POLYPLOIDY IN *DROSOPHILA MELANOGASTER* WITH TWO ATTACHED X CHROMOSOMES

L. V. MORGAN
Columbia University, New York City

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INTRODUCTION

From a mosaic of *Drosophila melanogaster*, with a yellow female region in which, as the subsequent history showed, the two X chromosomes were attached to each other by their ends, a so-called "double-yellow" stock has been maintained. The two X's of the original fly both carried the differential for the recessive mutation, yellow body color, and since the X chromosomes remained attached, the stock consists of yellow females that are derived from eggs with attached X's, fertilized by Y sperm, and of males like their father that are derived from eggs with a Y chromosome, fertilized by X sperm. The two classes occur in equal numbers. Occasionally in the cultures an XX X female occurs, which develops from an XX egg fertilized by X sperm. She is gray in body color if her father has been a wild-type fly, and has characteristic irregularities of form and of eyes and wings by which 3X flies with two sets of autosomes are recog-

TABLE 1

Offspring of double-yellow females.

P_1		F_1				
Number	Mate of $\widehat{yy}$ ♀	$\widehat{XX}$ Y $\widehat{yy}$ ♀♀	$\widehat{XX}$ X Super-females	Patroclinous ♂♂	XX Gray ♀♀ one detached X from mother	Yellow ♂♂ detached X from mother
850bb	$w^e f$	*n*	0	*n*	..	1
856oa	Wild-type	71	0	80	..	1
770	$w^e c_t f$	*n*	4	*n*	..	..
813	Wild-type	84	0	71	0	0
850	" "	78	1	71	0	0
851	" "	21	0	14	0	0
852	$y\ w$	59	0	86	0	0
853	$y\ w$	35	0	56	0	0
854	X-ple	74	4	63	0	0
855	X-ple	11	0	8	0	0
856	$y\ b_r\ w^e$	73	0	68	0	0
75a	Mass, $w^e\ s_n$	75	55	60+	0	0
75b	Mass, $w^e\ s_n$	26	23	32	1?	0
15	Mass, $c_v\ c_t f$	138+	35(+?)	55+	1 or 2	0
1500	$c_v\ c_t f$	22	0	..	1	1(+?)
260	Mass, $c_v\ c_t f$	71	33	36	0	0
260a	Wild-type	42	..	35	..	..
260b	" "	36	..	39	..	..
771a	" "	98	15	125	0	0
771b	" "	66	4	56	0	0
771c	" "	148	7	125	0	0
771d	" "	105	5	100	0	0
32a, b, c, d, e	" "	536	5	556	0	0
54a, b, c, d, e	" "	485	3	543	0	0
144	" "	*n*	..	*n*	..	2
18	" "	*n*	..	*n*	..	2
18a	" "	*n*	0	*n*	0	0
18b	" "	*n*	0	*n*	0	0
18t	$f\ B$	*n*	0	*n*	$0\frac{B}{+}$	0
19	$w^e\ c_t\ vf$	24±	..	21	3	3
19b	"	*n*	1	*n*	0	0
19c	"	*n*	1	*n*	0	0
19m	$f\ B$	139	0	23	$2\frac{B}{+}$	0
184	B	14	0	62	$11\frac{B}{+}$	14
7-24*	w^a	4419	15	4257	9	4

* Eighteen generations of cultures which produced also 9 patroclinous apricot ♀♀.

nized. There is a fourth possible individual, one that would arise if an egg with a Y chromosome were fertilized by Y sperm; but a fly so constituted has not appeared and presumably dies. The number of 3X females varies from none to a few in a generation (see table 1); but in some cultures (75a, mass culture, and others) the number of 3X females is equal to that of other classes.

FEMALES WITH ATTACHED X'S

The genetic results in regard to sex-linked characters are those that are to be expected, according to the known behavior of chromosomes, if two like X chromosomes are attached, and sections of metaphase plates have also shown that the X's are present, joined at one end (cf. figure 1b). More details will be added to some of the results already reported (L. V. MORGAN 1922).

The autosomes were tested by three short experiments to find whether the race was normal for autosomal crossing over. The characters black, purple, vestigial were used for the second chromosome. A double-yellow female was crossed to a black, purple, vestigial male, and the yellow daughters, heterozygous for these II-chromosome recessive characters, were backcrossed to the recessive males. The second chromosome proved to be normal for crossing over as far as the test went (table 2). In a total of 361 flies, the percentage of recombination of black and purple was 6.4 where 6.0 was expected, and the percentage for the loci of purple and vestigial was 12.5, which was expected.

TABLE 2

Crossing over in autosomes II and III in double-yellow females.

CHROMOSOME	LOCI	PERCENT EXPECTED	PERCENT FOUND	NUMBER OF FLIES
II	b-p_r	6.0	6.4	361
	p_r-v_g	12.5	12.5	"
III	p-s_s	8–14	12.6	819
	s_s-e^s	9–15	14.1	"
	e^s-r_o	18–22	14.1	"
	s_t-s_r	14–20	23.6(♂♂only, 22.6)	1761
	s_r-e^s	8.7	8.2	"
	e^s-r_o	18–22	18.0	"
	r_o-c_a	9.6	9.9	"

The third chromosome was similarly tested with two different stocks. Accurate data on crossing over in the third chromosome show differences in behavior in the region including the middle of the chromosome, and without a control the expectation for a given experiment can be known only within certain limits. With peach, spineless, sooty, rough, in a total of 819 flies, the percentage of recombination of peach and spineless was 12.6, where the expectation varies from 8 to 14, the percentage for spineless and sooty was 14.1 and the expectation is 9 to 15. The expectation for sooty and rough is 18 to 22, and the much lower figure 14.1 was obtained.

In a second test with scarlet, stripe, sooty, rough, claret, involving 1761 flies, the percentage for sooty and rough came up to 18.0, nearer to expectation but again low; this was partly offset by a percentage (in the same experiment) higher than expectation for scarlet and stripe, for which 23.6 was found and 14 to 20 is expected. 23.6 was based on counts of all the flies, but since the females were all yellow, and since stripe does not always show as clearly in yellow flies as in wild-type flies, the percentage for the scarlet-stripe section was calculated also from counts of males only; 22.6 was then obtained. For the other two sections the percentages were close to expectation; for stripe and sooty, 8.2 was found and 8.7 expected; for rough and claret, 9.9 found and 9.6 expected. On the whole, it may be concluded that crossing over in the third chromosome, as in the second, is normal in flies with attached X chromosomes. Crossing over in the X chromosome could not be observed because the two attached X's were alike and because the 3X flies were sterile.

PATROCLINOUS FEMALES

An exceptional kind of female, one homozygous for the sex-linked characters of the father, has been found rarely (about fourteen times) among the offspring of double-yellow females. They come presumably from Y eggs. One of these flies had wings which were cut out irregularly on the inner margin, a disturbance often found with certain unusual relations among the chromosomes. The fly died, and four other patroclinous females died or failed to breed even when transferred to fresh food with several mates.

Three other patroclinous females mated to males unlike themselves gave, in F_1, males like themselves and heterozygous daughters, and had, therefore, separate X chromosomes. One certainly, and perhaps two, produced a non-disjunctional daughter and a non-disjunctional son, showing the presence of a Y chromosome.

Another patroclinous female, which was apricot, mated to forked bar, gave no forked bar sons, but as many apricot daughters as there were sons or daughters of the regular classes. Four of the apricot daughters were tested, but they gave no exceptional offspring. Non-virginity might explain the classes but not the ratios obtained in F_1.

BREAKING APART OF THE ATTACHED X'S

Occasionally the two X chromosomes of a double-yellow fly become detached, and an egg with a single yellow chromosome gives rise to a yellow male or to a wild-type female with normal body color if the father was not yellow, or to a not-gray heterozygous bar female if the father was bar. Flies with the detached yellow-bearing chromosome are often found singly as though the break between the chromosomes had not occurred early in the germ track; but there are also a few records of more than one fly with a detached chromosome from a pair mating. No. 19 (table 1) gave as many as six, three yellow males and three wild-type females which produced yellow sons, proving that they had received a single yellow-bearing chromosome. Two of the double-yellow sisters of the six flies with a detached chromosome were mated separately to brothers (19b and 19c) and none of the offspring had a single yellow-bearing chromosome, but a very nearly related double-yellow female (19m), outcrossed, gave two gray (heterozygous bar) females which proved each to have a single yellow chromosome. An unrelated culture, No. 18, gave at least two yellow males, and two pairs of F_1's (18a and 18b) and one double-yellow F_1 female (18t) outcrossed gave no more "breaks."

The largest number of "breaks" recorded from one pair of flies is in culture No. 184, a double-yellow female mated to a bar male, a pair from a stock that was giving a high ratio of males to females. The number of yellow males in F_1 was equal to the number of double-yellow females, and the number of F_1 heterozygous bar females with wild-type body color was nearly as large. Two of the females were tested and proved to be heterozygous for yellow and for bar, giving yellow sons and bar sons. No other cultures of the stock (which was being watched for other purposes) showed similar ratios, although there were other kinds of exceptional individuals, as will be described later.

The data do not indicate that the breaking apart of the chromosomes depends on an inherited condition in the attached-X females.

In order to find out whether the break between the chromosomes takes place at the original point of attachment, a number of the detached chromosomes were tested. If the chromosomes broke apart at a point other than that between the original chromosomes, one resulting chromosome would have a deficiency at one end and the other a corresponding duplication, presumably at the like end. Since it was not at the time known by which end the chromosomes are attached, it was desirable to test both ends. The locus for yellow is very near the "left" end of the chromosome, and if the detached chromosome had the differential for yellow, there should not be a deficiency except possibly a very short one to the left of yellow. A duplication of the yellow locus could not be detected by the presence or absence of the yellow color because both chromosomes carry the differential for yellow. If there were a duplication as far as the locus for the dominant mutation, abnormal (3 units to the right of yellow), a female heterozygous for the detached chromosome and for abnormal would probably not show abnormal, because the character is incompletely dominant, and at the locus for abnormal, the fly would have two wild-type differentials and one for abnormal. The right end of the chromosome could not be easily tested, especially beyond the locus for bar. It was thought that in a female heterozygous for the detached chromosome and for forked and bar, a normal heterozygous bar eye and a not-forked condition would be proof that there was no duplication in that individual in the detached chromosome at bar, or deficiency at bar, or deficiency at the near locus of the recessive mutation, forked. The test is, however, not very useful, because a fly with a deficiency or a duplication for a distance much shorter than from the end of the chromosome to and including bar would probably (judging from known cases) have wing disturbances and lessened viability, and what the behavior of bar would be is not certain. The test with forked bar is therefore useful only to show whether flies with the detached chromosome give normal offspring of the classes and in the ratios expected.

The tested males and females came from various cultures in which the fathers had different sex-linked characters other than yellow.

Two yellow males were double-mated, each to a forked bar and to an abnormal female. The first matings in both cases gave in F_1 regular heterozygous bar females, not-forked, and the second matings gave F_1 females that were "abnormal." One of the males was also backcrossed to a heterozygous bar daughter and gave regular yellow females and the other expected classes. The detached yellow-bearing chromosomes were in every way normal. Eleven other yellow males showed normal behavior for the loci for yellow, abnormal (when the mating succeeded), forked and bar, and showed no somatic disturbances. Six yellow males failed to breed.

No more males were tested, since the chances of picking up a chromosome with any disturbance would be greater in a female, because a male zygote with a deficiency or duplication would probably die, but a female might survive, owing to the presence of the other X chromosome, which is normal. Of females with a detached X chromosome received from a double-yellow mother, which were tested, fifty-nine gave yellow sons and other expected classes of males and females in an estimated 1:1 sex ratio. It was not determined whether or how often the maternal Y chromosome passed into the egg with the detached X chromosome. Fourteen not-yellow daughters of double-yellow females failed to breed.

In all, 72 tested flies showed no unusual condition at the loci for yellow, forked and bar, and those that were tested for the locus abnormal were regular. From examination of samples of the F_1 generation and from genetic tests of a few of the F_1's, it appears that the tested flies behaved as do normal flies which have a single (i.e., unattached) X chromosome carrying the differential for yellow.

Eight other females found at different times among offspring of double-yellow females have shown various types of unusual behavior, not, however, at least in some of them, concerned with the breaking apart of the attached chromosomes.

TRIPLOID FEMALE

One of the flies which did not give expected genetic results was a large heterozygous-bar female (No. 182) of wild-type but slightly yellowish color, found by T. H. MORGAN in a culture of double-yellow females and bar males. She was mated to a male with the recessive characters scute, echinus, crossveinless, vermilion, garnet and forked, a combination called "z-ple."

Among the offspring (table 3), there were, besides males and females, a number of intersexes.[1] Forty of the offspring were mated; the females proved to be of three kinds: first, those that gave 100 percent non-disjunction in the next generation like the double-yellow race (though not all of these were yellow); second, those whose two sex chromosomes were separate X's, as in common females; and third, those that repeated the behavior of the mother, and gave again three kinds of females, and males and intersexes. The race was carried on by mating females that gave intersexes; these females may often be recognized by the large size and

[1] For descriptions of the characteristics of intersexes see BRIDGES (1921, 1922). Intersexes are most easily recognized when the abdomen is like that of a female, because sex combs are always present. But the abdomen is sometimes almost like that of a male. Classification is then based on other variable conditions, such as a broadness of the head and thorax, or disturbances of the wings which are sometimes cut out on the inner margin, or compressed laterally, sometimes with incomplete venation. When the eye is heterozygous bar it is not bean-shaped, as it often appears in females, but it is narrower and more truly bar-shaped.

TABLE 3

F_1 offspring of P_1, heterozygous bar (y/y/B) 3n ♀ × "z-ple" ♂. Percentages of classes of F_1 offspring from 3n females with two attached X chromosomes and one free X chromosome, mated to 2n males.

P_1	F_1					
182	Females			Intersexes		Males
	$\widehat{XX}X3A$	$\widehat{XX}Y2A$	XX2A	$\widehat{XX}Y3A$	XX3A	XY2A
y y / B	y: y y / z-ple	y: y y / —	B/+: B / z-ple	y: y y / —	B: B / z-ple	B: B / —
	1 (tested)	6 (5 tested)	8 (7 tested)	1 (mated, failed) (2♂♂ or intersexes?)	or	16 (4 tested)
by	Wild type: y + / z-ple	Wild type: y + / —	y + B / z-ple	Wild type: y + / —	y + B / z-ple	yB: y + B / —
"z-ple" sc ec cv v g f / —	3 (tested)	9 (3 tested)	1 (tested)	2 (1 mated, failed)	20 (9 mated failed)	2 (1 tested)
	B/+: y B / z-ple					
	1 (tested)					
	1 (failed to breed)					
Total 1474	9.0%	30.1%	18.4%	4.8%	20.7%	17.0%

granular surface of the eyes, and, when bar is present, by a very broad type of bar.

Another line which gave apparently parallel results was discovered by STURTEVANT. It was derived from the double-yellow stock through a patroclinous female with a new origination of the attached-X condition. It was evident from the behavior of both lines that three X chromosomes were present in the mothers of intersexes, but no part of the X chromosome seemed to be primarily responsible for the results that were found. STURTEVANT concluded that they were probably to be accounted for by the presence of three instead of two sets of autosomes, as in the $3n$ individuals described by BRIDGES (1922). The hypothesis was confirmed by cytological evidence, when preparations of the ovaries of a fly (from the stock from No. 182), which was selected as being of the class of mothers of intersexes, showed three complete sets of chromosomes (figure 1a).

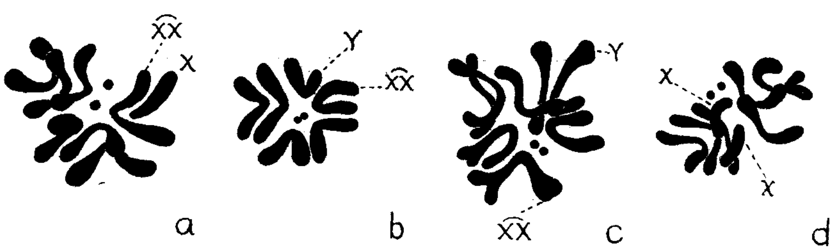


FIGURE 1.—Chromosome groups of: *a*. $3n$ female with two attached X's, one free X, and three sets of autosomes. *b*. $2n$ non-disjunctional female with two attached X's and a Y, descendant of a $3n$ female. *c*, *d*. Intersexes with three sets of autosomes, offspring of $3n$ females, *c*, with two attached X's and a Y, *d*, with two free X's.

The three sex chromosomes consist of two attached X chromosomes (as found in the double-yellow stock) and of one free X chromosome. The kinds of individuals that are expected from such a female mated to a $2n$ male are shown in table 4. BRIDGES (1922) has discovered that from $3n$ females (with separate X's) no flies survive which have three of one of the two large autosomes and two of the other large autosome; that is, autosomes II and III are either both diploid or both triploid. The behavior of autosome IV is different, and in the present account, $3n$ stands for 3 X, 3 II, 3 III, irrespective of the number of IV's that are present. The eggs are of four kinds: those with two attached X chromosomes and two sets or one set of autosomes, and those with a single free X chromosome and two sets or one set of autosomes (table 4). The four kinds of eggs fertilized by X sperm (with one set of autosomes) give the flies whose sex chromosomes are diagrammatically represented in the upper row in table 4, and the four kinds of eggs fertilized by Y sperm give the combinations represented in the lower row.

Of the offspring of No. 182 four wild-type females* and one female with a broad type of heterozygous bar eye (table 3, column 2) were mated to $2n$ males, and gave in the next generation, males, females and intersexes, which proved that the F_1 females, like their mother, were $3n$ flies.

Five of the six F_1 yellow females (table 3, column 3) were tested and gave in F_2 yellow females and males like their father, that is, 100 percent non-disjunction, the result of having attached X chromosomes (both carrying yellow). They evidently came from eggs which received the

TABLE 4

Diagram of sex chromosomes of the different classes of F_1 offspring of a $3n$ female with two attached X chromosomes and one free X chromosome, mated to a $2n$ male.

$\widehat{XX}\,X\,3A$ × $XY\,2A$

eggs / sperm	$\widehat{XX}\,2A$	$\widehat{XX}A$	$X\,2A$	XA
XA	$3n$ ♀	super ♀ (rare, sterile)	intersex	♀
YA	intersex	♀ non-disjunctional	super ♂ (rare, sterile)	♂

attached X chromosomes of the mother, fertilized by Y sperm. Figure 1b is drawn from a division plate of a descendant of such a female from another culture, and the sex chromosomes are shown to be two attached X's and a Y. In cytology and genetic behavior they are like females of the double-yellow stock. Out of nine other wild-type females, three were tested (table 3, column 3) and gave 100 percent non-disjunction. All of their sons were like their mates, but their daughters were of two kinds, wild-type and yellow,—crossing over between the X chromosomes was taking place, as will be described later.

Eight other heterozygous bar females (table 3, column 4) had two separate X's, since seven of them gave bar sons, z-ple sons and sons of crossover classes between bar and z-ple, and one gave yellow bar sons, z-ple sons and crossovers. They behaved genetically like common females,

* The first fly in column 2 has been wrongly named y (=yellow) in table 3.

and undoubtedly came from eggs with the free X chromosome carrying bar (in one case also yellow) fertilized by X sperm which carried the differentials for the characters of z-ple.

The constitution of the intersexes in regard to sex chromosomes can be inferred from the phenotypically similar females, since the intersexes differ from females only in the number of autosomes. Like the $2n$ females they are of two kinds, one with two attached X's from their mother and a Y chromosome from their father (table 3, column 5), and the other kind (table 3, column 6) with one X from each parent. Preparations of metaphase plates of intersexes from No. 182 were not made, but from other suitable matings, the two kinds of intersexes could be inferred from the phenotypes. Figures 1c and 1d are drawn from chromosome groups of intersexes from two different cultures. In both, the autosomes are triploid. Figure 1c is from a wild-type intersex whose $3n$ mother had differentials for forked and bar in the free chromosome and no mutant differential in the attached X's. She was mated to a wild-type male and the F_1 intersexes that were bar showed that they were of the class that received the free bar chromosome from their mother and had therefore two separate chromosomes (unless they were of the very small class of crossovers). The not-bar intersex, whose chromosomes were drawn, should have attached X's from the mother and a Y chromosome from the father, the kinds of chromosomes which the figure shows. Figure 1d is drawn from a chromosome group of a wild-type intersex of different parentage. The $3n$ mother had a differential for bar in the attached X's and no mutant differential in the free X chromosome. She was mated to a wild-type male. Her wild-type (non-crossover) intersex offspring should be those that had a single X chromosome from their mother and an X from their father; two separate X's are found in the sections. Eleven F_1 intersexes of No. 182 were mated as males or as females, according to the type of abdomen, and all failed, showing the usual sterility of intersexes. Five males (table 3, column 7) were mated to different kinds of females and proved to be fertile and gave expected classes of offspring.

Many generations of $3n$ flies have been bred, and it has been found that, although the number of offspring of No. 182 was not large, all of the non-crossover and crossover classes which are derived from a $3n$ female in which two of the X chromosomes are attached were represented, except the rare 3X 2A "super-females" and X 3A "super-males."

That the $3n$ females were such has been shown by the counts of chromosomes in the metaphase plates and also by the regular occurrence of $3n$ females and of intersexes in successive generations. The X chromosomes were marked, and their behavior, which was similar to that of the X chromosomes of the first culture, furnishes specific genetic evidence that there were three of them in the $3n$ females. In some cultures the second chromosomes also were marked and the results have added genetic proof of the presence of three autosomes in the $3n$ females and intersexes. A $3n$ female was mated to a male which carried in one second chromosome the differentials for curly and black, and also for a factor which prevents crossing over in the region between them; curly is a dominant character and black is recessive. In F_1, the curly $3n$ flies had two wild-type second chromosomes from their mother and one with curly and black from their father. $2n$ offspring of such an F_1 female, which was mated to a black male, were either curly and black or not-curly and not-black. Some of the female offspring with three sets of autosomes were curly and not-black; they had one chromosome with black from their father, one with curly and black from their mother, and one wild-type chromosome with neither of the mutant differentials, from their mother. They bred as $3n$ females and, mated to black, gave again in F_3 some curly not-black females and intersexes, classes that could occur only as the result of the presence of three sets of autosomes. The line is continued by repeating the last kind of mating. The interference with crossing over makes it certain that a curly not-black offspring of a black father has developed from an egg which had two second chromosomes, one curly because the fly is curly, and one wild-type because the fly is not-black.

The percents of different classes of F_1 offspring of $3n$ females have been calculated from records of 17 cultures which had been followed primarily to ascertain the kinds of flies in F_1 rather than the exact number of each kind. In a total of 996 flies, 8 percent were $3n$ females, 30.0 percent attached-X females, 17.5 percent separate-X females, 4.6 percent attached-X intersexes, 22.2 percent separate-X intersexes and 17.5 percent males.

In order to check the numerical results just quoted, more complete counts were made of 9 cultures of which the doubtful females were tested by breeding. The $3n$ mothers had differentials for forked and bar in the free X chromosome, and the normal allelomorphs of both in the attached X chromosomes; the second chromosomes were one curly black, one black and one wild-type; they were mated to forked bar black males. The offspring that could be classified without testing were: not-bar females and intersexes which had attached X's; forked bar females and intersexes which had separate X's; not-forked, heterozygous bar, curly, not-black females which were $3n$ females; and males. The flies of one remaining phenotype, which was not-forked heterozygous bar, were either $3n$

females, crossover females, or intersexes. The last two might have attached X's or separate X's and the females were bred and rated by their offspring. Nearly all the tests succeeded and the counts were the basis of the following percentages: the total number of flies was 478, of which there were 10.2 percent of $3n$ females, 30.7 percent of attached-X females, 20.2 percent of separate-X females, 5.2 percent of attached-X intersexes, 17.5 percent of separate-X intersexes and 15.8 percent of males. The results are fairly in accord with the first set of figures and the disparity between them relates as much to percentages of males, females and intersexes as to the percentages of different kinds of females and of different kinds of intersexes (the latter being not accurate in either set of counts, since the crossovers could not be tested). Among the flies of the first group of cultures there were 55.5 percent of females, 26.8 percent of intersexes and 17.5 percent of males. Those of the second group were 61.1 percent of females, 22.7 percent of intersexes and 15.8 percent of males. The percentages in table 3 have been calculated from both groups together.

CROSSING OVER IN TRIPLOID FEMALES

With the introduction into the double-yellow stock of the third complete set of chromosomes, crossing over of an attached X chromosome could, for the first time in the history of the stock, be detected, since the third

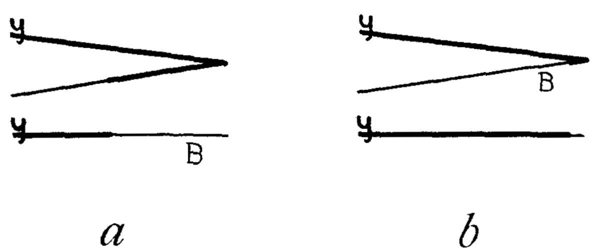


FIGURE 2.—Two possible crossovers between two attached chromosomes, both carrying yellow, and a free chromosome carrying bar.

X did not carry yellow, and the extra autosomes made possible the breeding of females with three X's. The F_1 males of No. 182 were of two classes, the non-crossover class bar, like their grandfather, and one kind of crossover, namely, yellow bar, but not the usual complementary class, which, in this case, would be wild-type. The explanation is found in the fact that the free chromosome crosses over with one of the two attached chromosomes. (In No. 182, a chromosome carrying bar crossed over with an attached yellow-bearing chromosome, figure 2a.) One piece of the free chromosome becomes part of an attached chromosome, and it goes therefore to a female fly and never to a male as in ordinary crossing over. Eggs with crossover chromosomes, when fertilized by Y sperm, become, if they receive the free chromosome, males or super-males, and in the complementary class, receiving attached chromosomes, they become non-disjunctional females or intersexes, according to the number of autosomes in the egg. Some of the non-disjunctional F_1 females of No. 182 were yellow (showing no crossing over) and others were wild-type, and these have been proved by breeding to have had a yellow-bearing chromosome attached to a wild-type chromosome. It is therefore the left end (wild-type) of the bar chromosome that crossed over to the attached pair, and the left yellow-bearing end of the attached pair was shown by the presence of yellow bar F_1 males to have crossed over to the free chromosome (cf. crossover classes in table 3, columns 3, 7). Since these two complementary classes, namely, yellow bar males and non-disjunctional females heterozygous for yellow, prove that the left end of the attached chromosome crosses over to the free chromosome, it follows that the joined chromosomes are attached to each other by their right ends.

The fact of the attachment by the right end can be proved as well by the examination of the other complementary classes in F_1. Eggs with the single crossover chromosome, shown in figure 2a, fertilized by X sperm, become females or intersexes with separate X's (according to the number of sets of autosomes in the egg) and eggs with the attached crossover chromosomes fertilized by X sperm are the complementary class and become (according to the number of autosomes) 3X 2A females (which usually die), or 3X 3A females. Out of eight tested heterozygous bar (not-$3n$) F_1 females (table 3, column 4), seven proved to have received the free bar-bearing chromosome from the mother, and one had received a crossover yellow bar chromosome (the same combination that was found in the crossover males). Again, the left end of the free chromosome carrying bar was found to have crossed over in three of the tested wild-type $3n$ females (table 3, column 2), because in them the attached chromosomes were found by breeding to be one yellow-bearing and one wild-type chromosome.

In the individuals with a crossover chromosome, so far described, crossing over had taken place in the region between the loci for yellow and bar. In one other $3n$ female (which was heterozygous bar, table 3, column 2) crossing over had occurred to the right of bar as proved by the constitution of her attached X's. They were found by breeding to be a yellow-bearing chromosome attached to one carrying the differential for bar but not for yellow (cf. figure 2b).

In all the crossover chromosomes, at whatever level they crossed over, whether in eggs that gave rise to males, to females with separate X's, to non-disjunctional females with attached X's, or to $3n$ females, it was the left end of the free X that had interchanged with the left end of one of the attached X's. Abundant confirmation was found in subsequent cultures of males and tested females, and the records of the F_1 males of two cultures are given as further examples.

In the first example the tested $3n$ female had the differential for scute (a character at the left end) in one attached chromosome, and the differential for vermilion in the middle of the other attached chromosome, and had in the free chromosome the differentials for forked and bar (whose loci are toward the right end). Her sons were 5 non-crossover forked bar, one scute forked bar, and one vermilion forked bar. A female, with forked bar in one attached chromosome and scute, echinus, crossveinless, cut_6, $garnet_2$ in the other, had a free chromosome with differentials for yellow, and for hairy-wing, loci at the left end. The F_1 males were 6 non-crossover yellow hairy-wing, and 1 scute echinus, 1 wild-type, and 1 scute, echinus, crossveinless, cut_6.

CROSSING OVER BETWEEN THE ATTACHED CHROMOSOMES

It has been shown that an attached X chromosome of a $3n$ female may cross over with the free X chromosome; differentials for sex-linked characters can therefore be introduced into the attached pair of chromosomes. In this way a $3n$ female was obtained in which one of the attached chromosomes carried the differential for yellow and the other chromosome the differentials for scute, echinus, crossveinless, vermilion, garnet and forked, a combination already referred to as "z-ple." A wild-type $2n$ non-disjunctional daughter from an egg with attached chromosomes of this constitution gave in F_1, wild-type daughters, yellow daughters and daughters showing various combinations of the mutant characters. Crossing over was taking place, which involved the two attached X chromosomes carried by the females of the line. Some points in regard to crossing over of attached chromosomes were observed by breeding the wild-type females for a few generations.

RECESSIVES SHOWING MUTANT CHARACTERS DETERMINED BY DIFFERENTIALS IN THE LEFT END OF THE CHROMOSOME

The findings recorded in table 5 show that a wild-type fly with recessive characters in two attached chromosomes produces some daughters that are recessives. It is evident that interchange between two chromosomes attached to each other (e.g., those represented by AB, figure 3a) would not change the phenotype in F_1. Recessive individuals could be produced from two attached chromosomes only if the interchange took place after division of the chromosomes and then between the unlike not-joined strands of the double pair (e.g., between A and B′), resulting in two attached chromosomes, such, for example, as those represented by AB in figure 3b. (A′ and B′ are reversed in position as compared with A′ and B′ in figure 3a.) Since recessives in fact occur, crossing over must, sometimes at any rate, take place in the four-strand stage between the strands just named.[2] If crossing over took place as represented in figure 3b, the fly from an egg receiving the chromosomes AB would show those characters of z-ple that had become homozygous, namely, those determined by differentials in the left end of the chromosome, and would be scute, echinus, crossveinless. If at the same time crossing over took place between the attached strands to the left of a marked locus (such as *f*) the distribution of the differentials would be changed, but not the phenotype (as in AB, figure 3c). The second crossing over could be detected in the next generation.

RECESSIVES SHOWING MUTANT CHARACTERS DETERMINED BY DIFFERENTIALS IN THE RIGHT END AND MIDDLE OF THE CHROMOSOME

The phenotypes of the usual complementary classes when single chromosomes are in question occur in F_1 from attached X's (table 5), but both classes cannot be the result of a single crossing over of attached chromosomes. For instance, the chromosomes B′A′, which are complementary to AB (figure 3b), would not give rise to a recessive showing vermilion, garnet, forked, the supplementary z-ple characters in the right end of the chromosome B′; because this is attached to a chromosome with the wild-type allelomorphs. Those characters could appear in a fly if A and B′ crossed as before only in case a simultaneous crossing over occurred between B and A′ (figure 3d), such that A′ would receive from B a piece including the loci for the right-end characters, vermilion, garnet and forked in the example. The second crossing over must take place at a level to the right of the locus of the last character (forked), and as the fly is not yellow, it must be a single crossing over, as represented between B and A′, figure 3d.

[2] The same conclusion has been reached by ANDERSON and STURTEVANT from similar data not yet published, and by BRIDGES from a study of 3*n* females with separate X's.

The resulting chromosomes A′B′ would give the vermilion, garnet, forked phenotype. Because of the attachment of the right ends of the chromosomes, recessives involving the right-end characters are in the class of phenotypically double crossovers. They occur less frequently than scute, echinus, crossveinless flies having the characters determined by the left end. This is expressed in table 5 by the inequality in numbers of the classes showing supplementary blocks of recessive characters, since the counts are made from F_1's of females of like constitution. The smaller classes are probably heterogeneous, as will presently appear, though not in the way described as possible for the larger classes. The true complementary classes, when two attached chromosomes are involved, of which

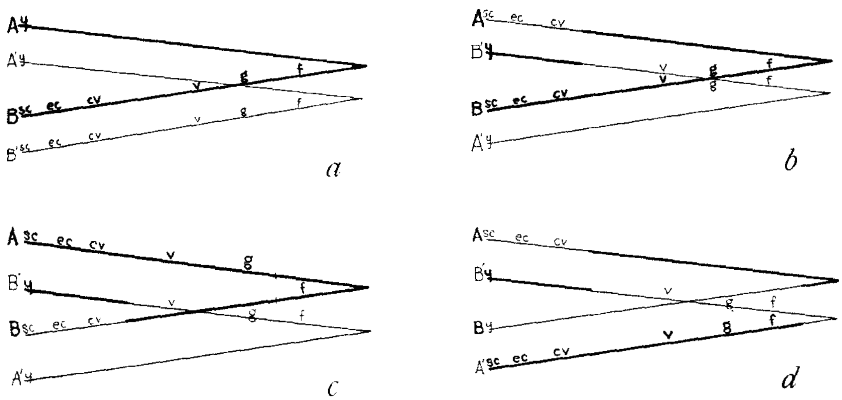


FIGURE 3.—Diagram of: *a*. Two attached chromosomes after splitting. *b*. Single crossing over near the left end between two unlike not-joined strands. An egg with chromosomes AB would produce a scute, echinus, crossveinless recessive. *c*. The same crossing over with a second crossing over toward the right between joined chromosomes. *d*. The same crossing over, with a second crossing over to the right between the other pair of not-joined chromosomes. A yellow fly with chromosomes A′B′ of figure *b* is the real complement of a recessive with chromosomes AB of figure *b*.

only one is marked, are recessives showing characters determined by the left end, and wild-type flies that have lost those characters and are heterozygous for the supplementary characters; the latter class can be recognized only by their F_1 offspring. On account of the presence of yellow in the second X chromosome, the class complementary to the recessives with characters of the left end in the flies recorded in table 5 is among the yellow flies (cf. figure 3b).

The phenotypes that are like double crossovers of single chromosomes (e.g., vermilion, garnet) might come about in two ways, either by a double crossing over between two unlike not-attached strands (figure 4a), or by two single crossings over at different levels that overlap, one between

TABLE 5

F_1 females from wild-type mothers with attached X chromosomes. P_1 11c to 101d, $\left(\frac{y}{s_c\ e_c\ c_v\ v\ g\ f}\right)$ ♀♀. *P_1, 49 m (bottom line), ♀ with same mutant characters differently distributed. F_1, 11d to 49n, ♀♀ lacking some of the characters of the right end.*

NUMBER	WILD-TYPE	y	s_c	e_c c_v v g f	s_c e_c	c_v v g f v	s_c e_c c_v	v g f	s_c e_c c_v v	g f	s_c e_c c_v v g	f	s_c e_c c_v v g f	e_c	c_v	v	g	e_c c_v	c_v v	v g	e_c c_v v	c_v v g	e_c c_v v g	
11c	5	3	..	..	..	..	3	1	..	..	2	..	..	..	..	..	..	..	..	..	1	..	..	
11e	42	16	..	..	2	..	1	..	..	2	4	..	..	..	..	1	4	1	..	1	1	..	1	1 *ygf*, 1 *yg*
11f	10	5	..	..	2	..	..	..	1	2	:	..	1	..	..	2	..	..	..	..	..	..	..	
11g	16	3	..	1	..	..	2	1	..	..	3	..	..	..	..	..	1	..	..	..	..	1	..	
12a	11	4	1	..	..	..	1	..	..	..	1	3	..	..	..	..	..	..	..	..	..	..		
17d	42	12	2	1	3	..	6	..	4	..	..	..	..	..	..	..	..	..	..	..	..	..	..	2 s_c e_c c_v g (f in F_2)
29c	29	12	1	1	1	..	1	..	..	..	..	2	..	1	..	1	..	..	..	1	..	..	..	
29d	17	6	..		..	..	1	..	1	..	..	1	..	1	..	..	..	1	..	1	..	..	..	
29e	26	15	..		1	..	2	..	..	1	2	1?	..	..	..	..	1	..	2	3	..	2	1	
30b	25	9	2		3	1	2	2	..	1	1	..	..	..	..	..	..	..	..	1	..	1	..	1 y v g f
30f	72	25	..		6	..	11	..	2	1	..	..	1	..	2	3	..	..	..	1	..	1	1	1 s_c e_c g, 1 s_c e_c vgf, 1 e_c v
30g	38	12	..	2	4	..	5	2	..	1	..	1	..	..	..	..	..	1	1	4	1	..	..	1 *ygf*
31d	52	23	2		..	1	4	4	..	..	1	1	..	..	..	1	..	..	..	1	..	..	1	
32a	37	6	1		2	1		..	10	..	..	1	..	..	2	..	..	1	..	..	..	..	..	1 s_c e_c c_v f
49a	31	5	2		1	..	4	1	1	1	3	..	..	..	..	..	..	..	..	1	..	1	..	
49j	59	14	..	1	4	..	6	3	2	1	2	..	..	..	..	..	..	..	2	1	..	.	1	1 s_c e_c c_v g
49r	39	21	1		3	..	8	..	1	..	5	1	..	..	..	3	6	1	1	..	..	..	..	1 s_c e_c vg, 1 *ygf*, $1\frac{B^*}{+}$
44c	13	11	..	1	2	1	4	..	2	..	..	..	..	..	..	..	2	..	..	4	1	1	..	
55c	23	12	1		1	1	8	2	..	1	1	1	..	..	..	..	..	..	1	1	..	..	..	
101a	37	6	1		2	1	11	..	2	2	2	..	..	..	..	1	..	..	..	2	..	..	2	
101b	13	8	1		..	..	1	..	..	..	1	..	1	..	..	..	..	..	..	1	..	1	..	
101d	18	4	..		1	..	2	1	..	..	..	..	..	..	..	1	..	..	..	..	1	..	..	1 *yf*
Totals	655	232	15	6	38	6	83	17	26	13	29	12	3	3	4	13	14	5	7	23	5	8	7	
11d	56	12	..											8										
17f	38	8	4																					
49h	45	15	2		10									2										
49n	45	16	1		15									2										

49m: 43 wild-type, 6s_c, 3y, 4 y e_c c_v, 3 y e_c c_v v, 3 y e_c c_v v (g?), 2 s_c g, 1 s_c gf, 1 e_c c_v v, 3 c_v v, 1 c_v vg, 1v, 2g, 3gf.

* Derived from an egg with a detached X chromosome, fertilized by X sperm carrying bar.

the two members in each of the two pairs of unlike not-joined strands (figure 4b). The differentials would be differently distributed in the chromosomes in the two events, and the amount of crossing over that had taken place would be in the first case twice in one pair of chromosomes, and in the second case twice in two pairs, which, with detached chromosomes, would count for two flies. Recessives showing right-end characters (figure 3d, A′B′) are, as shown above, cases of double crossovers and might arise in either of the two ways. That both processes do in fact sometimes take place is shown by the occurrence of flies which are yellow and at the same time homozygous for one or more recessive characters carried by the other chromosome of the mother. For example, the yellow, garnet, forked

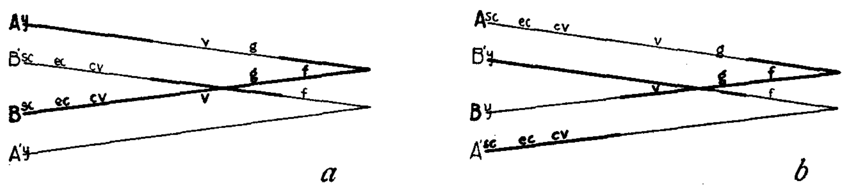


FIGURE 4.—Diagram of: *a*. Double crossing over between two unlike not-joined chromosomes. *b*. Single crossings over at different levels between the unlike chromosomes of both of the not-joined pairs. Resulting phenotypes with the chromosomes AB of *a* and of *b* are the same, but the distribution of differentials in the chromosomes is different.

fly whose mother was of the given constitution must have resulted from two simultaneous crossings over between two unlike not-joined pairs of strands, namely, one single crossing over and one double crossing over. Wild-type flies that have lost some of the mutant differentials of the middle or right end (as A′B′ in figures 4a and 4b) are of the class complementary to the double crossovers by either process; they can be recognized only by their F_1 offspring. For example, in table 5, P_1 females of cultures 49h or 49n are complementary to crossveinless, vermilion, garnet, forked flies.

REDISTRIBUTION OF DIFFERENTIALS

That a redistribution of differentials without change of phenotype and without loss of any mutant differential sometimes occurs is shown by cultures of which 49m (table 5) is an example. The redistribution might have come about either by crossing over between two unlike joined strands (figure 5a single, figure 5b double crossover) or by crossing over between two unlike strands not attached to each other, if at the same time an interchange took place at the same level between the other two unlike not-joined strands (figures 5c and d). The amount of crossing over would be twice as much in the latter case, but the constitution of the

F_1 flies (for example, those receiving the chromosomes AB of figure 5, a and c, or the double-crossover chromosomes AB of figures 5, b and d) would be the same and genetic tests would not show which had occurred.

The results from the breeding of the wild-type flies show that with the various possibilities of crossing over which produce the same phenotypes, the amount of crossing over can not be known without genetic tests of all of the F_1 flies and comparison of results from mothers that by their offspring are proved to have been of similar constitution.

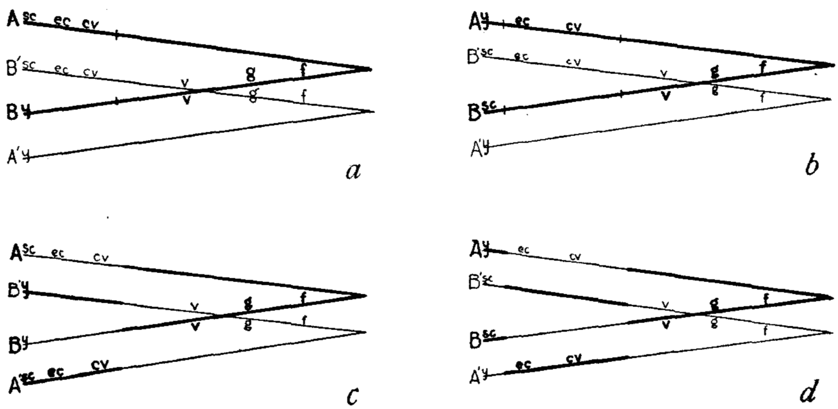


FIGURE 5.—Diagram to show redistribution of differentials in chromosomes AB. *a*, by single crossing over between two joined chromosomes, or *c*, same redistribution by single crossings over between unlike chromosomes of both of the not-joined pairs. *b*, and *d*. The same alternatives involving double crossings over.

CROSSING OVER AMONG THREE CHROMOSOMES

It has been shown that in $3n$ females crossing over takes place between an attached X and a free X, and that it takes place also between two attached X's; it sometimes occurs that a crossover chromosome is made up of parts of all three chromosomes; one example of this kind was a yellow, hairy-wing, broad, vermilion, garnet, forked, bar male which was

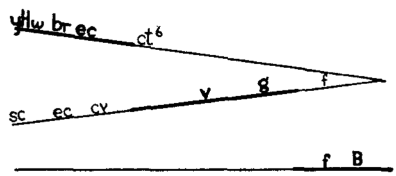


FIGURE 6.—Diagram of crossing over among three chromosomes.

probably also echinus (a character not always distinguishable when bar is present). The constitution of the X chromosomes of the mother is represented in figure 6, where the parts which made up the chromosome received by the egg, from which the male developed, are represented by heavy lines. Crossing over in the attached chromosomes between the loci for crossveinless and cut, and crossing over with the free chromosome at a point between the loci for garnet and forked would result in a chromosome of the kind to produce the male observed.

RECURRENCE OF ADDITIONAL CHROMOSOMES

At two different times, a $2n$ female of a non-disjunctional line, whose parentage goes back to the first $3n$ fly, has produced, besides her regular non-disjunctional offspring, a fly that was a $3n$ female, as proved by breeding. Other exceptions found in three non-disjunctional cultures were recognized as intersexes by the presence of sex combs and more or less female abdomens. Two of the three mothers of these intersexes were attached-X daughters of a $3n$ female, and the third was a daughter of their $3n$ sister. The first was mated to apricot and produced the expected non-disjunctional daughters and apricot sons, a number of 3X 2A daughters and two or three apricot intersexes. The second was wild-type and was mated to an eosin, ruby, bar male, and produced besides the non-disjunctional daughters and sons, one wild-type intersex. The third was wild-type and mated to eosin miniature; she produced, besides females (wild-type and crossover) and eosin miniature males, one intersex that was miniature but had red and not eosin eyes. The apricot intersexes suggest a comparison with patroclinous females, if in the P_1 male non-disjunction of autosomes as well as of the X chromosome occurred at some division. Non-disjunction of maternal autosomes, or of paternal autosomes in Y sperm, would result in the phenotype of the wild-type intersex, but neither of the assumptions explains the miniature not-eosin intersexes.

It may be noted here that in the various non-disjunctional lines from $3n$ females the same kinds of exceptional flies have been found that were described as having occurred in the double-yellow stock (table 1). They were 3X 2A super-females, and males and females with a detached maternal chromosome, and two patroclinous females, one of which was shown to be non-disjunctional with separate chromosomes.

TETRAPLOID FEMALE

Still another instance of increase in the number of chromosomes was found in a daughter of a $3n$ female, which proved to be a $4n$ female; she behaved genetically as would be expected if she had four X chromosomes and four sets of autosomes. She had eyes so slightly bar that they appeared to be normal except for a slight nick in the anterior edge. Her mother (23ea, table 6, column 1) carried in the attached chromosomes the differentials for yellow and the dominant character hairy-wing, and the differentials for forked and bar, and in the free chromosome the differential for miniature wing. The mother was mated to a miniature male and she produced flies of the usual classes of males and females and intersexes, and, in addition, the female with nicked eyes (28a). If this daughter (28a, table 26, column 2) were a $4n$ female she would be expected to have as sex chromosomes the attached pair like that of her mother (if no crossing over had occurred) and two free X chromosomes, both with the differential for miniature, if one came from her mother and one from her father. The two free X chromosomes would also both have the differential for miniature if she had received a double set of all of the paternal chromosomes.

The $4n$ female was mated to a male which was wild-type in respect to sex-linked characters. Her offspring (table 6) were all (with one exception) either $3n$ females or intersexes; none of them was a $2n$ female or a male. The regular eggs of a $4n$ fly would be expected to have two X chromosomes and two sets of autosomes, and these fertilized by sperm with one set of autosomes would give only offspring with three sets of autosomes. The eggs that were fertilized by X sperm should be $3n$ females, those by Y sperm should be intersexes (2XY 3A).

Twenty-one of the F_1 females were tested, and proved to be $3n$ females. They were of two kinds, heterozygous bar and wild-type (the character hairy-wing will for the present be disregarded). If their mother (P_1) had the supposed combination of X chromosomes, the heterozygous bar F_1's (table 6, column 4, of the non-crossover class) should have in the attached chromosomes one differential for forked and bar. The daughters of these (F_2, table 7) that received the attached chromosomes should be non-disjunctional heterozygous bar females (or forked bar or wild-type by crossing over). These would give in F_3 (table 7) daughters like themselves, a few crossovers, and sons like their mates.

On the other hand, the wild-type F_1 females should have (except one rare kind of crossover) 3 separate X's (table 6, column 5), and they should produce some non-disjunctional miniature daughters, from eggs with two separate miniature-bearing chromosomes, fertilized by Y sperm (F_2, table 7, below the double line). These should give in F_3, if mated to males with a differential for bar, heterozygous bar daughters, miniature males, and a few non-disjunctional individuals, i.e., miniature females and males like the father.

TABLE 6

Parentage and F_1 offspring of a 4n female with two attached X chromosomes and two free X chromosomes. P_1, heterozygous hairy-wing heterozygous bar $\left(\frac{y\,H_w}{B}\ \frac{m}{m}\right)$ *4n ♀ × wild-type ♂.*

Parentage of P_1 ♀	P_1	F_1						
23ea	28a	Females			Intersexes			Males
$\widehat{XX}X$ 3A yHw / fB ; m by XY 2A m ; Y	$\widehat{XX}XX$ 4A yHw / fB ; m ; m by XY 2A X ; Y	$\widehat{XX}XX$ 4A slightly B 1 (tested = 4n(?))	$\widehat{XX}X$ 3A $\frac{B}{+}$ ± $\frac{Hw}{+}$ ±yHw / ±m fB 17 (9 tested = 3n) Wild type yHw / yHw? m 1 (tested = 3n)	XXX 3A Wild type ± $\frac{Hw}{+}$ ±yHw m ; m 15 (11 tested = 3n)	$\widehat{XX}$Y 3A $\frac{B}{+}$ ± $\frac{Hw}{+}$ 4 ±yHw / fB ; Y	XXY 3A m ; m ; m ; Y 9 Wild type m ; + ; Y 8	$\widehat{XX}XX$ 3A slightly B 2 (failed to breed)	none

The records given in table 7 show that these expectations were realized. Tests of F_2 (or F_3) females from five different heterozygous bar F_1 females, showed that the F_1 heterozygous bar females had received attached chromosomes from their mother. (Miniature appeared in F_3 (or rarely in F_2) non-disjunctional females, when the attached chromosomes had received miniature by crossing over with a free chromosome in the $4n$ P_1 female.) Tests of F_2 miniature females, from three wild-type F_1 females, proved that these F_1's had received two free miniature chromosomes.

One of the not-bar F_1 females, 20q, had received attached chromosomes (as judged by the offspring) in which one crossing over had occurred with the free miniature-bearing chromosome at a level to the right of bar and (perhaps in the same generation) another crossing over with yellow hairy-wing (table 6, column 4). Non-disjunctional F_2 females were homozygous for yellow hairy-wing (44x and y) and heterozygous for miniature (see F_3).

Further evidence of the $4n$ condition is seen in the occurrence of F_1 intersexes that were miniature. The attached chromosomes of the mother carried bar, and these account for the bar intersexes (table 6, column 6). The father was wild-type and eggs that produced the intersexes homozygous for miniature (table 6, column 7) must have received two miniature-bearing chromosomes from their mother, and have been fertilized by Y sperm, that is, the miniature intersexes are accounted for if the mother had two free chromosomes (carrying miniature) in addition to the two attached chromosomes. The crossover class of intersexes with the two free chromosomes (table 6, column 7) should be wild-type; wild-type intersexes were found, but add nothing positive to the analysis. The genetic results prove the presence of two attached and two separate X chromosomes in the P_1 female, four X chromosomes in all; and the normal form and the fertility of the P_1 female and the classes in F_1 make probable without doubt the presence of four sets of autosomes in P_1.

Two other F_1 intersexes of a somewhat different type (table 6, column 8) were in all probability 4X 3A flies. The wings showed disturbances which are commonly found with an unbalance between X chromosomes and autosomes (as in 3X 2A and 2X 3A flies); the eyes were very slightly bar, not so distinctly bar as in $3n$ flies. Both were sterile. Lastly, among the F_1 females was one fly which, though much smaller, resembled the $4n$ mother in that the eyes were faintly bar or nicked, while the wings were normal. She produced one fly which was heterozygous bar of the degree typical for the $3n$ flies, and she herself was probably $4n$. BRIDGES has

TABLE 7

P_{11} heterozygous hairy-wing heterozygous bar $\left(\frac{yHw}{\quad B}\ \frac{m}{m}\right)$ *4n ♀: Test for two attached and two free X chromosomes by 100 percent of partial non-disjunction in F_3.*

$F_1(3n)$	$F_2(2n)$	F_3		
$\frac{\pm H_w\ B^*}{+\ \ +}$	Attached X's	♀♀	Crossovers	♂♂
20b	35d, $\frac{B}{+}$	$42\frac{B}{+}$	$3m\ \frac{B}{+}\ 1m$	Like father
20c	40b, $\frac{H_w B}{++}$	$\frac{\pm H_w B}{+\ +}$	$1\ yH_w\frac{B}{+}$	" "
	40e, fB	$18fB$		" "
	36g, $\frac{B}{+}$	$12\frac{B}{+}$	1 wild-type	" "
	58ε $\frac{H_w B}{+\ +}$	$\frac{\pm H_w B}{+\ \ +}$	$1\ yH_w\frac{B}{+}$	" "
$F_2(3n)$	58f, $\frac{H_w B}{++}$	$\frac{\pm H_w B}{+\ \ +}$	?	
20h, $\frac{H_w B}{++}$	58g, $\frac{H_w B}{++}$	0		15 " "
38y	58h, $y\frac{H_w B}{++}$	0		14 " "
20s	46a, $\frac{H_w B}{++}$	$21\frac{\pm H_w B}{+\ \ +}$	$2\frac{H_w}{+}mfB_1 2\frac{H_u}{+}m$	" "
	46j, $\frac{H_w B}{++}$	$\frac{H_w B}{++}$	$2\ yH_w\frac{B}{+}\ 1\frac{H_w}{+}$	" "
	46l, $\frac{H_w\ B}{++}$	$27\frac{\pm H_w B}{+\ \ +}$	$2yHw\frac{B}{+}\ 1\frac{H_w}{+}$	" "
20t	48a, $m\frac{B}{+}$	$1\ m\frac{B}{+}$		" "
	48d, $\frac{B}{+}$	$2\ \frac{B}{+}$		" "
$\frac{H_w}{+}$	44x, yH_w	yH_w	$1\ yH_w m$	" "
20q	44y, yH_w	yH_w		" "

Wild-type	Separate X's:	Mate	Regular	Non-disjunctional	Regular	Non-disjunctional
20ε	37a, m ×	vfB	$69\ \frac{B}{+}$	$1m$	$65m$	$2vfB$
20f	37j, m ×	v	Wild-type	$1m$	m	v?
	37k, m ×	vfB	$40\ \frac{B}{+}$	$1m$	$46m$	$4vfB$
	37 l, m ×	wild-type	Wild-type	$1m$	m	1 wild-type
20σ	40b, m ×	wild-type	Wild-type	$1m$	m	

* Phenotype.

previous records (unpublished) of two $4n$ females arising from $3n$ mothers with separate X chromosomes.

NON-DISJUNCTION IN MALES

If non-disjunction of one or more chromosomes should occur in a male at such a time that the sperm would have an increased number of chromosomes, the affected sperm would presumably produce an embryo if it fertilized an egg whose chromosomes were of a number to make a viable combination with those of the sperm. For example, if a sperm had two X chromosomes and fertilized a common egg with one X chromosome the result would be a poorly viable 3X 2A super-female. But if the male had been mated to a female with attached X's, the chances are even that the 2X sperm would fertilize an egg with a Y chromosome, and a viable combination would result, producing a patroclinous female with a Y chromosome. Patroclinous females should then be more numerous in 100 percent non-disjunctional lines than in the common lines with separate X's. As a matter of fact they have been more often reported from non-disjunctional lines although very little of the work on Drosophila has been done with those lines.

Another viable combination would be a sperm with diploid first, second and third chromosomes and an egg with a Y chromosome, resulting in a patroclinous intersex, such, perhaps, as the two or three apricot intersexes already referred to as offspring of an attached-X female. The same kind of sperm with an ordinary-X egg would produce a $3n$ female. The same kind of sperm fertilizing an egg with attached X's and two sets of autosomes from a $3n$ mother would produce a $4n$ female; the genetic records of the $4n$ female just described show that it might have been produced in this way. Sperms with diploid autosomes and a single X chromosome would produce, if they fertilized eggs with attached X's, the kind of $3n$ females found in non-disjunctional lines; those recorded were not marked to show whether the doubled autosomes came in with the egg or with the sperm. Sperm with diploid second or third autosomes, but not both, could not make a viable combination with any regular egg.

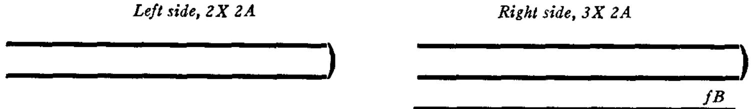
The mosaic from which the double-yellow stock originated was probably at the outset a super-female which became in part a fertile female by the elimination of an X chromosome. The fact that the sons of the mosaic were sterile is in accord with the explanation, since the absence of a Y produces sterility. MORGAN and BRIDGES (1919) have shown that a female fly may become in part male by elimination at some stage of development of one X chromosome, and similarly the loss of an X from one region of a 3X 2A embryo would produce a fly with the characters and behavior of the mosaic. The female region was patroclinous, for the father was yellow and there was no yellow in the parentage of the mother. The attachment of the chromosomes can be formally explained if at some division of the chromosomes the daughter halves failed to separate completely.

It may be recorded here that two other mosaics, with a yellow female region, and two yellow females were found in related cultures of the same pedigree as that of the first mosaic. The mosaics showed characters carried by the X chromosomes of the mother, but in these flies the results could not be explained by a single elimination. If in the yellow females there was a region with a maternal X chromosome, it failed to show, although the diagnostic character was a general body color. One of the yellow females and one of the mosaics bred as yellow females with separate X chromosomes.

COMPOSITES

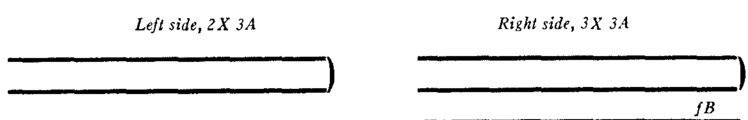
Three different $3n$ females each produced a fly which was mosaic for a sex-linked character. In one instance (65a) the mother had three X chromosomes without mutant differentials and had the differentials for dominant curly wing and recessive black body color in one of her three second chromosomes. She was mated to a forked bar homozygous black male. The mosaic offspring was altogether not black, its left eye was not bar, but somewhat rough and bulging. The right eye was rather broad heterozygous bar. The left wing was not-curly and normal. The right wing was too small and irregular to admit of judging the character curly, and the last right leg was abnormal. There were no sex combs and therefore the region including the fore legs, at any rate, showed neither intersex nor male constitution; the abdomen also was entirely female. The mosaic can be explained by elimination. If an egg with the attached chromosomes and one set of autosomes had been fertilized by X sperm which carried bar, a heterozygous bar super-female would have been produced, and such a 3X 2A condition might account for the abnormalities of the right side, and for the somewhat broad type of bar eye. After elimination of the free chromosome carrying bar, a region like that of the left side would develop, where the eye was wild-type and the wing was normal. The left side showed the regular $2n$ characteristics (except that the eye was slightly irregular) and the mosaic was probably a composite of super-female and female.

Left side, 2X 2A *Right side, 3X 2A*



The alternative explanation of a binuclear origin also fits with the facts: if two nuclei had each a pair of attached chromosomes, one fertilized by X sperm would give conditions found in the right side, the other with Y sperm would give those found in the left side. Only the region including the left side could be fertile and it was hoped that the gonads might be included in it and that the presence or absence of a Y chromosome in that region could be demonstrated by testing the fertility of male offspring. By this test one or the other of the alternative explanations could be disproved. Unfortunately, the fly failed to breed. Evidence for the binuclear explanation from the second-chromosome characters was negative.

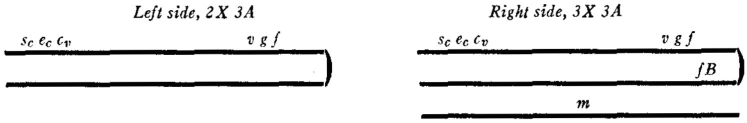
In another culture of the same 3*n* stock a fly mosaic for bar was found (72a), which can be explained if it was a composite of intersex and 3*n* female. The mother was heterozygous bar because her free X chromosome carried bar; this did not therefore affect her 3*n* offspring which received from their mother an attached pair of chromosomes with no mutant differentials. The mother had one second chromosome which carried curly black, one with black and one wild-type, and was mated to forked bar black. One of her offspring was black all over with both wings curly; there were no sex combs. The left eye was wild-type and the right eye was broad heterozygous bar, which, with the normal condition of the fly, suggested that it was in the main a 3*n* female. Elimination of the paternal X carrying forked bar would leave an intersex (2X 3A) region and might account for the round eye, which was rough as in an intersex. If so, the intersex region included only the left part of the head, because all of the right side and the left side of the thorax with no sex comb and the left wing were normal, and the abdomen was normal female. The fly was mated and proved to be 3*n* in the region which included the genital organs.



As before, the binuclear explanation would apply and the evidence from the autosomes is negative.

A 3*n* fly (No. 10a), in which the left side of the thorax was intersex, was the offspring of a heterozygous bar 3*n* female. Mated to a wild-type male the composite bred as a 3*n* female in which one of the attached chromosomes carried the differentials for the combination z-ple, and the other those for forked and bar. This had been the constitution of her mother's attached chromosomes, which she inherited without crossing over. Her father was miniature. The eyes were broad heterozygous bar; the right side of the thorax with no sex comb, the right wing and the female abdomen showed no recessive characters and were normal, as expected in a $3n$ fly of the given parentage. The genetic behavior was also in accord. The left side of the thorax, on the other hand, was forked, and the left wing had thickened veins and was shorter than the right and somewhat compressed as in an intersex; also, there was a sex comb. If the paternal X chromosome had been eliminated, the region affected should be intersex and should be forked because the two attached chromosomes were homozygous for forked. The mosaic of intersex and female characteristics and of phenotypic characters and the genetic behavior are all in accord with the interpretation that a $3n$ female became composite in regard to the number of chromosomes by elimination of one X chromosome.

Left side, 2X 3A *Right side, 3X 3A*



Again there is no evidence which discriminates between this and the binuclear interpretation.

OTHER EXCEPTIONAL FLIES OF THE DOUBLE-YELLOW STOCK

The $3n$ female, No. 182, was related to the non-disjunctional culture already referred to as No. 184 (table 1), in which there were many offspring, both male and female, with a detached yellow-bearing chromosome. At about the same time there were found three other heterozygous bar females in different related cultures of the same stock of double-yellow females and bar males. Two of them gave the regular results for females with a (detached) yellow-bearing chromosome and a bar chromosome. The third, No. 185, gave mixed results. She was backcrossed to a bar male and the offspring were 26 bar, 39 heterozygous bar, 3 yellow and 3 wild-type females, and 42 bar, 33 wild-type, 5 yellow and 13 yellow bar males.

The three yellow F_1 females were tested and proved to have attached X chromosomes, both carrying yellow. The three wild-type F_1 females were tested and proved also to have attached X chromosomes: in one of them one chromosome was wild-type and one carried yellow; the wild-type chromosome would have resulted if crossing over had taken place with a bar chromosome, the only kind present except yellow. In the other two wild-type F_1 females, neither of the attached chromosomes carried yellow; if crossing over was the means by which yellow was lost, both strands of a free chromosome had crossed over to attached strands, an occurrence not found in the records of No. 182. There is no evidence that two free chromosomes were present.

Three of the heterozygous bar F_1 females were tested and all had two separate X chromosomes. One of the females had a yellow-bearing and a bar chromosome as found in females with a detached X chromosome from the mother and an X chromosome from the father. But the other two heterozygous bar F_1 females both had a wild-type chromosome and one carrying bar. If their mother had herself been derived from a detached-X egg, these two daughters would be explained as receiving a crossover (wild-type) chromosome from their mother, but since she had six daughters with attached X's (the three yellow and three wild-type described above), she herself must have had attached X's.

The wild-type chromosomes then remain to be explained; they appeared not only in two out of three tested heterozygous bar daughters, but in 33 sons. It has been shown that the mother had attached X's and that yellow had been lost in some daughters with attached X's, possibly by crossing over with a free chromosome carrying bar. A wild-type free chromosome might have resulted from a double crossing over with an attached X chromosome by which the section with the locus for bar in the free chromosome was replaced by a section carrying the wild-type allelomorph from an attached X. But the offspring with a wild-type chromosome are far too numerous to be the result of so rare a kind of crossing over.

The loss of yellow from some of the attached chromosomes suggests that crossing over had taken place in them, which is known only in $3n$ flies. Whether or not the mother was $3n$ the data do not show; if she was, it may well be that intersexes among her offspring were overlooked; but even allowing for intersexes in the classes where they might have occurred, the count of the F_1 offspring is far from typical, as well as the kind of crossing over that appears to have taken place. No explanation adequate for other exceptional flies will account for the various results found in F_1 of No. 185.

Five other exceptional females of double-yellow stock that have given results different from any others so far known will not be described here.

A new occurrence of a $3n$ female (No. 199) which was the wild-type offspring of a double-yellow female and an apricot, cut, vermilion, forked male, has recently been found. The $3n$ condition was recognized by the characters and kinds of F_1 offspring, including intersexes. Further evidence was given by the results in F_2 of breeding a wild-type daughter to a forked bar male. Not being bar, the wild-type daughter must have received her two X chromosomes from her mother,—the attached chromosomes,—and not being yellow, there must have been in the mother a third X chromosome with which one yellow attached chromosome had crossed over, namely, the apricot-bearing chromosome. The daughters of the wild-type female were of the expected classes; the largest class was wild-type, the smaller classes were yellow and apricot.

SUMMARY

In lines of *Drosophila melanogaster* with two attached X chromosomes, the offspring are daughters like their mother and sons like their father, in a 1:1 ratio. The females have, in addition to the two attached X chromosomes, a Y chromosome. One-fourth of the offspring are expected to be super-females (XX X) and, though poorly viable, a few in fact appear.

Occasionally the attached chromosomes break apart and an egg receives a single X chromosome and gives the same results as an ordinary egg. There is no evidence that the breaking apart of attached chromosomes depends on an inherited factor.

Rarely individuals appear that are patroclinous females, having two paternal X chromosomes and a Y chromosome. They develop presumably from a Y egg fertilized by non-disjunctional XX sperm.

A very few single intersexes have been found among the offspring of females with attached X chromosomes.

Triploid females also rarely occur in the cultures. They have two attached X chromosomes and a free X chromosome. Crossing over takes place between the attached X's and the free X. The resulting chromosomes are, on the one hand, a free chromosome with a piece of the left end of one chromosome of the attached pair, and, on the other hand, attached chromosomes with an equal piece of the left end of the free chromosome crossed over to one of them.

From crossing over between marked chromosomes it is proved that the attached chromosomes are joined by their right ends.

From non-disjunctional lines in which the attached chromosomes are not alike, it is found that crossing over may take place between the attached X chromosomes.

A tetraploid female with two attached X chromosomes and two free X chromosomes was found among the offspring of a triploid female.

Three composite flies have been found among the offspring of triploid females. They were composed of a super-female and a female region, or of a triploid and an intersex region.

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