

# CROSSING OVER NEAR THE SPINDLE ATTACHMENT OF THE X CHROMOSOMES IN ATTACHED-X TRIPLOIDS OF *DROSOPHILA MELANOGASTER*

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## THE PROBLEM

A study of crossing over in triploid females of *Drosophila melanogaster* (BEADLE 1934) with two of the X chromosomes attached showed that crossing over near the spindle attachment is higher between the two attached chromosomes than between either of the attached chromosomes and the free chromosome. In more distal regions (beyond forked) the chromosomes were found to be involved in crossovers at random. Since these facts have a bearing on the mode of prophase pairing of chromosomes, it was thought worth while to check the experiment insofar as it concerned crossing over in the proximal portions of the chromosomes. Since only a relatively small fraction of the genetic length of the X chromosome was to be studied, the experiment could be much simplified over the original. Because of the less complicated genetic set-up in the X chromosomes it was possible to plan the experiment so that it would yield information on any relation which might exist between autosomal disjunction and detected crossing over in the X chromosome. That such a relation might be important was suggested by results obtained by Doctors J. SCHULTZ and H. REDFIELD of this laboratory (unpublished) in studies of free X chromosome triploids.

The results confirm the finding of the previous experiment that crossing over near the spindle attachment apparently does not involve chromosomes at random, but they go farther in indicating that the interpretation of this fact must take into account a relation shown to exist between recovered crossovers in the X chromosome and the type of disjunction of the autosomes.

## PLAN OF THE EXPERIMENT

In planning an experiment which would give the desired information, several considerations were of importance. It was desirable to carry out the experiment in such a way that the phenotypic frequencies of the progeny of triploids of the proper constitution could be directly used as a measure of crossing over in the several types of gametes to be studied. The alternative, testing for genotypic constitution of individuals getting more than one X chromosome strand from their mothers, must be resorted

to if any considerable portion of the X chromosome is to be followed but has the serious disadvantage that its use is excluded in the case of intersexes, which are sterile. Intersex classes are relatively weak and their viability is therefore strongly influenced by many of the characters ordinarily used in genetic studies. Since it was highly desirable to make use of intersexes, the minimum possible number of genes consistent with efficiency was used and these were selected after due consideration had been given to their effects on viability. The use of the *cIIIG* gene was avoided as a means of obtaining triploids of the proper constitution since it was known from previous experience (BEADLE 1934) that a high proportion of the triploid daughters of  $\widehat{XXY}$  *cIIIG* females are  $\widehat{XXX}$ Y in constitution; the Y chromosome is an undesirable complication.

Triploids of the desired constitution were built up by first making up a stock of  $\widehat{XXX}$  triploids of the constitution *v f cr/v f B/v f cr*. This stock could be maintained and multiplied with little danger of change due to

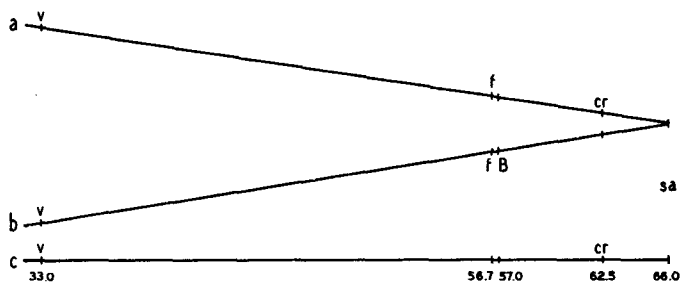


FIGURE 1.—Genetic constitution of the triploids used for a study of crossing over. The three chromosomes are designated a, b, and c. Standard map positions of the genes used as markers are indicated. The spindle attachment end is indicated by "sa" at the right.

crossing over. The only single crossover which could give a phenotypically undetectable change in constitution is a reciprocal between the attached chromosomes in the forked (*f*)-carnation (*cr*) interval. Such a change would not decrease the usefulness of the stock and the fact of its having occurred could be detected in the next generation. Such females were mated to vermilion (*v*) carnation males. Vermilion forked Bar (*B/+/+* which can be distinguished from *B/B/+*) triploids were selected from this mating. They were of the proper constitution for the experiment (figure 1). Such females were mated to forked carnation males and allowed to lay eggs for a seven day period after which they were discarded. At the end of this seven day period the cultures were removed from the 25° C incubator to room temperature (about 20°) and allowed to remain until the counts were completed. The progeny were classified at one or two day intervals for eleven days.

The triploid females used in the experiment were homozygous for the vermilion gene, the males were not-vermilion; hence any individual in the progeny with two X chromosomes (females and intersexes) which showed vermilion got both of these chromosomes from the mother. Thus the female and intersex progeny could be classified phenotypically as having either attached or free X chromosomes.

The genetic constitution of the triploids is such that crossing over between the spindle attachment and forked (or Bar since *f-B* crossing over is negligible in frequency) can be detected in any of the three possible combinations of two chromosomes. In certain cases the interval under control can be separated into two, spindle attachment-carnation (region 1) and carnation-forked (region 2). Unless otherwise stated, crossover values in the discussion of the data refer to the total of the two regions, that is, spindle attachment-forked.

PROGENY OF  $\widehat{XXX}$  TRIPLOIDS

A summary of the progeny of 123 triploid females is given in table 1. These data are more useful in indicating the types of individuals produced by  $\widehat{XXX}$  triploids than are those from the previous experiment already referred to both because the numbers are larger and because the genetic set-up is such that more information is available.

TABLE 1  
*Summary of the progeny of 123  $\widehat{XXX}$  females.*

CLASS	NUMBER
Regular males	996
Patroclinous males	4
XX females	1186
$\widehat{XX}$ females	1968
3n females	871
Matroclinous 3n females	1
XX intersexes	1854
$\widehat{XX}$ intersexes	700
Superfemales	55
Matroclinous superfemales	3
Supermales	182
Patroclinous supermales	13
Total	7833

It is well known that the distribution of chromosomes at meiosis in the free-X triploid of *Drosophila* is not random but deviates in such a way that the tendency is for equal numbers of chromosomes to be distributed to the two poles at the first division. This is shown clearly by the data published by BRIDGES and ANDERSON (1925) and which are given in table 2

of this paper. The same deviation from a random distribution is shown in  $\widehat{XXX}$  triploids but it is much less extreme (table 2). The proportion of triploid females and diploid males from  $\widehat{XXX}$  triploids is higher than from  $XXX$  triploids (BEADLE, l.c.) and is, of course, an expression of the difference in the deviation from random distribution of chromosomes in the

TABLE 2  
*Comparison of frequencies of different types of gametes from  $XXX$  and  $\widehat{XXX}$  triploids.*

EGG	SPERM	ZYGOTE	FREQUENCY		RELATIVE FREQUENCY	
			$XXX^*$	$\widehat{XXX}$	$XXX$	$\widehat{XXX}$
2X, 1A	YA	2n female	1116	1968	7.2	1.7
1X, 1A	XA	2n female	154	1186	1.0	1.0
2X, 2A	YA	Intersex	104	700	1.0	1.0
1X, 2A	XA	Intersex	898	1854	8.9	2.7

\* Data from BRIDGES and ANDERSON (1925).

two types of triploids. The cause of the deviation is unknown but the difference between  $\widehat{XXX}$  and  $XXX$  triploids shows that both the mass of chromatin and the number of independent units (chromosomes) influence the distribution.

The summary given in table 1 shows that  $\widehat{XXX}$  triploids produce several types in addition to those regularly found in the progeny of triploids. These are: matroclinous (for the X) triploids and matroclinous superfemales (from  $\widehat{XXX}$ , 2A and  $\widehat{XXX}$ , 1A eggs), and patroclinous males and patroclinous supermales (from noX, 1A and noX, 2A eggs). The experiment with  $\widehat{XXX}$  triploids referred to above showed that patroclinous males are rather frequent among the progeny of  $\widehat{XXX}Y$  triploids (13.7 percent of all males). With this fact in mind, tests were made for fertility of the patroclinous males in this experiment. Of 3 tested 2 were sterile and 1 was fertile. There is evidence therefore that at least one of the 123 triploid parents carried a Y chromosome. Probably few or no others were of this constitution, otherwise there should have been an appreciable number of patroclinous males. It is interesting to compare the relative frequencies of  $\widehat{XXX}$ , 2A and  $\widehat{XXX}$ , 1A and those of noX, 2A and noX, 1A gametes. The first two types are recovered in matroclinous triploids and in matroclinous superfemales. The ratios of these to regular triploids and superfemales must be used to correct for the violent difference in viability of the two sex types. The relative frequencies, so corrected, are 1:48 for  $\widehat{XXX}$ , 2A and  $\widehat{XXX}$ , 1A gametes. NoX, 1A and noX, 2A

gametes are recovered in patroclinous males and in patroclinous supermales. The ratio of these two types (excluding the one culture the mother of which was shown to be  $\widehat{XXXY}$ ), corrected for viability differences, is 1:24. These ratios are based on relatively small numbers but nevertheless indicate extreme deviations from the 1:1 relation expected with independent assortment of the chromosomes. They show that the frequency of large deviations from an approximately equal distribution of the chromosomes to the two poles of the first meiotic spindle is very much lower than that expected with random distribution.

#### CROSSING OVER

Since intersex classes are to be used in measuring crossing over in certain types of gametes produced by triploids, it is necessary to examine the data to see whether or not viability complications might invalidate comparisons between values based on normal and those based on intersex classes. The experiment is so arranged that crossovers recovered in  $\widehat{XX}$ , 2A gametes can be directly compared in the two sex classes, triploids and  $\widehat{XX}$  intersexes. These two classes are differentiated by the sperm and should, aside from viability differences, give similar results. The frequencies of different crossover types in these classes are given in table 3. The percentages of detected crossovers between the chromosomes of the three combinations are summarized as follows:

CHROMOSOMES	TRIPLOIDS	INTERSEXES	DIFFERENCE	S.E.
a-b	3.04	3.57	0.53	0.82
b-c	7.01	7.29	0.28	1.31
a-c	7.23	7.30	0.07	1.33

The values for a-b crossovers represent only one half the frequency of potentially detectable crossovers. They are presented in this way because of uncertain classification of one type of crossover (*v f BB*) in the  $\widehat{XX}$  intersexes. It can be seen that the values from the two sex classes agree very well; all of the differences are considerably less than their standard errors. This agreement means that there are no appreciable differential inviability effects in the different phenotypes used as measures of crossover frequencies, and it is therefore legitimate, in this experiment, to compare crossover values based on intersex classes with those based on normal sex classes.

#### *Crossover Values in Different Types of Gametes*

There are three combinations of two chromosomes in which crossovers can be detected in the experiment as set up (a-b, a-c, and b-c in figure 1).

The relative frequencies of these in different classes of individuals are given in table 3. Crossovers between the two attached chromosomes

TABLE 3  
Progeny of  $\widehat{XXX}$  triploids ( $v f cr/v f B/v cr$ ) mated to  $f cr$  males.

PROGENY CLASS	CHROMOSOMES	REGION	PHENOTYPE	NUMBER	PERCENT
Males (996)	c	0	$v cr$	844	
	c-b	1	$v f B$	45	4.51
		2	$v f B cr$	24	2.41
	c-a	1 or 2	$v f cr$	81	8.13
	c-b	1 and 2	$v$	2	0.20
XX females (1186)	c	0	$cr$	1026	
	c-b	1	$f B$	54	4.55
		2	$f B cr$	28	2.36
	c-a	1 or 2	$f cr$	75	6.32
	c-b	1 and 2	+	2	0.17
			$f$	1*	0.08
Males and XX females (2182)	c	0		1870	
	c-b	1		99	4.53
		2		52	2.38
	c-a	1 or 2		156	7.14
	c-b	1 and 2		4	0.18
	c-b-a	1 and 2		1*	0.05
XX intersexes (1854)	c	0	$cr$	1725	
	c-b	1	$f B$	32	1.73
		2	$f B cr$	32	1.73
	c-a	1 or 2	$f cr$	64	3.45
	c-b	1 and 2	+	1	0.05
Triploids (871)		0	$f B$	694	
	a-b	1	$f cr$	17	1.95
		2	$f$	7	0.80
		1 or 2	$f BB$	28	3.22
	b-c	1	$cr$	43	4.95
		2	+	18	2.06
	a-c	1 or 2	$B$	61	7.00
			$B cr$	2*	0.23
			$BB$	1†	0.11
	$\widehat{XX}$ intersexes (700)		0	$v f B$	566
a-b		1	$v f cr$	15	2.14
		2	$v f$	10	1.43
		1 or 2	$v f BB$	7	
b-c		1	$v cr$	33	4.72
		2	$v$	18	2.57
a-c		1 or 2	$v B$	51	7.30

TABLE 3 (Continued)

PROGENY CLASS	CHROMOSOMES	REGION	PHENOTYPE	NUMBER	PERCENT
$\widehat{XX}$ females (1968)		0	<i>vfB</i>	1657	
	a-b	1	<i>vf cr</i>	53	2.70
		2	<i>vf</i>	23	1.17
		1 or 2	<i>vfBB</i>	67	3.40
	b-c	1	<i>v cr</i>	40	2.03
		2	<i>v</i>	32	1.65
	a-c	1 or 2	<i>vB</i>	92	4.68
			<i>vfB cr</i>	3†	0.15
			<i>vB cr</i>	1*	0.05

\* From  $\widehat{XXX}$  parent of the constitution *v f/v f B cr/v cr*—should be added to c-a single crossovers.

† a-b-c double crossover with the b-c crossover between *f* and *B*—treated as a-b single.

‡ b-a-b or b-c-b doubles in regions 1 and 2—because a-b crossing over is more frequent in this type of gamete and because b-a-b doubles are detected with twice the frequency of b-c-b doubles, these are treated as b-a-b crossover.

(a-b) will be considered first. The detected crossover frequencies (one half the total exchange frequency between the two designated chromosomes) for different types of gametes are summarized as follows:

GAMETE	SPERM	ZYGOTE	PERCENT CROSSOVERS	S.E.
$\widehat{XX}$ , 2A	XA	3n female	6.08	0.85
	YA	$\widehat{XX}$ intersex	7.14	1.40
$\widehat{XX}$ , 1A	YA	$\widehat{XX}$ female	7.43	0.59

The value for  $\widehat{XX}$  intersexes, 7.16, is obtained by multiplying the homozygosis value for chromosome a by two; homozygosis for Bar (chromosome b) cannot be measured because of difficulties of classification for this gene in intersexes. The values are in fair agreement. The average of the two values for  $\widehat{XX}$ , 2A gametes differs from the value for  $\widehat{XX}$ , 1A gametes by 0.81 percent, a value less than its standard error. Thus there is no indication that crossing over between the two attached chromosomes is any different in the two types of gametes in which it can be measured.

The frequencies of detected crossovers between one of the attached chromosomes and the free chromosome (a-c or b-c) are:

GAMETE	SPERM	ZYGOTE	CHROMOSOMES	PERCENT CROSSOVERS	
					S.E.
1X, 1A	YA or XA	2n male or female	a-c	7.19	0.75
			b-c	7.27	
			Total	14.36	
1X, 2A	XA	XX intersex	a-c	3.45	0.62
			b-c	3.56	
			Total	7.01	
$\widehat{XX}$ , 1A	YA	$\widehat{XX}$ female	a-c	4.73	0.62
			b-c	3.68	
			Total	8.41	
$\widehat{XX}$ , 2A	XA	3n female	a-c	7.23	1.19
			b-c	7.01	
			Total	14.24	
	YA	$\widehat{XX}$ intersex	a-c	7.30	1.35
			b-c	7.29	
			Total	14.59	

In contrast to those for crossing over between the two attached chromosomes, it is clear that these values show striking differences depending on whether one or two sets of autosomes were present in the egg. The gamete types 1X, 1A and  $\widehat{XX}$ , 2A give values about twice the magnitude of the comparable values for 1X, 2A and  $\widehat{XX}$ , 1A gametes. The difference between 1X, 1A and 1X, 2A values is 7.6 times its standard error; that between  $\widehat{XX}$ , 1A, and  $\widehat{XX}$ , 2A is 5.5 times its standard error. There can be no question as to the statistical significance of these differences. It is clear, then, that crossing over between attached and free chromosomes in regions near the spindle attachment is in some way related to autosomal disjunction. An inspection of the separate values for regions 1 and 2 which are based on smaller numbers and therefore less reliable than the combined values, suggests that the differences are greater in region 1 (nearest the spindle attachment) than in region 2; the data are inadequate to establish clearly this relation.

*Crossover values between different homologs*

For comparison of crossover values between the two attached chromosomes with those between attached and free chromosomes the data can be summarized as follows:

GAMETE	SPERM	ZYGOTE	PERCENT CROSSOVERS		DIFFERENCE	S.E.
			A-B	A-C OR B-C		
$\widehat{XX}$ , 1A	YA	$\widehat{XX}$ female	7.43	4.20	3.23	0.67
$\widehat{XX}$ , 2A	XA	3n female	6.08	7.12	1.04	1.04
$\widehat{XX}$ , 2A	YA	$\widehat{XX}$ intersex	7.14	7.30	0.16	1.56



These values are comparable and represent detected crossing over (one half total exchange) for one combination of two chromosomes; the "a-c or b-c" values are the average of the two. It can be seen that in  $\widehat{XX}$ , 1A gametes crossing over between the attached chromosomes (a-b) is significantly higher than between attached and free chromosomes (a-c or b-c); the difference is 4.8 times its standard error. It is a noteworthy fact that such a difference does not exist in  $\widehat{XX}$ , 2A gametes. In fact the observed differences are in the opposite direction but are clearly not statistically significant; in one case the difference is equal to its standard error, in the other it is much less.

*Comparison of triploid and diploid exchange values*

A comparison of crossing over near the spindle attachment of the X chromosome in triploids with that of diploids is complicated by the relation between crossing over in this region in the triploid and the type of disjunction of the autosomes. It is evident that the triploid values will vary

TABLE 4

*Homozygosis values in XX daughters of triploids used in this experiment.*

CONSTITUTION	PROGENY	CROSSOVER REGION	NUMBER	PERCENT
$\frac{vf}{vf}cr$	$vfB$	0	3416	
$\frac{vf}{vf}B$	$vfcr$	1	47	1.30
	$vf$	2	63	1.74
	$vfBB$	1 or 2	94	2.60
$\frac{vf}{v}cr$	$vcr$	0	1138	
$\frac{v}{v}cr$	$vfcr$	1 or 2	41	3.48

widely depending on the type of gamete in which they are measured. A question also arises as to the choice of a diploid control. Crossing over in diploid  $\widehat{XX}$  daughters of the triploids used in the experiment has been measured. This was done in non-crossover  $\widehat{XX}$  daughters and also in  $\widehat{XX}$  daughters in which the b chromosome had been replaced, by crossing over in region 1, with the c chromosome. The data are given in table 4. The two values agree but both are lower than standard values (see BEADLE and EMERSON 1935, for discussion of this difference). The total exchange in the triploid calculated in various ways, together with the standard diploid value (twice standard map distance) and the value obtained in the  $\widehat{XX}$  diploid control (twice or four times homozygosis values, depending on whether homozygosis for both or one chromosome could be detected) are:

	Percent Exchange
Standard diploid	18.6
$\widehat{XX}$ diploid	12.6
$\widehat{XX}$ , 2A gametes in 3n daughters	40.6
$\widehat{XX}$ , 2A gametes in intersexes	43.5
$\widehat{XX}$ , 1A gametes in $\widehat{XX}$ daughters	31.7
$\widehat{XX}$ , 2A and X, 2A gametes	27.2
Average of all gametes	35.9

It is clear that there is a marked regional increase near the spindle attachment in the triploid as compared with the diploid. These results corroborate those previously obtained (BEADLE 1934).

From the experiment it is possible to determine whether or not the two marked regions are increased proportionately in the triploid. This can conveniently be done by comparing the ratios of crossover values for the two regions in the triploid and diploid. The ratios (regions 1:2) are:

Standard	1:1.7
$\widehat{XX}$ diploid	1:1.3
Triploid	1:0.6

The ratio for the triploid represents the unweighted average of the ratios for the four types of gametes. In all types of gametes the triploid shows a higher proportion of crossovers in region 1 than does the diploid. It is therefore quite clear that the increase in crossing over in the triploid falls off progressively with distance from the spindle attachment (the distal regions of the chromosome are of course not considered here).

#### DISCUSSION

##### *Relation of autosomal disjunction and X chromosome crossing over*

The evidence presented above demonstrates that there is a relation in attached-X triploids between disjunction of the autosomes and crossing over near the spindle attachment between attached- and free-X chromosomes. This relation is such that attached and free chromosomes show low crossing over in gametes which result from disjunction which gives as near equal numbers of chromosomes to the two poles of the first meiotic spindle as is consistent with the formation of gametes which can give viable zygotes with normal spermatozoa, and high crossing over in gametes from disjunction which distributes two members of each of the three major

groups of homologues to one pole and one member of each to the other pole of the spindle. These two types of disjunction are illustrated in figure 2. From the fact that the chromosomes of triploids are not distributed to the two poles of the first meiotic spindle at random it is known that there must be a correlation in the orientation of different potential trivalents on the equatorial plate. The evidence presented here shows that such selective orientation of the  $\widehat{XXX}$  trivalent with respect to the autosome trivalents is influenced by crossing over near the spindle attachment, between attached and free chromosomes, in such a way that if crossovers occur in this region, the  $\widehat{XXX}$  trivalent is less likely to be selectively oriented than if such crossovers are absent. A possible explanation of the facts can be made on the basis of two assumptions, (1) a general and non-specific force



FIGURE 2.—Representation of the two types of disjunction differentiated in the present study. A—1X, 2A:XX, 1A type which gives low crossing over between attached and free chromosomes. B—XX, 2A: 1X, 1A type which gives high crossing over between attached and free chromosomes.

of repulsion acting at and before metaphase (LILLIE 1905, KUWADA 1929, DARLINGTON 1932) and (2) a relation between exchanges and metaphase association of homologous chromosomes (DARLINGTON 1931). If exchanges are assumed to hold homologs together at the point of crossing over during their orientation on the equatorial plate, then, following an exchange near the spindle attachment between one attached and the free-X chromosome, the members of the  $\widehat{XXX}$  trivalent will be so associated that they will bear approximately the same relation to the general mass of autosomes regardless of their orientation. If, on the other hand, the free-X is distally associated with one attached chromosome, a change in orientation of the  $\widehat{XXX}$  trivalent will considerably change the relation of its mass to that of the autosomes; hence such a type of  $\widehat{XXX}$  trivalent can easily be imagined to be selectively oriented with respect to the autosomes. In a similar way, the difference between X chromosome disjunction in triploids with free-X's and those with two of the X's attached can be accounted for.

It is evident that the relation discussed above has a bearing on the problem of measuring crossing over in spindle attachment regions in triploids. The reciprocal relation to that shown here, namely, that between auto-

somal crossing over and X chromosome disjunction has not been investigated but should be taken into account in any attempt to measure accurately crossing over in spindle attachment regions.

*Prophase pairing of chromosomes*

The fact that in  $\widehat{XX}$ , 1A gametes the two attached chromosomes cross over more frequently with each other than does either with the free chromosome was previously interpreted (BEADLE 1934) as indicating that prophase pairing is by twos and that the two attached chromosomes have a pairing advantage. The fact that this relation does not hold in all types of gametes necessitates a reconsideration of the interpretation. Considering all types of gametes the net difference in crossing over is in favor of the attached chromosomes, that is, the average of the high and low values for attached and free chromosome crossing over is less than the average value for crossing over between the two attached chromosomes. These values are:

a-b	6.9
a-c or b-c	5.5
Difference	1.4
Standard error	0.6

The difference is not large but is probably statistically significant. However the evidence for the interpretation previously given is considerably weakened by the additional information now available and the question as to the nature of prophase pairing in triploids of *Drosophila* must, as far as evidence in this organism goes, remain open.

SUMMARY

Crossing over was studied near the spindle attachment of the X chromosomes in triploids of *Drosophila melanogaster* with two attached chromosomes. The experiment was so arranged that crossovers could be detected in four types of gametes, namely, 1X, 1A; 1X, 2A;  $\widehat{XX}$ , 1A; and  $\widehat{XX}$ , 2A.

The results suggest that crossing over between the two attached chromosomes is constant in the two types of gametes in which it can be studied. On the other hand, crossing over between either of the attached chromosomes and the free chromosome is markedly different in different types of gametes. It is low in those gametes which have as near half the total number of major chromosomes as is possible (1X, 2A and  $\widehat{XX}$ , 1A) and is high in those whose chromosome numbers are lower or higher than this (1X, 1A and  $\widehat{XX}$ , 2A).

Non-random distribution of chromosomes at meiosis in triploids of *Drosophila* must mean that there is a correlated orientation of chromosomes on the equatorial plate. The evidence summarized above shows that this selective orientation is influenced by crossing over.

The relation of the results to the problem of measuring crossing over in triploids and to the question of the nature of prophase pairing of chromosomes is discussed.

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