A_a		5	16		7	43		17	44
B_1		3	14		9	13		8	28
B_2		1	13		8	12		10	27
Total	1,5	14	63	2,5	39	104	3,6	50	147
A_1, A_2	3,7	13	30	4,7	19	47	6,7	2	11
A_a		16	42		25	111		5	19
B_1		5	27		11	50		4	7
B_2		7	14		4	29		1	4
Total	3,7	41	113	4,7	59	237	6,7	12	41
A_1, A_2	4,5	6	20	5,6	5	15			
A_a		11	19		6	11			
B_1		3	12		1	8			
B_2		2	9		1	9			
Total	4,5	22	60	5,6	13	43			
A_1A_2	4,6	17	52	5,7	10	19	3,4,5,6		
A_a		14	61		11	36			
B_1		8	35		1	16			
B_2		11	31		1	13			1
Total	4,6	50	179	5,7	23	84	3,4,5,6		1

	regular classes			non-crossovers			double and quadruple crossovers		
Total		6746	10168		6240	8463		506	1705

	non-disjunctional ♀♀	patroclinous ♂♂	percent of total	percent of (X^0+patr.♂♂)
A_1A_2	5	inviable		
A_a	1	210	3.47	10.0
B_1	0	inviable		
B_2	1	86	3.0	5.6
Total	7			

L. V. MORGAN

TABLE 11
Control of Experiment A_1 and A_2. Female offspring of

$$\frac{sc \quad cv \quad v \quad f}{(sc) \; ec \quad ct^6 \quad g^2 \quad bb} \; ♀ \times X_8♂. \text{ For each type, the second class contains } ec.$$
$$2 \quad 3 \quad 4 \quad 5\;6\;7$$

TYPE	NUMBER OBSERVED		TYPE	NUMBER OBSERVED		TYPE	NUMBER OBSERVED	
0	558	557	2,6	12	22	5,7	11	8
2	71	94	2,7	19	12	6,7	5	2
3	65	80	3,4	2	3	2,3,7		1
4	162	150	3,5	10	5	2,4,6	1	
5	107	99	3,6	14	14	2,4,7	1	1
6	104	111	3,7	11	9	2,5,7	1	2
7	63	97	4,5	5	11	3,5,7		1
2,3	2	1	4,6	22	22	4,5,7		1
2,4	4	9	4,7	17	13	4,6,7		1
2,5	15	11	5,6	1	2	3,4,5,7		1

Total non-crossover	1115	Total single and triple	1213	Total double and quadruple	295

Grand total 2623

TABLE 12
Experiment D_a;

$$♂ \text{ offspring of } \frac{X^0 y \quad cv \quad v \quad f}{X^0 y \; ec \quad ct^6 \quad g^2} \; ♀ \times bb \; Y \; ♂.$$
$$1 \quad 2 \quad 3 \quad 4 \quad 5 \;6\;7$$

Experiment D_b;

$$♂ \text{ and } ♀ \text{ offspring of } \frac{X^0 y \quad cv \quad v \quad f}{X^0 y \; ec \quad ct^6 \quad g^2} \; ♀ \times X_8♂ \text{ or } X_7 \; ♂.$$
$$1 \quad 2 \quad 3 \quad 4 \quad 5\;6\;7$$

EXPERIMENT	APPARENT TYPE	NUMBER OBSERVED		TYPE	NUMBER OBSERVED		TYPE	NUMBER OBSERVED	
D_a	0	48	46	2,3	0	0	3,6	0	0
D_b		1336	1032		1	0		7	7
Total	0	2462			1			14	
D_a	2	0	2	2,4	1	0	4,5	0	0
D_b		4	6		2	6		3	7
Total	2	12			9			10	
D_a	3	0	0	2,5	0	1	4,6	1	0
D_b		6	8		9	4		8	5
Total	3	14			14			14	

TABLE 12 (Continued).

EXPERIMENT	APPARENT TYPE	NUMBER OBSERVED		TYPE	NUMBER OBSERVED		TYPE	NUMBER OBSERVED	
D_a	4	0	0	2,6	0	1	5,6	1	0
D_b		11	14		4	6		4	6
Total	4	25			11			11	
D_a	5	0	0	3,4	0	0	non-disjunc-		
D_b		10	11		4	1	tional ♀♀		2
Total	5	21			5				
D_a	6	0	0	3,5	0	0	gynandro-		
D_b		9	5		4	5	morphs		1
Total	6	14			9				

Values deduced from apparent type 0 and apparent singles

TYPE	0	1,2	1,3	1,4	1,5	1,6	1,7	2,7	3,7	4,7	5,7	6,7
NUMBER	2457	1	1	6	8	9	5	11	13	19	13	5
Total	regular classes	2646			non-cross- overs		2457		double crossovers		189	

F_1's of females mated to X_7 males

Total		2121	Patroclinous ♂♂	254	Per- cent	12.0

TABLE 13

Classes observed in experiments in which eggs were counted on the first day and on the fourth day after laying.

	NON-CROSSOVERS		CROSSOVERS		PATROCLINOUS ♂♂	EGGS TOTAL INVIABLE		NON-DISJUNCTIONAL ♀♀
J_1 $X^0 y/\Delta$-49 $cm^{\pm}bb$ $\times y \Delta$-49 $cm\,bb$ first 6 days	y 2317	cm 3157	$y\,cm$ 0	+ 0	$y \Delta$-49 $cm\,bb$ inviable	3383	217	+ 1
J_2 $X^0y/g^2 \times yg\,bb$ first 6 days	y 1302 754	g^2 1535 849	$y\,g^2$ 136 73	+ 393 224	$y\,g^2\,bb$ 135 74	3326	1040	+ among crossovers
J_3 g^2/Δ-49 $\times g^2$ first 6 days						3498	112	