A COMPARATIVE STUDY OF CROSSING OVER IN ATTACHED-X CHROMOSOMES OF DROSOPHILA MELANOGASTER[1]

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Received May 23, 1955

THE work of L. V. MORGAN (1925) demonstrated that crossing over in attached-X chromosomes of *Drosophila melanogaster* could be systematically studied if one could obtain attached-X chromosomes which were properly marked. From her experiments with these attached chromosomes, MORGAN concluded that at least sometimes crossing over occurred when four chromatids were present. In this same paper MORGAN indicated that by observing the various kinds of attached-X offspring of an attached-X mother, one could determine the genotype of the heterozygous mother.

The first experiment designed for the systematic study of crossing over in attached-X chromosomes was performed by ANDERSON (1925). This piece of work succeeded in demonstrating the following pertinent points: (1) Crossing over in attached-X chromosomes appeared to proceed normally and regularly in spite of the fact that the two chromosomes were obliged to behave as a unit. (2) Single exchanges between non-sister chromatids occurred at random. (3) Crossing over regularly occurred at the four strand stage and only two of the four chromatids crossed over at any one level. (4) The homozygosis frequency of the recessive allele carried by the X-chromosomes decreased in a regular fashion as the position of the alleles approached one end of the chromosome, at which end, it was inferred, the centromere must be located. In addition to the above mentioned points, ANDERSON demonstrated in detail the method of progeny testing whereby the genotype of an attached-X female could be determined.

EMERSON and BEADLE (1933) published the results of an experiment the purpose of which "was to obtain more accurate data concerning the relative frequencies of different crossover types." These data supported ANDERSON'S conclusions and in addition made possible a comparison of the frequency of 2-strand and 4-strand double crossover types. In order to determine the ratio of these types, the investigators incorporated the data of ANDERSON and a large block of data from STURTEVANT (1931). The combined data demonstrated a 1:1 ratio between 2-strand and 4-strand double exchanges for those cases in which the first detectable exchange nearest the spindle fiber is non-reciprocal; *i.e.* the exchange occurs between chromatids which are destined to be attached to different centromeres at the second meiotic division. This 1:1 ratio indicated that the first exchange in no way determined what type of second exchange would occur. In other words, there was an absence of chromatid interference.

BEADLE and EMERSON (1935) then performed another experiment using attached-X

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chromosomes which were marked from scute to carnation. All previous conclusions were confirmed. When allowance was made for the possible presence of lethal mutations, it was found that the 2-strand and 4-strand double exchanges occurred in equal frequency. Furthermore, data were obtained concerning the occurrence of 2-strand and 3-strand double exchanges in those cases where the first detectable exchange was reciprocal. A reciprocal first exchange occurs between two chromatids which are destined to be attached to the same centromere during the second meiotic division. In the absence of chromatid interference these 2-strand and 3-strand doubles should occur in equal frequency. BEADLE and EMERSON found this to be true.

Up to this time, all the work performed with attached-X chromosomes tended to indicate that exchanges occurred at random between non-sister chromatids, and that when multiple exchanges occurred, there was an absence of chromatid interference. However, BONNIER and NORDENSKIÖLD (1937) claimed to have demonstrated an excess of non-reciprocal single exchanges and in the case of double exchanges they believed that there was an excess of 4-strand doubles. Thus they claimed to have demonstrated chromatid interference. The work of BONNIER and NORDENSKIÖLD has not been repeated, and no further significant studies have been performed which utilize the advantages made available by the use of attached-X chromosomes.

It should be noted at this point that in the investigation concerned with crossing over in attached-X chromosomes, all the investigators except BONNIER and NORDENSKIÖLD performed their experiments by utilizing chromosomes which were marked in essentially the same manner. That is to say, the recessive markers were carried by the chromosomes in an alternate fashion at succeeding loci. Many of the data were obtained from flies with various recessive phenotypes, and precautions had to be taken to prevent the introduction of a bias caused by viability differences. BONNIER and NORDENSKIÖLD varied the scheme by placing all the mutant alleles on one chromosome and all the wild alleles on the other. If one performs the experiment in this way, it is possible to base one's conclusions on data derived from progeny tests of flies which are all of a wild type phenotype.

The decision was made to perform another experiment utilizing the method of BONNIER and NORDENSKIÖLD in an attempt to affirm or deny the presence of chromatid interference. It was soon discovered that an autosomal inversion could be inserted into the properly marked attached-X females. Therefore, the original study was expanded so as to be a comparative study of crossing over in attached-X chromosomes in the presence of an autosomal inversion and in the absence of one. DREW SCHWARTZ (1953) has uncovered new evidence which indicates that crossing over can occur between sister strands, and it seems especially desirable at this time to re-examine or add new data to the existing evidence upon which much of our knowledge of crossing over is based.

SYNTHESIS OF ATTACHED-X FEMALES

In order to synthesize attached-X females which had all the recessive mutant alleles located on one chromosome, a marked chromosome had to be inserted into the attached-X complex. The method used in this study was to make attached-X

chromosomes by taking advantage of crossovers which occur between a duplication carried by an X-chromosome, and the homologous segment of a normal chromosome. The X-chromosome bearing the duplication B^s (Bar of STONE) was first synthesized by MULLER (1936). Females heterozygous for the duplication are viable and fertile. When a crossover occurs between the duplication and the homologous segment in the normal chromosome, an attached-X chromosome is formed. Furthermore, when the crossover occurs, the resulting attached-X females are free of the B^s and hence have a wildtype phenotype. By making the proper crosses, one can pick out newly made attached-X females by selecting those females that lack the Bar phenotype.

The required exchange apparently occurs very infrequently unless some stimulus to crossing over is applied. The third chromosome Dichaete inversion, *In(3)DcxF*, was introduced into females which were already heterozygous for the duplicated X-chromosome, and under the stimulus of this inversion, attached-X females began to appear at the rate of approximately one in 900.

The above rate of production of the attached-X type of females was sufficient for the purpose of this experiment. Females were produced which were heterozygous for the duplicated X-chromosome and another X-chromosome bearing all the recessive mutant alleles to be used as the markers for this experiment. The occurrence of the type of crossover which makes an attached-X would then simultaneously make the complex heterozygous for the recessive alleles. All the recessive alleles would be located on one chromosome unless an exchange in the duplicated region was followed by another exchange distal to this region of duplication. In that event, the markers just to the left of the distal exchange would be transferred to the other X-chromosome or completely eliminated from the attached-X complex. As long as double exchanges of this type did not occur too frequently, it was no cause for alarm because the progeny testing which followed would soon betray the actual genotype of the resulting attached-X female.

For reasons which will be discussed later, only that portion of the chromosome within about 40 units of the spindle fiber attachment was marked for study. This portion of the chromosome was well marked from the locus of pentagon to the locus of forked. The markers which were used, their symbols, and their localization according to BRIDGES and BREHME (1944) are as follows: (1) pentagon^3, *ptg*^{3}, 23.2 (2) vermilion, *v*, 33.0 (3) miniature, *m*, 36.1 (4) garnet^2, *g*^{2}, 44.4 (5) scalloped, *sd*, 51.5 (6) forked, *f*, 56.7. No two neighboring loci are more than 9.8 map units apart. With loci so closely spaced there should not be an appreciable amount of undetected double exchanges occurring between the neighboring loci. Females which are homozygous for all six mutant alleles are sterile, but hemizygous males are fertile. No sterility trouble was encountered, since the males were used to introduce the marked chromosome into the situation in which an exchange between it and the duplicated X-chromosome would produce an attached-X female.

Since the Dichaete inversion is present, it is expected that half of the synthesized attached-X females will carry the inversion and the other half will be free of it. By utilizing both types of attached-X females, it was possible to make a comparative study of crossing over. It should be pointed out that the dominant phenotype of the Dichaete inversion does not interfere with the recognition of phenotypes resulting from homozygosity of the sex-linked recessive alleles.

PRODUCTION OF ATTACHED-X PROGENY

When the technique outlined above yielded an attached-X female, the fly was transferred to a one ounce creamer which was approximately half full of food, well yeasted, and covered with a layer of wheat germ. The female was allowed to lay for a period of six days. The fly was then transferred to a fresh creamer and allowed to lay for four days more. Another transfer was followed by another laying period of four days, and finally the fly was transferred once again and allowed to lay until death. Attached-X females that were also phenotypically Dichaete required some special handling. Since the wings of such a female are always wide spread, the flies frequently became stuck to the medium or to the sides of the creamer. Therefore, half of each wing was removed, as a result of which they frequently survived long enough to yield an appreciable number of offspring. The purpose of the wheat germ was two-fold; it served both to fortify the medium with B vitamins and to take up the excess water.

The progeny furnished the data which revealed the process of crossing over as it had occurred in the parental female. It would have been possible to use the progeny of original attached-X females as the source of additional progeny and repeat this procedure generation after generation until the study was completed. However, this method may lead to the accumulation of recessive lethals in the attached-X chromosomes and thereby eliminate certain crossover classes of progeny. For this reason, new attached-X's were synthesized afresh, and in general, but not always, the data were derived from their immediate progeny.

COLLECTION OF PROGENY FROM PARENTAL FEMALES

Every parental female which is heterozygous for the marker genes listed above will give rise to a group of progeny which is phenotypically variable. Some flies will be wild type in appearance and may be homozygous or heterozygous for the wild type alleles. Some will be homozygous for one recessive allele and consequently show that characteristic phenotype. Others will be homozygous for a combination of recessive alleles and consequently will show a different recessive phenotype. The offspring of the parental females were collected as they emerged. If any one of the attached-X progeny had a recessive phenotype, this fact was recorded and the fly was discarded. If the emerging fly had a wild type phenotype, this fact was recorded also, and then the fly was placed in a fresh creamer with Muller-5 males in order to progeny test it. By collecting progeny from such a female it is possible to determine its exact genotype and to decide what sort of crossover, if any, had taken place.

RECIPROCAL VERSUS NON-RECIPROCAL EXCHANGES

Reciprocal and non-reciprocal exchanges lead to derivatives which are specific for the type of exchange. They may be identified upon progeny testing the offspring of such a female. In figure 1 note that the reciprocal exchange yields either a heterozygous crossover attached-X (1) or a heterozygous non-crossover attached-X like the mother (2). A non-reciprocal exchange yields two attached-X's which are homozygous beyond the point of exchange. In one case the mutant alleles become homozygous (3) and in the other case it is the wild type alleles which become homozygous

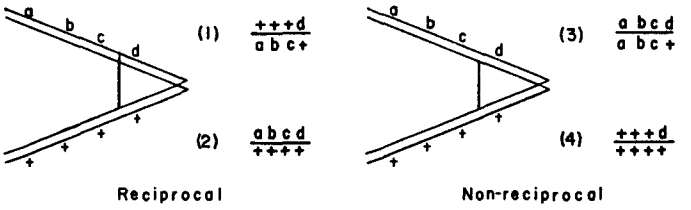

FIGURE 1.—The possible attached-X products of a reciprocal and a non-reciprocal exchange are shown above. The products of a reciprocal exchange are heterozygous to the left of the exchange point while the products of a non-reciprocal exchange are homozygous.

(4). If single exchanges occur, and these at random between non-sister chromatids, and if only wild type flies are progeny tested, then the two types +++d/abc+ and +++d/++++ should be found with equal frequency.

The results obtained from progeny testing 1638 wild type offspring are listed in table 1. For each region between two marker genes, the frequency of reciprocal and non-reciprocal exchanges was totaled and listed in table 2. It should be emphasized that in those cases where double exchanges occurred, only the rightmost exchange was noted because it is not always possible to identify the type of crossover which has occurred at the second point of exchange. The occurrence of double exchanges introduces a bias into the expected 1:1 ratio between reciprocal and non-reciprocal exchanges; the act of listing only the right-most exchanges does not alleviate the bias. However, the extent of the bias is proportional to the frequency of double exchanges, and the bias will be in the direction of an excess of non-reciprocal exchanges. As will be shown later, double exchanges were not frequent enough to cause an appreciable deviation from the expected 1:1 ratio of reciprocal to non-reciprocal exchanges. This topic will be pursued further when double exchanges come under discussion.

It is obvious from an examination of table 2 that the agreement between observation and expectation is very good. The reciprocal and non-reciprocal exchanges occur in equal frequency. No comparison for the region from forked to the spindle fiber can be made because reciprocal exchanges in this region remain undetected.

Before proceeding further it might be well to stop and ask if the data from which these exchanges were obtained are homogeneous. It happens that the 1638 wild type offspring of table 1, and hence the total of 604 reciprocal and non-reciprocal exchanges, were obtained from a total of 16 parental females. In order to test for homogeneity, the observed numbers of reciprocal and non-reciprocal exchanges produced by each parental female were compared with an expected ratio of 1:1. Since the total exchanges were obtained from 16 parents, we have 16 separate chi-squares which can now be summed; the degrees of freedom also number 16.

The sum of the chi-squares equals 10.59 for 16 degrees of freedom. The chi-square value of the total 298 reciprocal: 306 non-reciprocal equals 0.11 for one degree of freedom. This corresponds to a P value between 0.7 and 0.8. This chi-square value with its one degree of freedom can be subtracted from the sum of 10.59 to yield a chi-square of 10.48 for 15 degrees of freedom which is a measure of heterogeneity or

TABLE 1

The genotypes of wild type females as revealed by progeny tests

Attached-X genotype	+/+*	D/+†	Attached-X genotype	+/+*	D/+†	Attached-X genotype	+/+*	D/+†
plg v m g sd f / + + + + + +	982	465	*plg* + + + + *f* / + + + + + +	1	1	*plg v* + + + + / + + + + *sd f*		1
+ + + + + + / + + + + + +	42	28	*plg* + + + + + / + + + + + *f*	3		*plg v m* + *sd f* / + + + *g* + +	1	
plg + + + + + / + *v m g sd f*	87	60	*plg v* + + + *f* / + + *m g sd* +	1	1	*plg v m* + + + / + + + + *sd f*		2
+ *v m g sd f* / + + + + + +	103	43	+ + + + + *f* / + + *m g sd* +	1	2	*plg* + + *g sd f* / + *v m* + + +		1
plg v + + + + / + + *m g sd f*	42	12	*plg* + + + + *f* / + + + + + +	1		+ + + *g sd f* / + *v m* + + +	2	1
+ + *m g sd f* / + + + + + +	37	15	*plg v* + + + + / + + + + + *f*		2	*plg* + + *g sd f* / + + + + + +		4
plg v m + + + / + + + *g sd f*	63	45	*plg v m* + + + / + + + + + *f*	1		*plg* + + + + + / + + + *g sd f*	4	2
+ + + *g sd f* / + + + + + +	56	55	*plg* + + + *sd f* / + *v m g* + +	1	2	*plg* + + + + + / + + *m g sd f*	1	
plg v m g + + / + + + + *sd f*	48	30	+ + + + *sd f* / + *v m g* + +	1	1	*plg* + + + + + / + + + + + +	8	7
+ + + + *sd f* / + + + + + +	54	32	*plg* + + + *sd f* / + + + + + +	3	2	*plg v* + + + + / + + + + + +		2
plg v m g sd + / + + + + + *f*	45	23	*plg* + + + + + / + + + + *sd f*	3	1	*plg v m* + + + / + + + + + +		5
+ + + + + *f* / + + + + + +	37	19	*plg v* + + *sd f* / + + *m g* + +		1	*plg v m g* + + / + + + + + +	1	
plg + + + + *f* / + *v m g sd* +	4	1	+ + + + *sd f* / + + *m g* + +		1	+ + *m* + + + / + + + + + +	1	
+ + + + + *f* / + *v m g sd* +	2		*plg v* + + *sd f* / + + + + + +	2		Total	1638	867

* *In(3)DcxF* not present.
† *In(3)DcxF* present.

TABLE 2

The regional frequencies of reciprocal and non-reciprocal exchanges

		ptg-v	*v-m*	*m-g*	*g-sd*	*sd-f*	*f-sp.f.*	Total
In(3)DcxF absent	R*	87	42	65	51	53	52	298
	NR†	103	38	60	62	43		306
	Total	190	80	125	113	96		604
	χ^2	1.35	0.20	0.29	1.07	1.47		0.11
In(3)DcxF present	R*	60	12	47	35	27	42	181
	NR†	43	15	61	38	22		179
	Total	103	27	108	73	49		360
	χ^2	2.81	0.33	1.82	0.12	0.51		0.01

* R = Reciprocal exchange.

† NR = Non-reciprocal exchange.

disagreement among the groups. The *P* value is again between 0.7 and 0.8 and the data can be considered homogeneous.

If we add an autosomal inversion to the parental attached-X females, what effect will this have on the crossing over? Will the addition of an autosomal inversion alter the comparative frequencies of the two types of exchanges, or will they still occur in the expected ratio of 1:1? Table 2 indicates that there is not a significant deviation from the expected 1:1 ratio. The actual frequencies of exchanges between any two loci are altered by the addition of an inversion so that, in general, the amount of crossing over increases (table 6), but no modification in the ratio of reciprocal to non-reciprocal exchanges has occurred.

Since the exchanges discussed above were found among the offspring of 19 different parental females, the question again arises as to whether or not the separate blocks of data are homogeneous. For the total of 181 reciprocal to 179 non-reciprocals the heterogeneity chi-square has a value of 20.52 for 15 degrees of freedom. The *P* value lies between 0.20 and 0.10 and therefore is not significant.

DOUBLE EXCHANGES

With a source of attached-X females marked as they are in this experiment, it is possible to obtain directly the relative frequencies with which certain recognizable double exchanges occur. The result can then be compared with expectation. Figure 2 has been constructed in order to demonstrate the derivation of ratios that should be found to exist if double exchanges occur at random. This figure is patterned after one constructed by EMERSON and BEADLE (1933).

It should be emphasized that the function of figure 2 is to demonstrate the various double crossover types. In the diagram the exchanges have occurred between the markers *c-d* and *a-b*. The double exchanges could occur differently within the marked section of the chromosome, for example, between *b-c* and *a-b*. The configuration of the derivatives of the double exchanges would be quite similar to those shown in figure 2 except that a smaller piece of the chromosome has been shifted about by the double exchange.

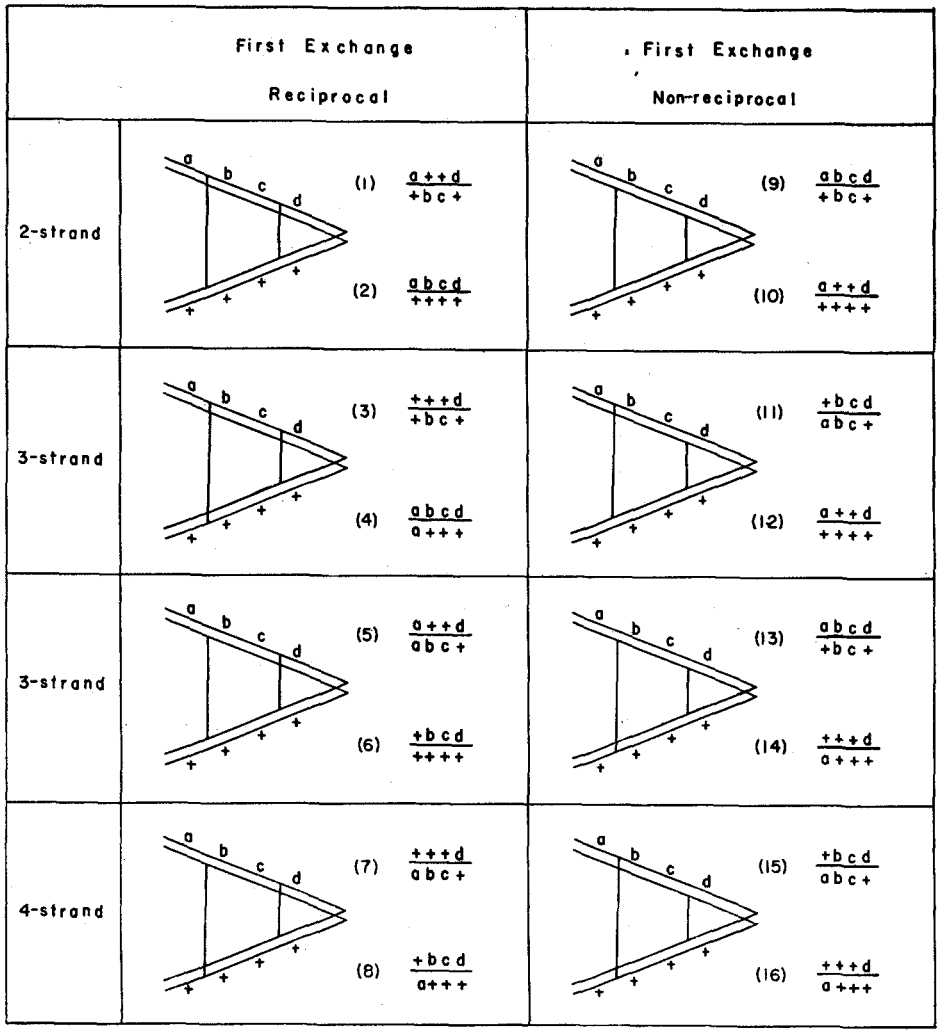

FIGURE 2.—The crossover types recovered from the eight different double exchanges which can occur between the non-sister chromatids of attached-X chromosomes.

The rightmost exchange, that is, the exchange nearest the centromere, will be referred to as the first exchange. As noted previously, this exchange may be reciprocal or non-reciprocal with equal frequency. Once the first exchange is determined, the remaining exchange can occur in such a way as to form a 2-strand, 3-strand, or 4-strand double exchange within the tetrad. If double exchanges occur at random, 2-, 3-, and 4-strand double exchange tetrads should exist in a ratio of 1:2:1.

Note those cases in which the first exchange is reciprocal. From these tetrads listed in figure 2, it is possible to recognize certain derivatives as resulting from the occurrence of double crossing over. The types $a{+}{+}d/{+}bc{+}$ (1) and $+{+}{+}d/{+}bc{+}$ (3) are two such derivatives. All other attached-X derivatives from a reciprocal first

exchange resemble non-crossovers, single crossovers, or bear a recessive phenotype and hence would not be further tested. Therefore, if attached-X female progeny with a wild phenotype are selected and tested, the double crossover types (1) and (3) should be found to exist in a ratio of 1:1. Type (1), $a++d/+bc+$, is a derivative of a 2-strand double exchange; type (3), $+++d/+bc+$, is derived from a 3-strand double exchange.

Another ratio which can be tested is one which should be found to exist in those cases where the first exchange is of the non-reciprocal type. If this happens to be the case, the double exchanges which are recognizable as such are the types $a++d/++++$ (10) and (12), and $+++d/a+++$ (14) and (16); all other double exchanges in which the first exchange is non-reciprocal result in attached-X females which have a recessive phenotype. By progeny testing wild type flies, we should find again a 1:1 ratio between the two types $a++d/++++$ and $+++d/a+++$.

One other ratio can be tested in those cases where the first exchange is non-reciprocal. Figure 2 shows that numbers (9), (11), (13) and (15) all bear the recessive phenotype *bc*. This phenotype can appear every time a double exchange occurs in which the first exchange is non-reciprocal; the recessive phenotype of these flies enables one to recognize them as having been derived from a double exchange. If this phenotypic class is then progeny tested, the two genotypes $abcd/+bc+$ and $abc+/+bcd$ should be found in a ratio of 1:1 if doubles occur at random. Most of these doubles bearing a recessive phenotype were picked up in the course of progeny testing wild type flies; their phenotype indicated that they were the product of a double exchange. They cannot be treated on a par with the other double exchanges. Inasmuch as doubles do not occur frequently in such a short segment of the chromosome, this comparatively large class of doubles formed a valuable contribution to the study.

Among the wild type flies, one more ratio remains to be tested. If we compare double exchanges in which the first exchange is reciprocal to double exchanges in which the first exchange is non-reciprocal, we should observe a 1:2 ratio. Thus types (1) and (3) versus types (10), (12), (14), and (16) should be found to exist in a ratio of 1:2.

The data pertinent to the ratios we have been considering are summarized in tables 3 and 4. In table 3 will be found a summary of data in which double exchanges have resulted in females homozygous for one or more mutant alleles. These double exchanges are equivalent to the types (9), (11), (13), and (15) of figure 2. In all future discussion the identical types (9) and (13) which result in the first case from a 2-strand double exchange and in the second case from one of the 3-strand doubles, will be referred to as a type-1 double and symbolized as $abcd/+bc+$. The remaining identical types, (11) and (15), will be referred to as a type-2 double and symbolized as $abc+/+bcd$. When we are concerned with those doubles that have a wild phenotype, the same procedure will be followed. The identical types (10) and (12), which may result either from a 2-strand or a 3-strand double exchange, will be referred to as a type-1 double and symbolized as $a++d/++++$. Types (14) and (16), which are identical and can come about as the result of a 3-strand or 4-strand double exchange, will be symbolized as $+++d/a+++$ and referred to as a type-2 double.

TABLE 3

The frequencies of type-1 and type-2 double exchanges obtained from offspring homozygous for one or more mutant alleles

Phenotype of offspring	Genotype of parent	Type-1 double		Type-2 double	
		+/+*	D/+**	+/+*	D/+**
v m g	$\frac{plg\ v\ m\ g\ sd\ f}{+\ +\ +\ +\ +\ +\ +}$	14	3	18	4
m g sd	$\frac{plg\ v\ m\ g\ sd\ f}{+\ +\ +\ +\ +\ +\ +}$	5	1	4	2
"	$\frac{plg\ +\ +\ +\ +\ +}{+\ v\ m\ g\ sd\ f}$	0	—	1	—
"	$\frac{+\ v\ m\ g\ sd\ f}{+\ +\ +\ +\ +\ +\ +}$	—	0	—	1
v m	$\frac{plg\ v\ m\ g\ sd\ f}{+\ +\ +\ +\ +\ +\ +}$	13	4	9	2
"	$\frac{plg\ v\ m\ g\ +\ +}{+\ +\ +\ +\ +\ +\ +}$	3	—	1	—
"	$\frac{plg\ v\ m\ g\ sd\ +}{+\ +\ +\ +\ +\ f}$	1	—	1	—
m g	$\frac{plg\ v\ m\ g\ sd\ f}{+\ +\ +\ +\ +\ +\ +}$	3	0	4	1
g sd	$\frac{plg\ v\ m\ g\ sd\ f}{+\ +\ +\ +\ +\ +\ +}$	7	3	5	2
"	$\frac{plg\ +\ +\ +\ +\ +}{+\ v\ m\ g\ sd\ f}$	—	1	—	1
"	$\frac{+\ +\ m\ g\ sd\ f}{+\ +\ +\ +\ +\ +\ +}$	0	—	1	—
v	$\frac{plg\ v\ m\ g\ sd\ f}{+\ +\ +\ +\ +\ +\ +}$	4	3	1	0
"	$\frac{plg\ v\ m\ g\ sd\ +}{+\ +\ +\ +\ +\ f}$	—	2	—	0
"	$\frac{plg\ v\ m\ +\ +\ +}{+\ +\ +\ g\ sd\ f}$	—	0	—	1
m	$\frac{plg\ v\ m\ g\ sd\ f}{+\ +\ +\ +\ +\ +\ +}$	2	0	0	1
g	$\frac{plg\ v\ m\ g\ sd\ f}{+\ +\ +\ +\ +\ +\ +}$	3	2	1	1
"	$\frac{plg\ +\ +\ +\ +\ +}{+\ v\ m\ g\ sd\ f}$	1	—	0	—
sd	$\frac{plg\ v\ m\ g\ sd\ f}{+\ +\ +\ +\ +\ +\ +}$	0	4	1	0
"	$\frac{plg\ v\ +\ +\ +\ +}{+\ +\ m\ g\ sd\ f}$	1	—	0	—
	Total	56	23	47	17

* *In(3)DcxF* not present.
** *In(3)DcxF* present.

TABLE 4

A summary of the double exchanges obtained from attached-X females

	First exchange reciprocal		First exchange non-reciprocal					
			Type-1		Type-2		Type-1:Type-2	
	2-strand a++d/ +bc+	3-strand +++d/ +bc+	a++d/ ++++	abcd/ + bc+	a+++/ +++d	abc+/ +bcd		
In(3)Dcxf absent	7	6	7	56	12	47	63	59
In(3)DcxF present	6	5	7	23	8	17	30	25

Table 4 contains the sum of the data found in table 3 and table 1. In those cases where the first exchange is reciprocal, 2-strand type, $a++d/+bc+$, and the 3-strand type, $+++d/+bc+$, would be expected to appear in equal frequency; the observed values are 7 and 6. In those cases where the first exchange is non-reciprocal, the type-1 and the type-2 doubles should be found to exist in a ratio of 1:1. The observed frequencies are 7 and 12. To these 19 cases one is able to add the sum of the type-1 and type-2 doubles of table 3. This results in a total of 63 type-1 ($a++d/++++$ and $abcd/+bc+$) and 59 type-2 ($+++d/a+++$ and $abc+/+bcd$). The agreement with an expected 1:1 ratio is very good.

One ratio remains to be tested. By restricting ourselves to the wild type data obtained by progeny testing, we should find that double exchanges in which the first exchange is reciprocal should be just one half as frequent as double exchanges in which the first exchange is non-reciprocal. In other words, we expect a ratio of 1:2. The observation is 13 to 19. The chi-square value of the deviation from expectation equals 0.74 and is not significant.

Double exchanges which have occurred in attached-X females bearing an autosomal inversion have been considered in the same fashion. From the progeny tests of wild type flies, the number of 2-strand, 3-strand, type-1 and type-2 double exchanges are obtained. The results of the progeny tests are enumerated in table 1 and the frequencies of the double exchanges in question have been collected from this table and summarized in table 4. The type-1 and type-2 double crossover types which are homozygous for one or more recessive mutants are listed in table 3 and are also summarized in table 4.

When the first exchange is reciprocal, the observation is 6 of the 2-strand type and 5 of the 3-strand type. When the first exchange is non-reciprocal, the observation is 7 of the type-1 and 8 of the type-2. To this total of 15 can be added the type-1 and type-2 doubles obtained by progeny testing flies which were homozygous for one or more mutant alleles and which had been derived from a double exchange as indicated by their phenotype. There were 23 cases of the type-1 double and 17 cases of the type-2 double. These 40 additional flies plus the previously obtained 15 flies gives a total of 55. Of this total, 30 were derived from the type-1 double exchange and 25 were derived from the type-2 double exchange. The deviation from an expectation of 1:1 is not significant.

It was pointed out earlier when we were discussing the frequency of the two types of single exchanges that the occurrence of doubles introduced a bias into our expected 1:1 ratio. If only the rightmost crossover was noted in the case of a double exchange, the bias was in the direction of an excess of non-reciprocal exchanges. The explanation for that statement can now be given. If doubles occur at random and only the rightmost exchange is noted, then the expectation of reciprocal to non-reciprocal single exchanges is 4:5. Observation of figure 2 will clarify this statement. Since only wild type flies were progeny tested, the types (1), (3), (7), and (8) would be observed and listed as reciprocal exchanges. The types (6), (10), (12), (14), and (16) would correspond to non-reciprocal first exchanges. The ratio is 4 reciprocal to 5 non-reciprocal. Hence when doubles do occur, the expected 1:1 ratio will be biased toward a 4:5 ratio to an extent which is dependent upon the frequency of double exchanges. Table 4 indicates that double exchanges were not very frequent and could not cause an appreciable deviation from a 1:1 ratio in this data.

THE CALCULATION OF CROSSING OVER PERCENTAGES

If one utilizes the material summarized in table 1 and table 5, it is possible to estimate the exchanges which occurred between any two loci. Table 1 lists the results of successful progeny tests on 1638 of 2006 wild type flies; table 5 lists the various frequencies of the 451 flies which had become homozygous for one or more mutant alleles. For purposes of the calculation it has been assumed that all 2006 wild type flies survived the progeny tests, and that the frequency of their various genotypes can be accurately estimated from the 1638 successful progeny tests. The crossover values are therefore based on 2006 + 451 = 2457 flies or 4914 chromosomes since each female carried two tested chromosomes.

TABLE 5

A summary of the attached-X offspring which had become homozygous for one or more mutant alleles

Phenotype of offspring	Parents +/+* number	Parents D/+† number	Phenotype of offspring	Parents +/+* number	Parents D/+† number
ptg v m g sd f	48	26	*m g sd f*	4	5
ptg v m g sd	47	12	*m g sd*	1	6
ptg v m g	55	70	*m g*	2	4
ptg v m	82	65	*m*	0	0
ptg v	57	20	*g sd f*	3	9
ptg	116	85	*g sd*	2	1
v m g sd f	15	14	*g*	1	1
v m g sd	8	4	*sd f*	1	10
v m g	5	13	*sd*	0	1
v m	2	1	*f*	1	4
v	1	0	*ptg sd f*	0	1
			Total	451	352

* *In(3)DcxF* not present.

† *In(3)DcxF* present.

TABLE 6

Crossover frequencies in attached-X chromosomes

	ptg-v	*v-m*	*m-g*	*g-sd*	*sd-f*	*f-sp.f.**	Total
Non-inversion attached-X	10.8	4.5	6.7	5.4	5.1	5.5	38.0
Inversion attached-X	12.2	3.4	9.8	8.1	4.4	8.1	46.0
Standard	9.8	3.1	8.3	7.1	5.2	9.3	42.8

* Based on the frequency of non-reciprocal exchanges.

The exchanges which occurred in the exceptional interval forked to the centromere had to be calculated by summing all the observed exchanges and multiplying the value by two. This procedure had to be followed because only the non-reciprocal exchanges yield chromosomes which are known to result from an exchange in this region. The reciprocal exchanges which occur cannot be identified. On the basis of the assumption that reciprocal and non-reciprocal exchanges occur in equal frequency, the total number of exchanges should equal twice the number of observed non-reciprocal exchanges.

The calculated crossover frequencies along with the standard frequencies are listed in table 6. In general it is the regions near the centromere which deviate the most and these deviations are in the minus direction. This is the same phenomenon which was observed by BEADLE and EMERSON (1935). In fact the crossover values calculated here are quite similar to values observed by these two investigators.

FREQUENCIES OF HOMOZYGOSIS

Since a non-reciprocal exchange results in homozygosity of loci to the left of such an exchange, it is apparent that the frequency of homozygosity of any particular locus is a function of the frequency of exchanges which occur between that locus and the centromere. A non-reciprocal exchange yields with equal frequency homozygosity for either the mutant alleles or the wild type alleles; therefore it is possible to obtain two homozygosis values for each locus. The homozygosis values of the wild type alleles can be obtained from table 1; the values for the mutant alleles are obtained from table 5. For example, in table 5 there are listed 72 cases of homozygosity for forked. These 72 cases appeared among the 2457 offspring of the attached-X parents. The frequency of homozygosis therefore equals 2.93%. The homozygosis value of the wild allele of the forked locus is a little more difficult to obtain. In table 1 are listed 52 cases of homozygosity out of a total of 1638 successful progeny tests of wild type flies. There was a total of 2006 wild type flies out of 2457 total progeny. Therefore, the frequency of homozygosity in the 2006 wild type flies would have been $52/1638 \times 2006$. The homozygosis frequency of the total of 2457 flies would therefore equal $52/1638 \times 2006/2457 = 2.59\%$. In a similar manner the homozygosis frequencies have been calculated for each allele. The frequencies are listed in table 7 for the case where the female parents are free of the inversion, and for the case where the parents are heterozygous for the autosomal inversion.

TABLE 7

Homozygosis frequencies of wild and mutant alleles

		ptg	*v*	*m*	*g*	*sd*	*f*
Non-inversion attached-X	Wild alleles	16.7	12.5	10.7	7.8	4.7	2.6
	Mutant alleles	16.5	13.2	10.9	7.8	5.2	2.9
Inversion attached-X	Wild alleles	17.7	15.2	14.0	9.2	5.7	3.8
	Mutant alleles	17.6	14.2	13.8	10.4	5.6	4.3

TABLE 8

A summary of exchange and non-exchange chromatids carried by attached-X females

	Non-crossover	%	Single crossover	%	Double crossover	%	Total
+/+*	2352	71.8	896	27.4	28	0.9	3276
D/+†	1171	67.5	539	31.1	24	1.4	1734
χ^2	2.93		5.51		3.06		11.50

* *In(3)DcxF* not present.

† *In(3)DcxF* present.

DISCUSSION

Table 2 contains the pertinent data relative to a discussion of single exchanges. We have already seen that reciprocal and non-reciprocal exchanges occur with equal frequency whether or not the inversion is present; how then do these two experiments differ otherwise? As might be expected, exchanges between chromatids are more frequent in the presence of the inversion. Table 8 furnishes a basis for this statement. The chromosomes carried by each attached-X female listed in table 1 have been considered singly and independently of each other; they have been listed as non-crossover, single crossover, or double crossover chromosomes. Once this operation was completed, a homogeneity test was performed, the results of which are summarized in table 8. The results of the two experiments are not homogeneous. When the inversion is present, the single and double crossover strands increase in frequency and the non-crossover strands decrease in number.

Another question that arises concerns the distribution of crossovers along the marked portion of the chromosome. Do the exchanges in the attached-X have the same distribution whether or not an autosomal inversion is present? The answer to this question can be found by referring to table 9 which contains a summary of a homogeneity test between these two sets of data. The chi-square value of 17.65 for 4 degrees of freedom corresponds to a P value of less than 0.01. The distribution of exchanges is not the same in the two experiments. It is obvious that the greatest portion of the chi-square value is caused by discrepancies between vermilion and miniature, and between miniature and garnet. The contribution by these two regions is equal to 15.54; furthermore, the divergence of these two regions is in opposite directions. There are too many exchanges in one section of the chromosome and too

TABLE 9

A comparison of the regional distribution of exchanges in attached-X chromosomes

	Region of Crossover					Total
	ptg-v	*v-m*	*m-g*	*g-sd*	*sd-f*	
+/+*	315	129	194	165	149	952
D/+†	190	51	162	108	76	587
χ^2	0.06	7.37	8.17	0.24	1.81	17.65

* *In(3)DcxF* not present.

† *In(3)DcxF* present.

few in the neighboring section. If we combine the two regions into one region within the markers vermilion to garnet (enclosing a standard map unit distance of 11.1) and perform another homogeneity test with 3 degrees of freedom, the significance disappears.

From these observations it is possible to conclude that if the only genetic markers in this region had been vermilion and garnet, one would have been reasonably convinced that the inversion had no effect upon the distribution of exchanges in this section of the chromosome. One wonders if other regions of the chromosome are not reacting in a similar fashion but that this has not been detected because the marked portions of the chromosome have been too gross.

STEINBERG (1936), STEINBERG and FRASER (1944), SCHULTZ and REDFIELD (1951), and MORGAN, REDFIELD and MORGAN (1943) have all reported regional differences in crossing over under the influence of an inversion. In a few cases at least the frequency of exchanges was about standard in one region and considerably greater in a close neighboring region. With observations such as these already on record, it does not seem too presumptuous to suggest that an inversion may cause regional inhibitions as well as regional increases in crossover frequencies (table 6).

It is interesting to note a possible correlation between these observations and the distribution of euchromatic and heterochromatic sections of the salivary chromosome. HANNAH (1951) has summarized a great deal of work on the localization of heterochromatin. From her summary it appears that none of the investigators who have used the phenomena of high breakability or ectopic pairing as criteria for the localization of intercalary heterochromatin have claimed a localization within the region 9C to 10F of the salivary gland chromosome. BRIDGES (1938) places the locus of vermilion at 10A of the salivary map and DEMEREC, KAUFMAN, FANO, SUTTON, SANSOME (1942) place miniature somewhere between 10C3–4 and 10E1–2. Both of these loci appear to lie within a portion of the salivary chromosome which seems to be free of intercalary heterochromatin. A discussion of Inversion(1)delta-49 found in BRIDGES and BREHME (1944) suggests that the locus for garnet lies beyond the region 11F4, possibly in region 12 or 13 of the salivary map. From the summary by HANNAH (1951) it appears that region 11 and 12 of the salivary map both contain heterochromatin. Therefore, it is possible that the region vermilion to miniature lies within a portion of the chromosome which is primarily euchromatic, and the region miniature

to garnet differs from the previous region by containing abundant intercalary heterochromatin.

One might now postulate that the increased frequency of crossing over between miniature and garnet is due to a stimulatory effect of the Dichaete inversion upon the intercalary heterochromatin localized between these two loci. The decrease in crossing over between vermilion and miniature may be due to an inhibitory effect of the inversion upon the euchromatin localized in this region of the chromosome. The observation upon which this postulate rests will have to be carefully checked.

It has been indicated earlier that double exchanges occur as expected whether or not an autosomal inversion is present; therefore, it is possible to combine the data. If we compare the double exchanges in which the rightmost exchange is reciprocal to those cases in which the first exchange is non-reciprocal, the observation becomes 24 to 34. The expected ratio is 1:2. A chi-square test yields a value of approximately 1.7 which is not statistically significant. The two types of doubles in which the first exchange is non-reciprocal are expected to occur in a ratio of 1:1. The observed numbers are 93 to 84 and the deviation from expectation is obviously not significant. The totals given by BEADLE and EMERSON (1935) may be added to this to give a grand total of 245 to 205. This total approaches significance at the five percent level.

A more detailed analysis of the data shows an interesting trend which has not been emphasized yet. If one enumerates all those double exchanges which have taken place within a short segment of the marked region of the chromosome, as opposed to those exchanges which have occurred within a larger region of the chromosome, it appears that there is a preponderance of the type-1 exchanges over the type-2 exchanges. In table 4 all those individuals with a vermilion, miniature, garnet, or scalloped phenotype have resulted from a double exchange within a shorter region of the chromosome than any of the other phenotypes listed in this table. Where the double exchanges have taken place in these short regions, the observation is 22 of type-1 to 7 of type-2. A 1:1 ratio is expected. A few more of these types of double exchanges are listed in table 1. They bring the total observation to 22 type-1 to 10 type-2. A chi-square test (with the application of Yates' correction) yields a value of 3.78. The difference between observation and a 1:1 expectation is on the borderline of significance at the five percent level. More data are necessary before a good demonstration of negative chromatid interference can be claimed.

It is possible to interpret this observation so that it is consistent with the theory of sister strand crossing over as stated by SCHWARTZ (1953). If meiotic exchanges occur only between the newly formed chromatids, as first postulated by BELLING (1931), then it follows that in the case of a double exchange both exchanges would involve the same two chromatids and would result in a 2-strand double crossover in which the first exchange was reciprocal. If a sister strand exchange occurred in one of the arms to the right of the first meiotic exchange, or an odd number of sister strand exchanges occurred as the sum of all such exchanges in both arms, the 2-strand double in which the first exchange was reciprocal would be converted into a 2-strand double in which the first exchange was non-reciprocal. Under those conditions where the meiotic crossovers are far apart, sister strand crossing over may occur between the two initial meiotic exchanges and destroy the appearance of the 2-strand double type. However,

as the two meiotic exchanges occur closer and closer together, it could be that there is less and less opportunity for a sister strand exchange to occur between them. It follows that under such conditions one would eventually obtain an excess of the 2-strand double type (type-1).

The work discussed here agrees in general with the work performed by BEADLE and EMERSON and disagrees sharply with that of BONNIER and NORDENSKIÖLD (1937). The latter investigators claimed to have demonstrated a significant excess of non-reciprocal single exchanges and, in addition, the action of chromatid interference. The data presented in this paper and the work of BEADLE and EMERSON indicate that single exchanges occur at random and if there is any chromatid interference, it must be of the negative type. The discussion below indicates that the data of BONNIER and NORDENSKIÖLD cannot be seriously considered as contradictory to the conclusions formulated here.

In accumulating the single exchanges, BONNIER and NORDENSKIÖLD multiplied all non-reciprocal exchanges by two because such an exchange yields homozygosity for both wild alleles and mutant alleles, and for every homozygous wild type fly recovered, there must have been a corresponding homozygous mutant type. On this basis non-reciprocal exchanges would be twice as frequent as reciprocal exchanges so reciprocal exchanges were then multiplied by two and a chi-square test was applied on the basis of a one to one expectation of reciprocal to non-reciprocal. In this way the chi-square values were doubled, and since they appeared to be significant, the investigators concluded that there was an excess of non-reciprocal exchanges. If the chi-squares are divided by two, the significance disappears.

If one uses the raw data presented by these investigators and enumerates the reciprocal and non-reciprocal exchanges following the method used by this author, there is an excess of non-reciprocal exchanges on the basis of a 1:1 expectation. However, it was pointed out earlier that if double exchanges become frequent, the 1:1 ratio becomes biased in the direction of a 4:5 ratio of reciprocal to non-reciprocal types. Since these investigators were studying practically the whole of the X-chromosome, from yellow to forked, double exchanges were frequent and consequently there should be found an excess of the non-reciprocal type. The actual observation lies between a 1:1 and 4:5 ratio as expected.

BONNIER and NORDENSKIÖLD concluded that there was an excess of 4-strand double exchanges and their raw data do indicate a large excess of the type-2 double. However, in many instances the double exchange which occurred had removed so many of the intervening markers that the progeny test could not determine accurately whether the remaining markers were on the same or different chromosomes. For example, a double crossover in which the first exchange is non-reciprocal and is located just to the left of forked and the remaining exchange is just to the right of yellow will yield an attached-X complex which is heterozygous for only yellow and forked, but the markers will be so far apart that the progeny test will be unable to determine whether or not they are on the same chromosome. In fact, type-1 doubles in which yellow and forked are on the same chromosome will be misclassified as type-2 doubles in which yellow and forked are on different chromosomes. It is very probable that at least some of the excess 4-strand doubles are due to a misclassification of this type.

There is one remaining point to be made. If we compare doubles in which the first exchange is reciprocal with those in which the first exchange is non-reciprocal, a 1:2 ratio is expected if doubles occur at random. From the data of BONNIER and NORDENSKIÖLD one can extract 27 of the first type and 73 of the second. A chi-square on the basis of a 1:2 expectation is not significant. If chromatid interference were at play, we would have expected a suppression of the class in which the first exchange is reciprocal because all of this class results from 2-strand and 3-strand doubles. A bias toward 4-strand doubles would rob this class and leave it deficient. If there is any chromatid interference put in evidence by these data, it must come into play only when the first exchange of a double is of the non-reciprocal type.

SUMMARY

In the course of this experiment, data have been collected which yield information on the process of crossing over as it occurs in attached-X females of *Drosophila melanogaster*. Two types of parental females were used. In one case the parents were heterozygous for the third chromosome inversion *DcxF*, and in the other case the inversion was not present. The attached-X chromosomes were heterozygous for pentagon[3], vermilion, miniature, garnet[2], scalloped, and forked; all the recessive mutants were located in one chromosome. The pertinent observations were as follows:

1. In the case of single exchanges one would expect the reciprocal and non-reciprocal type of crossover to occur with equal frequency if crossing over is at random between non-sister chromatids. A 1:1 relationship was demonstrated between the two types of exchanges. When the autosomal inversion was present in the parental females, the ratio of reciprocal to non-reciprocal exchanges remained the same, but the total exchange frequency was increased.

2. In the presence of the inversion, the distribution of exchanges along the marked segment of the chromosome was altered. In the region from vermilion to miniature (3.1 map units) the exchange frequency was markedly reduced while in the neighboring region miniature to garnet (8.3 map units) the frequency was greatly increased. It was indicated that the first region is primarily a euchromatic region while the region miniature to garnet appears to contain appreciable intercalary heterochromatin. This suggests that the inversion has stimulated the heterochromatin to crossover while crossing over in the euchromatin has been inhibited.

3. In general, the expected ratios of various types of double exchanges were obtained. These same ratios were observed when the parental females were heterozygous for the inversion. It was noticed, however, that as two exchanges occur at points which are closer and closer together, there is a tendency to obtain an excess of 2-strand double exchanges over the 4-strand type; *i.e.* there appears to be a negative chromatid interference under these conditions.

4. The observation of an excess of 2-strand double exchanges was shown to be consistent with the theory of sister strand crossing over.

5. The data presented in this paper are in reasonable agreement with the data of BEADLE and EMERSON but disagree sharply with the data of BONNIER and NORDENSKIÖLD.

ACKNOWLEDGMENTS

The author would like to express his sincere thanks to DR. ALOHA HANNAH for her many helpful suggestions, and to DR. CURT STERN for his stimulatory interest in the subject. The constructive criticism given by DR. CURT STERN, DR. R. E. CLAUSEN, and DR. E. R. DEMPSTER was greatly appreciated.

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