

CROSSING OVER IN THE X CHROMOSOMES OF TRIPLOID FEMALES OF *DROSOPHILA MELANOGASTER*¹

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INTRODUCTION

An investigation of crossing over in triploid females of *Drosophila* was undertaken, partly from an interest in the problem of crossing over where three instead of two homologous chromosomes are involved, and partly in hope that it would throw light on the mechanism of crossing over under the ordinary diploid condition. The advantage of using triploids is that over forty percent of their offspring come from eggs in which two homologous chromosomes, instead of one, have been derived from a single process of maturation. The constitution of the other homologues derived from that same maturation, but lost to the polar body, can only be deduced from the characteristics of the two that can be directly observed. Such an opportunity to analyse the products of a reduction division had previously been restricted to the few sporadic cases of so-called "equational

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non-disjunction" (BRIDGES 1916). Subsequently, two other means have arisen. One is the study of crossing over in cases in which two X chromosomes are permanently attached to one another (ANDERSON 1925, L. V. MORGAN 1925, A. H. STURTEVANT unpublished). The other is by artificially increasing the frequency of "equational exceptions" through use of X rays (unpublished results of ANDERSON). These four means of analysis give results that, so far as can be judged at present, are in substantial agreement. In this paper we intend to present only the facts immediately derived from the study of the $3n$ female, leaving the more general considerations for treatment when the data from all four methods of analysis are available for comparison. The present study is restricted to the X chromosomes, because of technical difficulties in the case of the autosomes. For previous information on $3n$ females, see BRIDGES 1921, 1922. A preliminary account of the present experiments and findings has been included in the Year Book of the CARNEGIE INSTITUTION OF WASHINGTON 1923, p. 286.

PLAN

The general plan of our test was to synthesize triploids of known constitution with a sufficient number of factor differences so arranged that any considerable portion of any X chromosome could be identified in the progeny. The progeny to be studied were the exceptional diploid females, since each of them carried two X chromosomes from their triploid mother. Previous data (BRIDGES) had shown that these form about 41 percent of the total offspring of triploid mothers. The total distribution is shown

TABLE 1
Offspring of $3n$ ♀ × bar ♂. (BRIDGES.)

EGG	SPERM	ZYGOTE	TOTALS	PERCENT
2X2A*	XA	$3n$ female	105	3.9
	YA	Intersex, exceptional	104	3.8
1X1A	XA	$2n$ ♀, regular	154	5.7
	YA	Male, regular	162	6.0
2X1A	XA	Superfemale	36	1.3
	YA	$2n$ ♀, exceptional	1116	41.1
1X2A	XA	Intersex, regular	898	33.1
	YA	Supermale	142	5.2

* The symbol A is used to designate a set composed of one representative of each kind of autosome.

in table 1. The productivity of the triploid is quite low, the total progeny averaging about 60 individuals, the number of exceptional females averaging about 25.

REQUIRED CONSTITUTION OF THE TRIPLOID

In order to identify crossovers, it was necessary to have each of the three X chromosomes of the triploid differing by known genes at intervals. Five points on the map (I, II, III, IV and V, figure 1) were selected, at or near each of which two different genes were located. For example, with regard to the leftmost of these points (I), one chromosome may be marked by the recessive mutant yellow, the second by the normal allelomorph of yellow, and the third by scute, a recessive that ordinarily shows no crossing over with yellow. The following chromosome map shows graph-

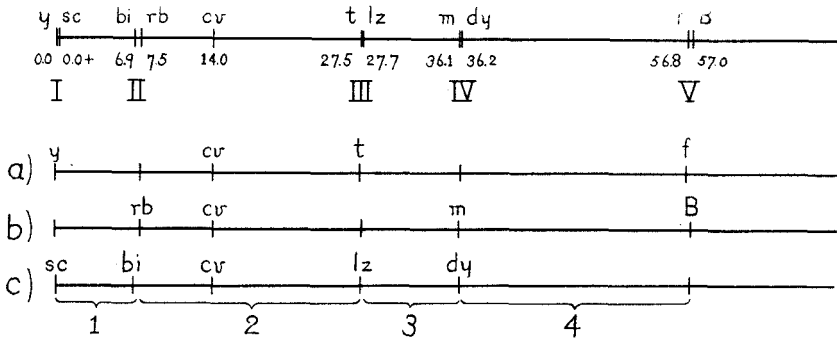


FIGURE 1.—Maps of the X chromosome showing standard loci of genes used in studies on crossing over in the triploid and a graphic representation of the constitution of the triploid females.

ically the location of the selected genes (figure 1, upper line). The distance between yellow and scute is 0.0 units, between bifid and ruby is 0.6 units, between tan and lozenge 0.2 units, between miniature and dusky 0.1 units, between forked and bar 0.2 units. These distances are all so short that crossing over within them can safely be neglected.

Three diploid stocks were planned to furnish these genes in combinations such that the viabilities would be approximately equal. In addition, each stock was made recessive for crossveinless (locus 14.0), to enable us to distinguish the exceptional offspring. The combinations selected were

- (a) Yellow crossveinless tan forked;
- (b) Ruby crossveinless miniature bar;
- (c) Scute bifid crossveinless lozenge dusky.

The total constitution of the required triploid is shown graphically in figure 1, lower three lines.

Two other stocks were needed as "testers." For these the two combinations yellow ruby miniature forked and bifid tan lozenge dusky were selected. Their purpose and use are described below.

SYNTHESIS OF THE REQUIRED TRIPLOID

The first step in the synthesis was to make up the five diploid stocks, a tedious but necessary process. This was facilitated somewhat by the use of L. V. MORGAN's "double-yellow" stock (L. V. MORGAN 1922) for multiplying males at certain stages. Although four months were required in making up these stocks, no unlooked-for difficulties were encountered.

As soon as males of the first stock, $y\ c_v\ t\ f$, were available, they were mated to triploid females. Their triploid daughters were again mated to $y\ c_v\ t\ f$ males. This process was repeated a third time in order to obtain a line of triploids homozygous for $y\ c_v\ t\ f$. Unfortunately, when these were obtained, they proved sterile. This difficulty was finally overcome by using one $c_v\ t\ f$ and two $y\ c_v\ t\ f$ chromosomes. The non-yellow triploid flies proved fertile and could be used. This stock we have designated as our triploid base. The absence of yellow in one of the chromosomes made it necessary to discard a third of the cultures in later stages of the synthesis when a test could be made for the presence of yellow.

These triploids were mated to ruby crossveinless miniature bar males. Their triploid daughters which showed bar were then mated to scute bifid crossveinless lozenge dusky males. Only about half of their triploid progeny should be of the required constitution. The not-bar triploids obviously lacked an intact chromosome "b" and hence were discarded. The others were crossed with the first tester stock, yellow ruby miniature forked. This test mating, as described below, enabled us to discard the remaining triploids that, because of crossing over, were not of the required constitution.

THE FIRST TESTER STOCK AND ITS USE

Since the data were to be obtained from the exceptional diploid offspring, the X chromosome of the male used at this stage must carry the normal allelomorph of c_v to make possible the recognition of exceptional offspring. As this chromosome was not to enter into any flies which were to be carried further, it might contain any other genes desired. This made it possible to use a stock which would help test the constitution of the triploid female to which it was mated. Since the $s_c\ b_i\ c_v\ l_2\ d_y$ chromosome had just been introduced into the triploid through the sperm in the last previous mating, it must be present intact in all of the triploids and need

not be tested for. The other two had had an opportunity for crossing over and might not be intact. Likewise, y might be absent, due to getting the wrong chromosome from the triploid base stock. The sons would give an indication of the constitution of the triploid, but would be very few in number. By introducing the proper recessive factors through the male, the more numerous intersexes could also be used for deducing the parental constitution. The presence of an intact chromosome "a" could be tested for by means of the end-points y and f . Likewise, chromosome "b" could be tested for by the two points r_b and m , and the presence of bar. The combination $y r_b m f$ was accordingly used as a tester. Cultures were discarded which failed to show all of the test points $y r_b m f$ and bar in the males and intersexes.

At this point another difficulty was encountered. Practically none of the triploids gave progeny when mated to males from the tester stock. Low fertility in the tester stock made this stock seem the probable source of the difficulty. So it was discarded, and a new $y r_b m f$ stock was built up from different sources. It proved much more fertile. The laborious synthesis of the a-b-c triploid then had to be repeated, starting from the triploid base stock, since the later stages could not be retained. Ultimately, several of the crosses were successful, and we were then able to proceed with the tests of the exceptional females that were produced.

THE SECOND TESTER STOCK AND ITS USE

The exceptional diploid females derived from the mating of the triploids with the first tester stock were readily identified by the presence of the character crossveinless. It was then necessary only to identify the constitution of their two X chromosomes. This could be done through their male offspring. Some difficulty was anticipated due to certain characters interfering with the classification of others. Thus, tan cannot be identified in the presence of yellow, nor dusky in the presence of miniature. Bifid was expected to interfere somewhat with miniature and dusky, and bar with lozenge. To overcome these anticipated difficulties a second tester had been made up, of constitution $b; t l_2 d_y$. Males of this stock were used for mating to the exceptional diploid females to be tested. This enabled us to positively identify these four characters in the female offspring. Also, and equally important, the use of the tester gave additional linkage data by which these mutants could be referred to their proper relations in the chromosomes of the mother. Bar, being dominant, could likewise be placed by study of the female offspring. The remaining genes could readily be placed from the male offspring. Counts were not made except in difficult

or unusual cases where there might be some doubt, or in cases where only a small number of offspring were produced.

Due to a shortage of flies of the second tester stock at one time, males from tan vermilion forked or from scute apricot crossveinless tan vermilion forked stocks were substituted in a few of the matings. These proved fairly satisfactory, especially since it was found in practice that bifid does not interfere with miniature and dusky, nor bar with lozenge.

CROSSING-OVER CONTROLS

It was foreseen that in triploid females the distribution of crossing over along the X chromosome might be found to be different from that in diploid females. In order that any observed difference might safely be attributed to the effect of triploidy, rather than to the effect of unknown crossing-over modifiers carried by the particular X chromosomes used, data were collected upon the crossing-over relations shown by these same chromosomes after they had passed through the triploid and emerged again in a diploid condition. Full counts were made of the male offspring of four females, two of which were of constitution a/b, one a/c, and one b/c. The data are given in table 2.

TABLE 2
Control data on linkage relations in chromosomes a, b and c.

TYPE	0	1	2	3	4	1,3	1,4	2,3	2,4	3,4
a/b	196	19	66	43	52	1	5	2	10	2
a/c	68	20	30	14	24	1	3	1
b/c	75	14	38	16	23	2	..
Totals	339	53	134	73	99	1	5	3	15	3

In addition, partial counts were made of twenty other cultures in the course of establishing the constitution of some of the more unusual exceptions. These counts are summarized in table 3. A summary of the combined data from tables 2 and 3 is given in table 4. The percentages of recombination obtained in these controls are the best basis of expectation

TABLE 3
Partial data on linkage relations in chromosomes a, b and c.

SECTIONS	TOTAL MALES	RECOMBINATIONS	PERCENT
(1) $y-r_b$	573	31	5.5
(2) r_b-l_s	684	170	24.9
(3) l_r-d_y	720	66	9.2
(4) d_y-B	862	135	15.6

TABLE 5 (continued)

3N MOTHER		E	J	A	F	D	I	H	K	TOTALS	
0-3	a-s _c b _i c _v l ₂ m B	1	1	4
	b-y c _v t d _y	1	1	
	b-s _c b _i c _v l ₂ f	1	1	
	c-r _b c _v f	1	1	
0-4	a-r _b c _v m	1	1	1	3	9
	b-y c _v t	1	1	2	
	b-s _c b _i c _v l ₂ d _y f	1	1	
	c-y c _v t B	1	1	..	2	
	c-r _b c _v m f	1	1	
0-1,2	a-s _c r _b c _v l ₂ d _y	..	1	1	4
	c-y r _b c _v t f	1	1	
	c-c _v m B	1	1	2	
0-1, 4	a-s _c r _b c _v m	2	2	3
	c-c _v t B	..	1	1	
0-2,4	a-r _b c _v l ₂ d _y B	1	1	3
	b-s _c b _i c _v t	1	1	
	c-y c _v m f	1	1	
0-3,4	c-r _b c _v B	1	1	1
2-2	s _c b _i c _v m B-r _b c _v l ₂ d _y	..	1	1	1
4-4	y c _v t-s _c b _i c _v l ₂ d _y f	1	1	3
	r _b c _v m-s _c b _i c _v l ₂ d _y B	1	1	
	y c _v t B-r _b c _v m f	1	1	
2-2,4	s _c b _i c _v m B-r _b c _v l ₂ d _y f	..	1	1	1
1-3	s _c r _b c _v m B-y c _v t d _y	1	1	2
	y r _b c _v m B-s _c b _i c _v l ₂ f	..	1	1	
4-1,2	y c _v t B-s _c r _b c _v l ₂ d _y	1	1	1
3-3	r _b c _v d _y -y c _v t m B	1	..	1	1
1-1,4	s _c c _v t f-b _i c _v l ₂ d _y B	1	..	1	1
Equationals											
0-0	a-a	1	1	1
0-1	a-y b _i c _v l ₂ d _y	..	1	2	3	10
	a-r _b c _v m B	1	1	
	b-b _i c _v l ₂ d _y	2	2	
	b-c _v t f	1	1	1	3	
	c-s _c c _v t f	1	1	
0-2	b-s _c b _i c _v m B	1	1	3
	b-r _b c _v l ₂ d _y	..	1	1	
	c-s _c b _i c _v m B	1	1	

TABLE 5 (continued)

	3N MOTHER	E	J	A	F	D	I	H	K	TOTALS	
		0-3	a-y c_v t m B	1	1	..
0-1,2	a-y b_i c_v m B	1	1	3
	c- s_c r_b c_v t f	1	1	
	c- b_i c_v t f	..	1	1	
0-1,3	c- s_c r_b c_v f	1	1	1
0-1,4	b- c_v t	1	1	1
0-2,4	b- s_c b_i c_v m f	..	1	1	1
0-1,2,4	c- b_i c_v m f	1	1	1
1-2	s_c c_v t f-y c_v l_2 d_y	..	1	1	2
	y b_i c_v l_2 d_y -y c_v m B	1	1	
1-2,4	b_i c_v l_2 d_y - s_c b_i c_v t B	..	1	1	2
	s_c c_v t f-y c_v m	1	1	
3-1,3	r_b c_v d_y - b_i c_v l_2 m B	..	1	1	1
	Totals	60	32	29	25	15	13	6	2	182	182

crossover chromosomes, y c_v t f, r_b c_v m B, and s_c b_i c_v l_2 d_y , respectively. The individual genes are given for all chromosomes derived from crossing over.

Table 6 presents the same data in a more schematic form, with the letters a, b and c substituted for the factor symbols. The letter indicates the original chromosome from which the particular gene was derived.

THE SOURCES OF THE GENES OF THE EMERGING CHROMOSOMES

The 182 exceptional individuals give a total of 364 distinct X chromosomes, each of which has emerged from an a-b-c triploid mother. A classification of these 364 chromosomes according to the source of the genes shows (summary in table 7) that 69 percent of the emerging chromosomes are identical with an original chromosome; that is, they are either a, b or c, each intact throughout. About 28 percent are composed of sections taken from some two of the three original chromosomes, from a and b, from a and c, or from b and c. These chromosomes have arisen through a process of crossing over. The kinds and frequency of the crossing over will be examined in detail in a following section. Finally, nearly 3 percent of the emerging chromosomes contain parts taken from all three of the

TABLE 6
Generalized constitutions of exceptional offspring of triploid females.

EXCEPTIONS, NON-EQUATIONAL			EQUATIONALS					
0	<u>aaaaa</u>	28		<u>bbbbbb</u>	1	0	<u>aaaaaa</u>	1
0	<u>bbbbbb</u>			<u>cccca</u>		0	<u>aaaaaa</u>	
	<u>aaaaa</u>	28	0	<u>ccccc</u>	2		<u>aaaaaa</u>	3
	<u>ccccc</u>		4	<u>aaaab</u>			<u>acccc</u>	
	<u>bbbbbb</u>	28		<u>ccccc</u>	1		<u>aaaaaa</u>	1
	<u>ccccc</u>			<u>bbbba</u>			<u>abbbb</u>	
	<u>bbbbbb</u>	2		<u>aaaaa</u>	1	0	<u>bbbbbb</u>	2
	<u>acccc</u>			<u>cbccc</u>		1	<u>bcccc</u>	
	<u>aaaaa</u>	4	0	<u>ccccc</u>	1		<u>bbbbbb</u>	3
	<u>cbbbb</u>		1,2	<u>abaaa</u>			<u>baaaa</u>	
	<u>aaaaa</u>	5		<u>ccccc</u>	2		<u>ccccc</u>	1
0	<u>bcccc</u>			<u>babbb</u>			<u>caaaa</u>	
1	<u>bbbbbb</u>	1		<u>aaaaa</u>	2		<u>bbbbbb</u>	1
	<u>acccc</u>			<u>cbbbc</u>			<u>ccbba</u>	
	<u>bbbbbb</u>	3	0	<u>ccccc</u>	1	0	<u>bbbbbb</u>	1
	<u>caaaa</u>		1,4	<u>baaab</u>		2	<u>bcccc</u>	
	<u>ccccc</u>	1		<u>aaaaa</u>	1		<u>ccccc</u>	1
	<u>abbbb</u>			<u>bbccb</u>			<u>ccbba</u>	
	<u>ccccc</u>	3	0	<u>bbbbbb</u>	1	0	<u>aaaaa</u>	2
	<u>baaaa</u>		2,4	<u>ccaac</u>		3	<u>aaabb</u>	
	<u>aaaaa</u>	4		<u>ccccc</u>	1		<u>aaaaa</u>	1
	<u>bbccc</u>			<u>aabba</u>		0	<u>acbbb</u>	
	<u>aaaaa</u>	3	0	<u>ccccc</u>	1	1,2	<u>ccccc</u>	1
	<u>ccbba</u>		3,4	<u>bbbab</u>			<u>cbaaa</u>	
	<u>bbbbbb</u>	2	2	<u>ccbba</u>	1		<u>ccccc</u>	1
	<u>aaccc</u>		2	<u>bbccc</u>			<u>bcaaa</u>	
0	<u>bbbbbb</u>	2		<u>aaaac</u>	1	0	<u>ccccc</u>	1
2	<u>caaaa</u>		4	<u>cccca</u>		1,3	<u>cbbaa</u>	
	<u>ccccc</u>	5	4	<u>bbbbbb</u>	1	0	<u>bbbbbb</u>	1
	<u>aaabb</u>			<u>ccccb</u>		1,4	<u>baaac</u>	
	<u>ccccc</u>	1		<u>aaaab</u>	1	0	<u>bbbbbb</u>	1
	<u>bbaaa</u>			<u>bbbba</u>		2,4	<u>ccbba</u>	

TABLE 6 (continued)

EXCEPTIONS, NON-EQUATIONAL				EQUATIONALS				
	<u>a a a a a</u>	1	<u>2</u>	<u>c c b b b</u>	1	<u>0</u>	<u>c c c c c</u>	1
	<u>c c c b b</u>		2,4	<u>b b c c a</u>		1,2,4	<u>b c b b a</u>	
0	<u>b b b b b</u>	1		<u>c b b b b</u>	1		<u>a c c c c</u>	1
3	<u>a a a c c</u>		1	<u>a a a c c</u>		1	<u>a a b b b</u>	
	<u>b b b b b</u>	1	3	<u>a b b b b</u>	1	2	<u>c a a a a</u>	1
	<u>c c c a a</u>			<u>c c c a a</u>			<u>a a c c c</u>	
	<u>c c c c c</u>	1	4	<u>a a a a b</u>	1		<u>b c c c c</u>	1
	<u>b b b a a</u>		1,2	<u>c b c c c</u>			<u>c c a a b</u>	
	<u>a a a a a</u>	3	3	<u>b b b c c</u>	1	1	<u>c a a a a</u>	1
	<u>b b b b c</u>		3	<u>a a a b b</u>		2,4	<u>a a b b c</u>	
0	<u>b b b b b</u>	2	1	<u>c a a a a</u>	1	3	<u>b b b c c</u>	1
4	<u>a a a a c</u>		1,4	<u>b c c c b</u>		1,3	<u>b c c b b</u>	

TABLE 7

Sources of emerging individual chromosomes.

a	b	c	ab	ac	bc	abc
89	81	82	33	29	40	10
252 (69.3 percent)			102 (28.0 percent)			(2.7 percent)

original chromosomes. These are the most interesting of the emerging chromosomes, since their production involved crossing over, and hence synapsis, of all three of the original chromosomes.

THE KINDS AND FREQUENCIES OF THE CROSSING OVER SHOWN BY THE EMERGING CHROMOSOMES

When the constitutions of the 364 emerging chromosomes are examined and classified with respect to the section or sections in which crossing over has occurred, it is seen (summary in table 8) that about 69 percent of the

TABLE 8

Crossovers shown by emerging chromosomes.

Sections...	0	1	2	3	4	1,2	1,3	1,4	2,3	2,4	3,4	1,2,4
Total	252	36	25	11	16	8	2	5	..	7	1	1
Percent	69.2		24.2	(88)			6.3	(23)				0.3

emerging chromosomes have undergone no apparent crossing over within the distance from yellow to bar. About 24 percent of the chromosomes have resulted from simple crossing over of some two of the original chromosomes. About 6 percent have arisen through double crossing over involving two or three of the original chromosomes. There was one case of triple crossing over that involved all three chromosomes. The nature of these multiple crossings over will be discussed in a following section in more detail.

When the cases of crossing over are summarized to show the total amount that occurred in each crossing-over section (summary in table 9), it is found that the amount of recombination due to crossing over in the

TABLE 9
Summary of crossing over in triploid females.

SECTIONS	TOTAL RECOMBINATION	RECOMBINATION PERCENT	CONTROL PERCENT	RATIO	STANDARD PERCENT
1	52	14.3	6.9	2.07 : 1	7.5
2	41	11.3	22.8	0.50 : 1	20.0
3	14	3.9	10.1	0.39 : 1	8.7
4	30	8.2	16.2	0.51 : 1	20.8

first section is over twice as high as the corresponding percentage for the diploid control data. Throughout the rest of the X the crossing over is only about half the normal amount. It seems probable that the crossing over in the extreme left end is even higher than the high general average for section 1, for two of the 364 chromosomes show crossing over between yellow and scute, and no case of crossing over between yellow and scute has been encountered in diploid material, though several thousand chances have occurred. These two crossovers have settled the serial order of yellow and scute (scute being to the right of yellow), and have given a useful double-recessive stock.

The fact is clear that in the triploid female there is a regional difference of marked extent in the deviations from standard amounts of crossing over for the X chromosome. This indicates that a unit of crossing over at the extreme left end of the X-chromosome map may correspond to a longer section of the chromosome than does a unit of crossing over in regions further to the right. This had already been indicated by the relatively high frequency of mutation per unit of map distance at the extreme left end of the map.

MULTIPLE CROSSING OVER AND COINCIDENCE

The double crossovers (summarized in table 10) are of two distinct types which we shall designate as "recurrent" and "progressive." In the

recurrent type the second crossover takes place between the same two chromosomes as the first, and, as a result, a mid-section from one chromosome becomes intercalated between the ends from another chromosome.

TABLE 10
Double crossing over and coincidence in triploid females.

SECTIONS	RECURRENT	PROGRESSIVE	TOTAL	COINCIDENCE	COINCIDENCE IN CONTROL	STANDARD COINCIDENCE
1,2	6	3	9	1.54	..	.20
1,3	1	1	2	1.00	.15	.45
1,4	4	1	5	1.16	.50	.95
2,318	.30
2,4	3	5	8	2.37	.59	.80
3,4	1	..	1	0.87	.30	.40
Total	15	10	25			

In the progressive type the second crossover takes place between chromosomes different from the first and as a result the emerging chromosome is derived from all three original chromosomes. As examples from table 5, the double-crossover chromosome $y c_v m f$ (a a b b a) is classified as recurrent, while $s_c b; c_v t B$ (c c a a b) is classified as progressive. The 1,2,4 triple crossover (b c b b a) can be analyzed into a 1,2 recurrent and a 2,4 progressive.

If the occurrence of one crossover does not prejudice the manner of occurrence of the second crossover these two types should be equally frequent. As the summary of table 10 shows, there were 15 recurrent and 10 progressive crossovers. This is not a statistically significant departure from equality. That half, or nearly half, of the double crossovers were of the progressive type signifies that, in general, two strands that have crossed over with each other were as free to cross over with a strand from the third chromosome as with a strand from one of the two original chromosomes. That is, all three chromosomes must be substantially on a par with one another in the manner of synapsis. This is good evidence that synapsis in the triploid female, generally, if not always, involves all three chromosomes equally throughout their length, and does not follow the type that seems to obtain for the sex chromosomes of an XXY female, namely, complete synapsis between two with the third left unsynapsed.

The coincidence values, calculated from the combined recurrent and progressive double crossovers, all involve high probable errors and are therefore not greatly to be relied upon (table 10). The surprising feature is their high values as compared with corresponding indices for control and for standard data. In three of the classes the occurrence of one cross-

over seems actually to have favored the occurrence of a second (coincidence over 1.0). A decrease in crossing over accompanied by a rise in coincidence shows that it is not simply the frequency of crossing over that is reduced (which would give lowered crossing over with unchanged proportion of doubles among those that do occur), but that the average distance apart of those that do occur is reduced.

THE GENES ASSOCIATED IN EMERGING CHROMOSOMES

Having examined the emerging chromosomes individually as to the source of their genes and as to the types of crossing over, we may next examine the characteristics of the chromosomes that have emerged together. From the types of "association" we should be able to deduce the types of distribution that have been produced by the two maturation divisions. Of the 182 exceptional flies that were examined as to the nature of the associated chromosomes, 28 (rightmost column of table 6) were found to have chromosomes that were identical for some part of their lengths. They possessed two representatives of certain genes of which the mother possessed only one. They are called "equational" exceptions, since they are of the type that would be produced by failure of the separation of sister strands of the tetrad (or hexad in this case).

The most significant point in connection with these equational exceptions is that they are not necessarily identical *throughout* their length. One may be an original chromosome throughout and the other be a crossover composed of a part of that same chromosome joined to a supplementary part from a second chromosome. Furthermore, in case one is a crossover the other is not likewise an identical crossover at the same point. *This means that at the time crossing over occurred each of the original chromosomes had already become split into two strands.* If crossing over occurred before each original chromosome had thus split, then the two strands must necessarily be identical throughout their length, and if one were derived by crossing over at a particular point between a given two original chromosomes, then the other must be an identical crossover at that point between the same two chromosomes. As the analysis shows, at the time of crossing over there are present in the triploid female six strands, and sister strands can undergo crossing over, each with a different other strand. This conclusion had already been indicated in the study of equational exceptions from diploid females (BRIDGES 1916) and is confirmed by the study of crossing over in attached-X females (ANDERSON 1925, L. V. MORGAN 1925, STURTEVANT, unpublished) and by the study of equational exceptions from X-rayed females (unpublished work of ANDERSON).

On random distribution of the six strands present before maturation, a given strand will be associated with its sister strand in one-fifteenth of the cases, and all equational cases should total three-fifteenths or 20 percent of the cases. This calculation disregards the fact that a given strand loses its original constitution through crossing over, so that it would be more correct to say that for any given point, e.g., the locus of yellow, the equationals are expected to be 20 percent of the total. When the associated chromosomes are tabulated with respect to each of the five points followed (table 11), it is seen that the values for all the different points are far below this expectation. That is, there is a strong tendency for genes in sister strands to pass to opposite poles rather than to remain together at the same pole. But this tendency is a graded one. At point V

TABLE 11
Associations of genes in emerging chromosomes.

	I	II	III	IV	V	EXPECTATIONS
aa	9	5	3	1	1	12.1
bb	8	1	2	2	1	12.1
cc	4	4	12.1
Subtotal	21	10	5	3	2	36.4
Percent	11.5	5.5	2.7	1.6	1.1	20.0
ab	51	60	59	60	56	48.5
ac	55	59	59	61	62	48.5
bc	55	53	59	58	62	48.5
Subtotal	161	172	177	179	180	145.6
Percent	88.5	94.5	97.3	98.4	98.9	80.0

(*f, B, +*) there were only two cases of association of sister genes, which gives 1.1 percent of association instead of the twenty percent expected. For the points further to the left the percentage is progressively higher, being 11.5 at point I (*y, s_c, +*). This seriation shows that the two ends of the X chromosome are markedly different with respect to separation of sister genes: at the extreme left the association is fairly frequent, but probably at the extreme right it does not occur at all. For all except two cases, crossing over has occurred to the right of the homozygous section, and in these two cases (the *y c_v t f* and the *c_v m B* equationals) crossing over between forked and the right end (some 13.5 units still further to the right than forked) may well have occurred. This indicates that the right end of the chromosome is the end governing the separation of the strands, for

which point the first² division is invariably reductional. The second division then invariably separates sister strands and the percentage of association for the extreme right end is zero. But whenever crossing over occurs, the part of one strand distal to the point of crossing over (that is, to the left) is replaced by a strand from another homolog. Then at the first division, the separation for the extreme right end, and for all points between it and the point of crossing over is reductional and for the remainder is equational. At the end of the first division in the triploid female the egg nucleus that will give an exception includes four strands, one of which, a a a b b, is a crossover between two original strands, and is accompanied by a full b b b b b strand. The other two strands are the strands reciprocal to the first, namely, a a a a a and b b b a a, or are both c c c c c. In case the other two strands are both c c c c c, then the second division will give either $\frac{a a a b b}{c c c c c}$ or $\frac{b b b b b}{c c c c c}$, either of which gives an ordinary-type exception. But in case the other two strands are the reciprocals then the second division will result in $\frac{a a a a a}{b b b b b}$, or $\frac{a a a a a}{a a a b b}$, or $\frac{b b b a a}{b b b b b}$, or $\frac{b b b a a}{a a a b b}$. Two of these equally probable possibilities result in equationals, one results in complementary crossover strands and one results in different non-crossovers. The further to the left a given point lies, the more numerous should be the cases in which crossing over between the given point and the extreme right end would occur and give opportunity for an equational exceptional to arise.

The reason that the separation begins at the right end is presumably that the separating mechanism, the spindle fiber, is attached to that end. This conclusion has also been reached by a study of the seriation in the frequency of equationals that occur in attached-X cases (ANDERSON 1925, L. V. MORGAN 1925, and unpublished work of STURTEVANT). We may therefore regard it as established that the spindle fiber is attached at the right end and that the left end is the free, outer, or distal end of the X chromosome.

THE CROSSING-OVER TYPES PRESENT IN ASSOCIATED CHROMOSOMES

As we have seen, a given gene that was present only once in the mother can come out in both X's of a daughter only if crossing over occurs between

² As far as the genetic data show, it could equally well be assumed that it is the second division that is reductional. To avoid lengthy discussion, we have here followed through only the case in which the reduction separation occurs at the first division, as is clearly the case in the male.

its locus and the point of spindle-fiber attachment, and accordingly the constitution of associated chromosomes with respect to the genes is not independent of their constitution with respect to crossing over. The analysis just given, together with table 11, was primarily concerned with the origin of the genes of the associated chromosomes. In the paragraph below, the associated chromosomes will be examined primarily with regard to the types of crossing over shown. There are four sections within each of which it can be determined whether a crossover has occurred or not, and in case it has, which chromosomes have contributed the parts of the emerging crossover chromosome. Table 5 is arranged according to the crossover formulae of the emerging chromosomes.

There were 84 cases in which the two associated chromosomes were both non-crossovers throughout their length.

There were 19, 17, 4 and 9 cases in which one chromosome was a complete non-crossover and the other chromosome was derived from the other two by crossing over in sections 1, 2, 3 and 4, respectively.

There were a few cases in which the second chromosome had come from double crossing over.

An especially interesting type of case is that in which the two associated chromosomes were both crossovers at the same point between the same two chromosomes, and were, in fact, complementary products of the same crossing over (2-2; 4-4; 2-2,4).

There were a few cases in which both chromosomes were crossovers, but in different sections (1-3; 4-1,2). One strand of a given chromosome (a) crossed over with one strand from a second chromosome (b) at one place, while the other a-strand crossed over with a strand from the third chromosome (c).

Finally, there is the special case, differing from the above only in that one a-strand crossed over with a b-strand in a certain section, while the other a-strand crossed over with a c-strand in that same section, though most probably not at the same point within the section (3-3; 1-1,4).

Within the equationals approximately the same types of chromosomes with respect to crossing over were encountered. In the one case where both chromosomes were identical non-crossovers throughout the sections followed, it is inferred that one of them had undergone crossing over to the right of point V, that is, between bar and the spindle-fiber attachment.

The $\frac{b\ b\ b\ b}{c\ c\ b\ b}$ individual likewise involved crossing over between point V and the right end.

These relations are generalized in table 6, where the space between consecutive symbols represents the crossing-over section, and the symbols give the source of the genes that emerge on the two sides of the crossing-over section. In table 12 is a summary of the cases of the different types with respect to each crossing-over section.

TABLE 12
Associations of crossovers in emerging chromosomes.

TYPE OF ASSOCIATIONS	FORMULA	1	2	3	4	TOTAL	PERCENT
Two non-crossovers, different	$\frac{a a}{b b}$	126	140	167	153	586	80.5
Crossover and dissimilar non-crossover	$\frac{a b}{c c}$	29	28	8	23	88	12.1
Crossover and similar non-crossover	(a) $\frac{a b}{a a}$	16	7	2	1	26	3.6
	(b) $\frac{b a}{a a}$	5	2	7	1.0
Two crossovers, complementary . .	$\frac{a b}{b a}$..	2	1	3	6	0.8
Two crossovers, different	$\frac{a b}{c a}$	1	..	1	..	2	0.3
Two non-crossovers, identical	$\frac{a a}{a a}$	5	3	3	2	13	1.8
Two crossovers, identical	$\frac{a b}{a b}$	0.0

Thus, if we let a represent a gene from any given chromosome (either a, b or c) and b represent a gene from either one of the other two chromosomes, then by far the most frequent type of association was that in which both strands were non-crossovers, but the second strand was from a different original chromosome than the first (formula $\frac{a a}{b b}$.)

The next most frequent case was that in which one strand was a crossover between two different original chromosomes and the other strand came from the third original chromosome ($\frac{a b}{c c}$).

Another type of case is that in which a crossover strand is associated with a non-crossover strand from one of the two chromosomes involved

in the crossing over. This type of association gives rise to equational exceptions. The above general type has two subtypes, namely, that in which the homozygous gene is to the left of the crossover point (formula $\frac{a b}{a a}$) and that in which it is to the right of the crossover point (formula $\frac{b a}{a a}$). On chance, these two types should be equal, but it was found that they were clearly unequal, in that 26 were $\frac{a b}{a a}$ while only 7 were $\frac{b a}{a a}$. A consideration of the spindle-fiber attachment has shown that equationals only arise when a crossing over occurs somewhere to the right of a given gene; therefore, the equationals should all be of the $\frac{a b}{a a}$ type, and none of the $\frac{b a}{a a}$ type. The assumed discrepancy is only apparent, for closer examination of these seven cases shows that in each of them the original situation had been disturbed by a crossing over of one or the other strand still further to the right.

The association of a crossover chromosome with a non-crossover similar to one of its own parts ($\frac{a b}{a a}$) should be half as frequent on chance as its association with the third chromosome ($\frac{a b}{c c}$). This holds approximately only for the leftmost section, and is about one-fourth for sections 2 and 3, while only one of the 24 crossovers of section 4 was of the $\frac{a b}{a a}$ type. An important comparison may be had when only the rightmost crossovers are considered. These are summarized in table 13. The ratios of the $\frac{a b}{c c}$ to the $\frac{a b}{a a}$ type of association are practically the same as when all crossovers are considered (table 14). The very low frequency of the $\frac{a b}{a a}$ type for region 4 may well indicate the absence of this type of association at the extreme right end, that is, near the spindle-fiber attachment. The gradually increasing frequency toward the left may be accounted for, at least in part, by the increasing possibility of crossing over further to the right in the chromosome which supplies the left-hand section of the observed crossover chromosome. In such cases the $\frac{a b}{a a}$ type of association may be

derived by the same process which gives rise to the $\frac{a\ b}{c\ c}$ type. Any such increase in the $\frac{a\ b}{a\ a}$ type of association should be accompanied by a slight decrease in the $\frac{a\ b}{c\ c}$ type. In the absence of interference, the percent of invalidity of the left crossover section, due to crossing over to the right

TABLE 13

Association of crossovers in emerging chromosomes; rightmost crossovers only.

TYPE OF ASSOCIATION	FORMULA	1	2	3	4	TOTAL
Two non-crossovers, different	$\frac{a\ a}{b\ b}$	84	113	142	153	492
Crossover and dissimilar non-crossover.	$\frac{a\ b}{c\ c}$	19	24	6	23	72
Crossover and similar non-crossover.	$\frac{a\ b}{a\ a}$	10	4	2	1	17
Two crossovers, complementary.	$\frac{a\ b}{b\ a}$..	1	1	3	5
Two crossovers, different	$\frac{a\ b}{c\ a}$	1	..	1

TABLE 14

Comparison of seriation of ratios of $\frac{a\ b}{c\ c}$ to $\frac{a\ b}{a\ a}$.

TYPE OF ASSOCIATION	1	2	3	4	TOTAL
All crossovers (table 12)	1.8	4	4	23	3.4
Right-hand crossovers (table 13)	1.9	6	3	23	4.2

of the observed crossover, should equal the percent of crossing over between the region and the spindle-fiber attachment. Allowing for six percent of crossing over to the right of forked and bar, the invalidity would amount to about 10 percent for the mid-point of region 4, 16 percent for region 3, 24 percent for region 2, and 36 percent for region 1. Interference would reduce the percentage, especially in the right-hand regions, whereas the high frequency of double crossing over where longer regions are involved, as shown by the extremely high coincidence values, should give a marked increase in the left-hand regions. Crossing over to the right of point V would similarly invalidate either chromosome.

Another type of association is that of complementary crossovers, $\frac{a\ b}{b\ a}$.

This type is expected to be half as numerous as the $\frac{a\ b}{a\ a}$ type, and a quarter as numerous as the $\frac{a\ b}{c\ c}$ type. In fact, it was far below either of these

expectations, having been only one-fifteenth as numerous as the $\frac{a\ b}{c\ c}$ type.

When only the right-hand crossovers are considered (table 13), the proportions remain the same. There is a suggestion of a seriation increasing from left to right.

An unknown, but probably small, proportion of the cases that are apparently $\frac{a\ b}{b\ a}$ may have been really examples of another type, namely,

one in which each of the two strands of a given original chromosome has crossed over somewhere within a given crossing-over section. Thus, if a strand from a given chromosome crosses over with the sister strand from the second within that same section, then it is possible for an $a\ b$ strand to be associated with a $b\ a$ strand without the two having been complementary products of a single crossing over. If each of two sister strands crosses over within a given section with a strand from a different other chromosome, then an $\frac{a\ c}{b\ a}$ type of association can arise. Two clear cases

of this were found, and this fact is a strong argument that all three X chromosomes were in synapsis throughout their length, and not simply that the left ends of two were synapsed and the right end of the third was synapsed with the right end of one of the first two. This argument is the stronger the shorter the section in which both strands crossed over with strands from different other chromosomes. Section 3 seems short enough to meet this requirement, and one of the two cases occurred there.

Cases in which both associated strands are identical non-crossovers ($\frac{a\ a}{a\ a}$) arise when there has been crossing over in some section to the right

of that considered. They are equationals. Only a few such cases were found, and this fact shows that most of the seriation present in the proportions of homozygosis (table 11) was not due to accumulated crossovers, but rather due to the seriation from right to left in the ratio

of $\frac{a\ b}{a\ a}$ to $\frac{a\ b}{c\ c}$ association.

A very significant feature is the total absence of the association of identical crossovers $\frac{a b}{a b}$. This class would be expected to occur freely if crossing over occurred before the splitting of the chromosomes, whereas the $\frac{a b}{a a}$ and $\frac{a b}{c a}$ classes would be impossible. The frequent occurrence of the latter classes together with the total absence of the $\frac{a b}{a b}$ class proves that crossing over does not occur until after each original chromosome has split into two strands, each of which then follows its own separate course during the crossing-over process.

The question arises as to the possibility of crossing over between the two strands that are derived from the same original chromosome. Since such crossovers do not lead to recombination of linked characters, they cannot be detected directly. If they occur with a frequency equal to that of crossing over between any two other strands, one-third of the total crossing over in the diploid would be undetectable, whereas in the triploid it would be only one-fifth. A systematic increase of approximately 20 percent in the observed crossing over in the triploid might then be expected. However, the specific changes in crossing over in the triploid, that is, the reduction in regions 2, 3 and 4, and the large increase in region 1 mask any difference that might be due to crossing over between sister strands.

SUMMARY

In order to study crossing over in the triploid condition, triploid females of *Drosophila melanogaster* were synthesized in such a way that five points in each of their three X chromosomes could be identified in their progeny. The X constitution of these triploids is represented graphically in figure 1 (page 420). Only the exceptional diploid daughters are included in the data. These contain two X chromosomes derived from their triploid mother and none from their father. The advantage of using the exceptional daughters is that their two X chromosomes were derived from a single oöcyte. The constitutions of 182 exceptional daughters were identified and are given in detail in tables 5 and 6.

Of the chromosomes present in the exceptional daughters, 69 percent were non-crossovers, 28 percent were crossovers involving two of the three original chromosomes, and about 3 percent were crossovers involving all three of the parental X chromosomes.

Crossing over in the region from yellow to bifid was twice as high as in the diploid controls. In the regions to the right of bifid it was only one-half as high.

Two types of double crossing over were found: a recurrent type in which the second crossover takes place between the same two chromosomes as the first, and a progressive type in which the second crossover takes place between chromosomes different from the first. The latter type gives a chromosome derived from parts of all three original chromosomes. Of the double crossovers obtained, 15 were recurrent and 10 progressive. This shows that synapsis generally involves all three X chromosomes and that a crossover in one region does not markedly prejudice which strands may cross over in another region.

Coincidence values are unusually high, indicating that double crossing over occurs more freely than in the diploid condition.

Of the 182 exceptional daughters examined, 28 had X chromosomes identical in some part of their lengths. Such females are known as equational exceptions. In only one case did the two X chromosomes seem identical throughout. The remaining cases were identical for a part of their length only. This shows that at the time crossing over occurred each of the original chromosomes had already become split into two strands.

The percentage of equationals was lowest for the right end, being 1.1 for point V ($fB+$). It increased progressively toward the left, reaching 11.5 percent for point I (ys_+). Random assortment should give 20 percent of equationals for any point. It is believed that the separation of strands is controlled by spindle fibers attached to the extreme right end, at which point the first division would be always reductional, and that equationals occur as a result of crossing over. The progressive increase toward the left, in the percentage of equationals, is interpreted as due to an accumulation of crossovers of the type which gives rise to equationals.

The crossovers present in associated chromosomes are classified and summarized in table 12 and discussed in the accompanying text. Several of the relative frequencies depart widely from the frequencies expected on random assortment. Further evidence is adduced to show that crossing over does not occur until each of the chromosomes has become split into two strands.

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