THE CONSTITUTION OF PRIMARY EXCEPTIONS OBTAINED AFTER X-RAY TREATMENT OF DROSOPHILA¹

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INTRODUCTION

The work of MAVOR (1924) on the effect of X-ray treatment of *Drosophila melanogaster* has established a method of artificially increasing the frequency of primary non-disjunction. Primary non-disjunction gives an opportunity to identify two chromosomes coming from the same maturation process. Since the increased frequency due to X-ray treatment makes the accumulation of data on primary non-disjunction less laborious, the experiment herein reported was planned to make use of this agency in the further analysis of the mechanism of crossing over. This experiment is one of a coordinated series of studies on the mechanism of crossing over of which there are already published studies on crossing over in triploids (BRIDGES and ANDERSON 1925) in cases of attached X chromosomes (L. V. MORGAN 1925, ANDERSON 1925) and in secondary exceptions from a high non-disjunction line (ANDERSON 1929).

These studies have already shown that crossing over occurs in a four strand stage and, further, that crossing over regularly involves only two of the four strands at any one level. The usual amount of crossing over occurs even when the chromosomes become permanently attached. Assortment of chromosomes in these cases is regular at the right or spindle-fiber end, being determined presumably by the spindle-fiber attachment. Assort-

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ment becomes more and more random as we proceed toward the left or distal end, reaching approximate randomness about 40 units to the left of the spindle-fiber attachment.

PLAN OF EXPERIMENT

Echinus cut^s garnet² ($c_e c_t g$) females were mated to scute crossveinless vermilion forked ($s_e c_v v f$) males. Virgin F₁ females were X-rayed within 28 hours after emergence. The X-ray treatments were administered at the UNIVERSITY OF MICHIGAN hospital. The flies were exposed for ten minutes at a distance of seven inches from the target, the tube being operated at a potential of about 70 kilovolts and a current of 5 milliamperes. Some groups of cultures were treated at about 130 kilovolts, the time of exposure being reduced somewhat. After treatment the flies were mated to yellow scute crossveinless bar ($y s_e c_v B$) or yellow forked bar ($y f B$) males.

Among the progeny of the rayed females, the regular offspring are bar females and not-bar males. The males show combinations of the recessive characters which were heterozygous in the mother. The exceptional offspring, due to primary non-disjunction, consist of not-bar females and yellow bar ($y s_e c_v B$ or $y f B$) males. These males are sterile. The exceptional females were mated to yellow scute crossveinless bar ($y s_e c_v B$) males to determine the genetic composition of both their X chromosomes. These two X chromosomes have both been derived from the egg and therefore came from a single maturation process.

A brief report dealing with the frequency of primary non-disjunction after X-ray treatment was presented before the MICHIGAN ACADEMY OF SCIENCE, ARTS, AND LETTERS in 1924 and a report on the proportion of secondary exceptions among the offspring of these primary exceptions was presented before the same academy in 1925 (ANDERSON 1924, 1925b).

FREQUENCY OF PRIMARY NON-DISJUNCTION AFTER X-RAY TREATMENT

In the course of these studies tabulations have been made for 57,877 offspring from X-rayed mothers. These are distributed as follows:

29716	regular (bar) females
27417	regular (not-bar) males
113	exceptional (not-bar) females
631	exceptional (bar) males

These numbers include those reported earlier (ANDERSON 1924). The percentages of exceptions are 0.4 for females and 2.3 for males.

Several hundred of the exceptional males were put up in cultures with virgin females to test for fertility. From one group of cultures a few of the exceptional males were fertile, indicating the presence of some secondary non-disjunction. This group of cultures are not included in the tabulations above. No other exceptional males tested gave offspring.

PHENOTYPIC DISTRIBUTION OF EXCEPTIONS

A total of 195 exceptional females, including the 113 listed above, were classified according to the presence of recessive characteristics which were heterozygous in the parents. Progeny were obtained from 95 of these. The remaining 100 failed to give offspring. Some died before adequate tests were made. Others were continued through several successive culture bottles and proved sterile. The distribution of phenotypes is given in table 1. Those which failed to give offspring are recorded separately from those

TABLE 1
Phenotypic distribution of primary exceptions following X-ray treatment.

PHENOTYPE	FERTILE	WITHOUT OFFSPRING	TOTAL
+	64	53	117
s_c	4	4	8
$s_c c_v$	6	4	10
$s_c c_v v$	3	3	6
$s_c c_v v f$	2	3	5
$c_v v f$	2	..	2
$v f$	3	..	3
f	1	2	3
e_c	1	8	9
$e_c c_t$	3	5	8
$e_c c_t g$	..	3	3
$c_t g$	1	4	5
g	2	3	5
v	1	6	7
$c_v v$	..	2	2
$s_c v f$	1	..	1
$e_c v f$	1	..	1
Total	95	100	195

which gave progeny and whose genotypic constitution was determined. A somewhat larger percentage of wild-type exceptions gave offspring, which may indicate only the greater viability of normal females as contrasted with those which are recessive for the characters involved, or it may indicate a higher percentage of sterility among equational exceptions than among non-equationals. It is possible that some of those recorded as echi-

nus were really triploid intersexes. It is also possible that a few of the fertile wild-type exceptions were secondary exceptions, since one test culture of exceptional males proved fertile.

Table 2 gives the frequency of homozygosis of each of the seven recessive characters which were heterozygous in the X-rayed mothers. The $s_c c_v v f$ chromosome became homozygous in the equationals more frequently than the $e_c c_t g$ chromosome. As the frequencies are rather low, this

TABLE 2
Frequency of homozygosis of recessive genes.

GENE	FERTILE	WITHOUT OFFSPRING	TOTAL	PERCENT
s_c	16	14	30	15.0
e_c	5	16	21	10.8
c_v	13	12	25	12.8
c_t	4	12	16	8.2
v	13	14	27	13.8
g	3	10	13	6.7
f	10	5	15	7.7

may have no significance. A more striking feature is the relatively high frequency of homozygosis of garnet and forked as contrasted with the low frequencies obtained for attached X chromosomes (ANDERSON 1925a, STURTEVANT 1930), triploids (BRIDGES and ANDERSON 1925), and exceptions from high non-disjunction (ANDERSON 1929).

GENOTYPIC CONSTITUTION OF EXCEPTIONS

Progeny tests were obtained on ninety-five of the exceptions. The genotypic constitutions of these exceptions are listed in table 3. In addition to the genotypic formula, a schematic representation of the constitution is also given in which a is used to represent any gene from the $s_c c_v v f$ parental chromosome, and b any gene from the $e_c c_t g$ parental chromosome.

TABLE 3
Genotypic constitution of primary exceptions following X-ray treatment.

PHENOTYPE	GENOTYPE	SCHEMATIC REPRESENTATION	CROSSING OVER	FREQUENCY
+	$s_c c_v v f$	$a a a a a a a$	0-0	48
	$e_c c_t g$	$b b b b b b b$		
+	$e_c c_v v f$	$b b a a a a a$	2-2	5
	$s_c c_t g$	$a a b b b b b$		
+	$e_c c_t v f$	$b b b b a a a$	4-4	2
	$s_c c_v g$	$a a a a b b b$		

TABLE 3 (continued)

PHENOTYPE	GENOTYPE	SCHEMATIC REPRESENTATION	CROSSING OVER	FREQUENCY
+	$\frac{e_c c_t f}{s_c c_v v g}$	$\frac{b b b b b a a}{a a a a a b b}$	5-5	1
	$\frac{e_c c_t g f}{s_c c_v v}$	$\frac{b b b b b b a}{a a a a a a b}$	6-6	2
+	$\frac{e_c c_t v}{s_c c_v g f}$	$\frac{b b b b a a b}{a a a a b b a}$	4, 6-4, 6	1
+	$\frac{c_v v f}{e_c c_t g}$	$\frac{b a a a a a a}{b b b b b b b}$	1-0	2
+	$\frac{s_c c_v}{e_c c_t g}$	$\frac{a a a a a a b}{b b b b b b b}$	6-0	2
+	$\frac{s_c c_v f}{e_c c_t g}$	$\frac{a a a a b a a}{b b b b b b b}$	4, 5-0	1
s_c	$\frac{s_c c_v v f}{s_c e_c c_t g}$	$\frac{a a a a a a a}{a b b b b b b}$	0-1	1
	$\frac{s_c c_v v f}{s_c c_t g}$	$\frac{a a a a a a a}{a a b b b b b}$	0-2	2
s_c	$\frac{s_c c_v g}{s_c e_c c_t v f}$	$\frac{a a a a b b b}{a b b b a a a}$	4-1, 4	1
$s_c c_v$	$\frac{s_c c_v v f}{s_c c_v c_t g}$	$\frac{a a a a a a a}{a a a b b b b}$	0-3	1
$s_c c_v$	$\frac{s_c c_v v f}{s_c c_v g}$	$\frac{a a a a a a a}{a a a a b b b}$	0-4	4
$s_c c_v$	$\frac{s_c c_v v}{s_c c_v c_t g f}$	$\frac{a a a a a a b}{a a a b b b a}$	6-3, 6	1
$s_c c_v v$	$\frac{s_c c_v v f}{s_c c_v v g}$	$\frac{a a a a a a a}{a a a a a b b}$	0-5	3
$s_c c_v v f$	$\frac{s_c c_v v f}{s_c c_v v f}$	$\frac{a a a a a a a}{a a a a a a a}$	0-0	2
$c_v v f$	$\frac{s_c c_v v f}{e_c c_v v f}$	$\frac{a a a a a a a}{b b a a a a a}$	0-2	2
$v f$	$\frac{s_c c_v v f}{e_c c_t v f}$	$\frac{a a a a a a a}{b b b b a a a}$	0-4	1
$v f$	$\frac{s_c c_v v f}{e_c v f}$	$\frac{a a a a a a a}{b b b a a a a}$	0-3	1
$v f$	$\frac{c_v v f}{s_c e_c c_t v f}$	$\frac{b a a a a a a}{a b b b a a a}$	1-1, 4	1
f	$\frac{s_c c_v v f}{e_c c_t f}$	$\frac{a a a a a a a}{b b b b b a a}$	0-5	1
e_c	$\frac{e_c c_t v f}{e_c c_v g}$	$\frac{b b b b a a a}{b b a a b b b}$	4-2, 4	1

TABLE 3 (continued)

PHENOTYPE	GENOTYPE	SCHEMATIC REPRESENTATION	CROSSING OVER	FREQUENCY
$e_c c_t$	$e_c c_t v f$	$b b b b a a a$	4-0	3
	$e_c c_t g$	$b b b b b b b$		
g	$s_c c_v g$	$a a a a b b b$	4-0	2
	$e_c c_t g$	$b b b b b b b$		
$c_t g$	$s_c c_t g f$	$a a b b b b a$	2, 6-0	1
	$e_c c_t g$	$b b b b b b b$		
v	$s_c c_v v f$	$a a a a a a a$	0-3, 5	1
	$e_c v g$	$b b b a a b b$		
$s_c v f$	$s_c c_v v f$	$a a a a a a a$	0-2, 4	1
	$s_c c_t v f$	$a a b b a a a$		
$e_c v f$	$s_c e_c c_v v f$	$a b a a a a a$	1, 2-4	1
	$e_c c_t v f$	$b b b b a a a$		

From the data of table 3 the frequency of homozygosis for each locus can be tabulated. This has the advantage over the phenotypic distributions in that the frequencies of homozygosis of the normal allelomorphs may be tabulated as well as that of the recessive genes. These frequencies are presented in table 4. The percentages are computed on the basis of 190

TABLE 4
Frequency of homozygosis for single loci summarized from table 3.

LOCUS	FREQUENCY	PERCENTAGE
s_c	22	11.6
e_c	19	10.0
c_v	17	9.0
c_t	17	9.0
v	17	9.0
g	13	7.0
f	14	7.4

instead of 95 to make them comparable with the percentages calculated for the phenotypic distributions. These percentages are more uniform than those of table 2, due to their not being influenced by the unequal frequency of homozygosis of the two chromosomes. A combination of the data from table 2, on the exceptions which gave no offspring, with the data of table 4 gives the following percentages of exceptions:

s_c —12.4	v —10.6
e_c —12.0	g — 7.9
c_v —10.0	f — 6.6
c_t —10.0	

The data on crossing over from table 3 are summarized in table 5. The control percentages given for comparison are the normal percentages of

TABLE 5
Summary of data on crossing over from table 3.

REGION	PERCENTAGE OF CROSSING OVER	CONTROL	RATIO TO CONTROL
$s_c - e_c$	3.7	6.8	.54
$e_c - c_v$	9.5	9.7	.98
$c_v - c_t$	2.1	8.4	.25
$c_t - v$	12.6	14.8	.85
$v - g$	4.3	11.2	.38
$g - f$	5.8	11.3	.51
Total	38.0	62.2	.61

crossing over shown by the same parental stocks (ANDERSON 1929). The observed percentages of crossing over in the exceptions are irregular but are all lower than the controls. The total crossing over is about sixty per cent of the normal.

ASSOCIATION OF CROSSOVERS

When only a single region is considered, a crossover may be associated with a non-crossover or another crossover. In the former case, either the left or the right side may be homozygous, that is, it may be ab/aa or ba/aa . When two crossovers are associated they may be complementary ab/ba or identical ab/ab . No case was observed of association of identical crossovers. This is further evidence that only two out of the four strands cross over at any one level.

The data on association of crossovers in single regions are summarized in table 6. There were 40 combinations of a crossover with a non-crossover to 16 combinations of complementary crossovers. This approaches the four to one ratio expected for random assortment.

TABLE 6
Association of crossovers.

REGION	TYPE OF ASSOCIATION		
	ab aa	ba aa	ab ba
1	4	1	1
2	5	3	5
3	2	2	0
4	7	7	5
5	5	1	1
6	1	2	4
Total	24	16	16

The rightmost crossovers are summarized in table 7. All cases where the *f* locus was homozygous are excluded. Among the rightmost crossovers there were only 19 *ab/aa* combinations to 14 *ab/ba* combinations. The data on attached X chromosomes (ANDERSON 1925a, STURTEVANT 1930) and on high non-disjunction (ANDERSON 1929) gave close approximations to the expected two to one ratio.

TABLE 7
Association of rightmost crossovers

REGION	TYPE OF ASSOCIATION	
	<i>ab</i> <i>aa</i>	<i>ab</i> <i>ba</i>
1	3	0
2	2	5
3	1	0
4	7	4
5	5	1
6	1	4
Total	19	14

INTERPRETATION

The data obtained on the X constitution of primary exceptions from X-ray treatment resemble in general the data on crossing over in attached-X cases (ANDERSON 1925a, STURTEVANT 1930) and on crossing over among the exceptions from high non-disjunction (ANDERSON 1929). One striking difference is noted, which calls for special consideration. This is the relatively high frequency of homozygosis of garnet and forked. In the attached-X and high non-disjunction cases the percentages of homozygosis were very low near the right or spindle-fiber end of the chromosome, converging toward zero at about 10 or 12 units beyond forked, a point which is believed to represent the end of the chromosome. This point practically coincides with the mapped position of bobbed, the rightmost known gene in the X chromosome. STERN (1926) has given independent evidence that the position of the spindle-fiber attachment is very close to the locus of bobbed. The absence of homozygosis at the spindle-fiber end in the attached-X case shows that distribution is regular at that point, being presumably determined by the spindle-fiber attachment. The attachment of the chromosomes prevents any separation at the reductional division. The random proportion of one reciprocal *ab/ba* to two equational *ab/aa* combinations among rightmost crossovers shows that of the four strands indicated in the diagram, *a* must cross over equally freely with each of the two

sister strands *b* and *b'*. This is further corroborated by the frequencies of homozygosis regularly increasing toward the left. For loci near the spindle-fiber end, the percentage of homozygosis approaches the calculated value of one-half of the map distance from the spindle-fiber end. Proceeding toward the left, it drops below this value due to multiple crossing over. At about the middle of the chromosome map, it approaches close to 16.7 percent, the percentage expected for random assortment. Since the percentage of crossing over is not altered by the attachment of the chromosomes, it is very probable that the mechanism of crossing over in these cases is the normal mechanism.

The exceptions from the high non-disjunction line showed the same decreasing frequency of homozygosis toward the right and the same random proportion between reciprocal and equational combinations of rightmost crossovers. These relations indicate that the non-disjunction occurs always at the reductional division while the behavior at the equational division remains normal, just as in the attached-X cases. The percentage of crossing over is much lower than among the regular offspring, especially for regions near the spindle fiber. However this only indicates that non-disjunction rarely occurs when the two X chromosomes have been intimately synapsed in the neighborhood of the spindle-fiber attachment. But when non-disjunction occurs the behavior is the same as in the attached-X cases.

Among the primary exception induced by X-ray treatment the percentages of homozygosis do not converge toward zero at the spindle-fiber attachment. In fact the data presented (tables 2 and 4) indicate that more than ten percent of these exceptions were homozygous for the rightmost end and of one or the other of the two parental chromosomes. The interpretation which seems most logical, is that these were due to non-disjunction occurring at the equational division. Exceptions formed in this way should be homozygous at the spindle-fiber end, and this homozygosis should only be disturbed where one of the two sister strands has crossed

over with a strand from the opposite chromosome. The curve of homozygosis for equational division exceptions should be the reciprocal of the curve for reductional division exceptions or for attached-X progenies. Homozygosis should be 100 percent at the spindle-fiber end, decreasing toward the left until the random expectation of 16.7 percent is reached. In a distribution including both kinds of exceptions, the effect of equational division exceptions would be most evident near the spindle-fiber end of the curve.

SUMMARY

Virgin females of constitution $s_c c_v v f / e_c c_t g$ were subjected to X-rays and mated to bar males. The exceptional daughters produced were bred to determine their X constitution.

Of the females 0.4 percent were exceptional, of the males 2.3 percent.

The chromosomes going into exceptional (XX) eggs showed about 60 percent of the normal amount of crossing over.

The percentages of homozygosis were as follows:

s_c —12.4

e_c —12.0

c_v —10.0

c_t —10.0

v —10.6

g — 7.9

f — 6.6

The comparatively high values for homozygosis of garnet and forked are believed to be due to non-disjunction occurring at both maturation divisions after X-ray treatment.

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