THE FREQUENCIES OF HOMOZYGOSIS OF FACTORS IN ATTACHED-X FEMALES OF *DROSOPHILA MELANOGASTER*

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INTRODUCTION

The first evidence that crossing over in *Drosophila melanogaster* occurs at a stage where each chromosome is divided or split into two strands was presented by BRIDGES (1916) in his interpretation of the origin of the rare "equational exceptions," that is, daughters which received two X chromosomes from their mother but were homozygous for a recessive gene carried in a heterozygous condition by their mother. Later L. V. MORGAN (1925), ANDERSON (1925), and STURTEVANT (in press) in their studies on crossing over in attached-X lines, ANDERSON (1929) by his determination of the genotypic constitution of exceptional daughters from a high non-disjunction line, and BRIDGES and ANDERSON (1925) in their analysis of crossing over in the X chromosomes of triploid Drosophila conclusively proved that crossing over in the X chromosome usually, if not always, takes place at a time when each conjugant chromosome is double. REDFIELD (1930) shows that in the third chromosomes of triploid Drosophila crossing over likewise takes place at a "double strand" stage. Data by DOBZHANSKY and STURTEVANT (in press) from their work on II-III translocations lead to a similar conclusion for the second chromosome. Nothing is known regarding the small fourth chromosome since crossovers rarely, if ever, are found.

ANDERSON (1925) studied crossing over between attached-X chromosomes heterozygous for the recessive genes cut (c_t), tan (t), garnet (g), and forked (f). He found that the frequency with which a recessive gene, heterozygous in the mother, appeared in a homozygous condition in the daughters was a function of its distance from the right or spindle-fiber end of the chromosome. Genes, such as forked, situated near the spindle fiber so that no, or little, double crossing over occurred between their loci and the fiber end, had a frequency of homozygosis equal to one-half their map distance from the attachment point. The progressive increase in homozygosis for factors located further to the left approached a value of 16.7 percent for the two left-most genes involved in his experiments, namely cut (c_t) and tan (t) for which he found percentages of 15.5 and 16.1 respectively. (If the left ends of the attached-X chromosomes assort at random with respect to the spindle fibers, 1 of the 6 possible combinations of the 4 strands, or 16.7 percent, will be homozygous for any recessive factor carried in the distal end.) His detailed analysis of the association of crossover and non-crossover strands for the different regions, derived from determining the genotypic constitution of exceptional daughters, showed that the assortment of strands approached that based on random expectation for the left-most, cut-tan, region involved.

It might be inferred from ANDERSON'S results that for genes located near the left or distal end of the X chromosome the frequency with which they appeared homozygous would closely approach 16.7 percent. But L. V. MORGAN (1925) found for the gene yellow (y), situated at the extreme left end, a frequency of 19.0 percent and for echinus (e_c) 5.5 map-units to the right of yellow, a frequency of 17.3 percent. Her numbers are, however, small and the excess of equationals is not statistically significant. STURTEVANT (in press) found a value of 17.1 percent for scute (s_c) in a total of approximately 25,000 flies. These apparently high values for y, s_c, and e_c indicated the possibility that the assortment or distribution of genes in the left end of the X chromosome was not perfectly random, and at the suggestion of Doctors A. H. STURTEVANT and E. G. ANDERSON an experiment was undertaken to definitely determine whether the frequency of homozygosis for the left end was in excess of 16.7 percent, or that expected on random assortment. I wish to express my sincere appreciation to both of them for their interest and advice and to Doctor STURTEVANT for supplying stocks with which to begin the experiment.

SYNTHESIS OF ATTACHED-X LINE

Females with attached-X's homozygous for the recessive gene forked (f) were made homozygous for a third chromosome recessive factor found by GOWEN (1922), called U.C.-G., which prevents or inhibits crossing over in all chromosomes and causes an approximately random distribution of the entire chromosome complement at the reduction division, and were mated to yellow[2] (y) males. Among the offspring of this cross several triploid females were obtained which carried one pair of attached-X's homozygous for forked and a free X-chromosome with the factor y. (Doctor STURTEVANT made these preliminary crosses.) These triploid females were mated to scute (s_c), apricot (w^a), echinus (e_c), ruby (r_b), not-forked males and the ensuing triploid daughters mated in individual cultures to yellow males. In the following generation, triploid daughters were mated to s_c, w^a, e_c, r_b, males and this alternating of breeding triploid females carrying attached-X's to the two classes of males continued until through the proper crossovers diploid females were obtained whose attached X-chromosomes were heterozygous for the recessive genes, y, s_c, w^a, e_c, r_b. It was necessary to test each diploid daughter individually in order to determine its genotypic constitution. Two forked females were found which gave equationals for the 5 genes. The forked daughters from these females were used in perpetuating the attached-X line. The map positions of the 5 genes are as follows:

y—0.0
s_c—0.+
w^a—1.5
e_c—5.5
r_b—7.5

No crossing over has ever been observed between y and s_c except in triploids. An attempt was made to introduce the normal allelomorph of forked into the attached-X line to give a check upon the normality of the right end of the attached pair, but due to the difficulty of introducing factors located so near the attachment point this plan was abandoned and the analysis begun with the 5 factors situated in the left end. Fortunately several reversions of forked to its wild-type allelomorph occurred in the attached-X line and it was possible to determine the frequency of homozygosis for this gene. All 5 of the mutant factors used are easily and accurately classified and have relatively slight effect upon viability. Where possible, as in the cases of the yellow and scute loci where yellow[2] and scute[1] were used, the more viable members of the allelomorphic series of genes were chosen.

DATA FROM ATTACHED-X FEMALES

The genotypic constitution of the attached-X females, which were finally secured, was $yf/s_c w^a e_c r_b f$. Since y and s_c may be considered as occupying the same locus there are 8 (2^3) possible combinations of the 4 different loci and these were all found during the course of the analysis and extensive data were obtained for 6 of the 8 types.

Forked females, heterozygous for the 5 mutant genes, were mated to Bar (B) or Beadex (B_x) males in individual culture bottles. Several stocks of Bar and Beadex males were used and the matings with different stocks arranged to avoid inbreeding and secure heterosis. In some cases the parental flies were transferred to fresh culture bottles after 7 days and a second progeny obtained. Without exception, counts were extended through a 9 or 10 day period in order to include as nearly as possible all the offspring from a given mating.

The 8 possible combinations of the 5 factors are:

1. $\frac{y \qquad\qquad f}{s_c\ w^a\ e_c\ r_b \quad f}$
2. $\frac{s_c \qquad\qquad f}{y\ w^a\ e_c\ r_b \quad f}$
3. $\frac{y\ w^a \qquad\qquad f}{s_c \quad e_c\ r_b \quad f}$
4. $\frac{y\ w^a\ e_c \qquad f}{s_c \qquad r_b \quad f}$
5. $\frac{y \qquad r_b \quad f}{s_c\ w^a\ e_c \qquad f}$
6. $\frac{y \quad e_c\ r_b \quad f}{s_c\ w^a \qquad\qquad f}$
7. $\frac{y\ w^a \quad r_b \quad f}{s_c \quad e_c \qquad f}$
8. $\frac{y \qquad e_c \quad f}{s_c\ w^a \quad r_b \quad f}$

The data from these combinations are presented in tables 2 to 9. The frequencies of homozygosis for the mutant genes, found by grouping together all flies from mothers of the same genotypic constitution excepting the forked locus, in the various combinations, are presented in table 1. The

TABLE 1

Percentages of flies equational for y $s_cw^ae_cr_b$. Data from females homozygous and heterozygous for forked are combined and given in percent.

COMBINATION	y PERCENT	s_c PERCENT	w^a PERCENT	e_c PERCENT	r_b PERCENT	NUMBER OF FLIES
$\frac{y}{s_c\ w^a\ \ e_c\ r_b}$	19.9	16.8	16.6	16.6	16.6	14,198
$\frac{s_c}{y\ w^a\ \ e_c\ r_b}$	17.1	18.8	16.9	16.6	16.5	4,399
$\frac{y \qquad r_b}{s_c\ w^a\ \ e_c}$	19.5	18.1	17.8	17.8	19.3	10,133
$\frac{y \quad e_c}{s_c\ w^a \quad r_b}$	19.4	18.6	18.6	18.7	18.4	3,953
$\frac{y \quad e_c\ r_b}{s_c\ w^a}$	18.0	18.9	19.0	17.2	17.5	4,157
$\frac{y\ w^a\ \ e_c}{s_c \qquad r_b}$	18.8	18.7	18.9	18.7	18.3	1,669
$\frac{y}{s_c}$	19.3	18.1				3,467

total number of flies obtained in 2 of the classes was so small that it was considered meaningless to determine the frequencies of homozygosis for the different loci and they have been omitted from the table. However, the percentages for y and s_c from females heterozygous for no other mutant factor have been included.

INTERPRETATION AND DISCUSSION OF DATA

These data show conclusively that for genes situated in the left end of the X chromosome the frequency of homozygosis is greater than that expected on random assortment of strands with respect to their fiber attachment. The average frequency for y in 40,306 flies, calculated from 6 different combinations in each of which at least 3,400 flies were counted, is 18.9 percent, a deviation from 16.7 which is statistically significant, the Dev./P.E. = 18.3.

The average value for s_c, determined in the same manner, is less, being 18.2 percent as compared with 18.9 percent for y. That this difference is undoubtedly due to the lesser viability of scute flies is substantiated by the frequencies of 19.3 and 18.1 percent for y and s_c, respectively, among the progeny from females heterozygous for only these 2 factors. For the s_c locus the excess above the theoretical expectation is also significant, the Dev./P.E. = 12.5.

The average percentages for w^a, e_c, and r_b, from the first 5 classes in table 1, are 17.8, 17.4, and 17.7, respectively. The average frequency of homozygosis for these genes is only slightly less than for y and s_c but the differences may be attributed to the fact that they occupy a somewhat closer position in the chromosome to the spindle fiber, and the data of MORGAN (1925), ANDERSON (1925 and 1929), and STURTEVANT show a decreasing frequency in homozygosis for genes lying progressively nearer the right end of the X chromosome.

The data show clearly that in the progeny of females carrying the mutant genes in an unbalanced condition, that is, only 1 mutant gene in one of the attached chromosomes and 4 mutant genes in the other, there is a decided and significant excess of females equational for the single gene. For example, the value for y in the combination ($y/s_cw^a\ e_cr_b$) was 19.9 percent as contrasted with 16.8 percent for s_c and slightly lower percentages for the other loci. The high value for y is in part accounted for by the comparatively low frequency of the contrary class of equationals. The corrected value is 19.3 percent. And in the arrangement ($yw^a\ e_cr_b/s_c$) there was a greater frequency of flies equational for s_c than for y or the other factors even though s_c has a more adverse effect upon viability than any of the other 4 genes. Their cumulative effect, however, was sufficient to appreciably decrease their frequencies of homozygosis.

The frequencies obtained in 5 of the balanced arrangements, that is, 2 genes in one chromosome and 3 in the other, are more uniform but the data show that flies homozygous for any 2 of the 5 mutant genes are more viable than flies equational for the remaining 3. The frequencies calculated from $(yw^a/s_c\ e_cr_b)$ mothers do not agree with this generalization but the total number of flies is small and the deviations may well be due to errors in sampling.

If, as the data suggest, an increase in the number of mutant characters lowers the viability it might be expected that the frequency of equationals derived from females heterozygous for an increased number of genes would be less than in these experiments. STURTEVANT, working with attached X's heterozygous for $s_c\ e_c\ c_v\ c_t\ v\ g\ f$, found for s_c, the left-most factor, a value of only 17.1 percent homozygosis.

It is impossible from these data to arrive at any definite conclusion regarding the crossing over mechanism that results in percentages of equationals greater than 16.7 but there are at least two plausible explanations, which need not be elaborated in the absence of complete data. If either the frequency of multiple crossovers is not sufficiently large or there be a disproportionate number of reciprocal as compared with diagonal crossovers, there will be a deviation from the expected percentages based on random assortment in the direction observed in these experiments. The answer awaits the analyses of the genotypic constitutions of daughters from mothers heterozygous for genes distributed along the length of the chromosome.

FORKED REVERSIONS

The attached-X line with which the experiment was begun was homozygous for the recessive gene forked. During the course of the investigations a total of 5 separate reversions of forked to its wild-type allelomorph were found. That only a single reversion occurred in any one culture suggests that they took place during or following the maturation divisions. Reversions of forked to its wild-type allelomorph are not frequent as MORGAN (1929) reported the first occurrence of such. Recently MULLER (1930) succeeded in inducing forked reversions through the agency of X-rays although he failed to find any reversions in his controls.

Since the reversions affected only one of the chromosomes in the attached-X's it was possible to determine the frequency of flies equational for the forked locus. The data from females heterozygous for the 6 genes are presented in tables 11 to 15. The combined data give a frequency of 4.9 percent of forked equationals among 3,665 flies, a value which closely agrees with those of 5.1 and 5.2 percent reported by STURTEVANT and ANDERSON. The frequencies of the other mutant genes are of the same order as from flies homozygous for forked. This strongly suggests the normality of crossing over in the right ends of the attached chromosomes used.

DETACHMENTS

In addition to the 41,975 exceptional females there occurred 39 regular offspring, which arose through a detachment of the attached-X's. Sixteen regular females and 13 regular males were found, where equal numbers of the 2 sexes are expected. That in some cases detachments take place in oogonial cells, prior to the maturation divisions, is suggested by the occurrence of more than one regular offspring in some of the cultures, several bottles containing 2 and one culture giving rise to 3 regular individuals. The frequency of regular individuals in the progeny of attached-X females is 1 in approximately 1450 flies. ANDERSON (1925) found the proportion of regular offspring to be 1 in about 1300 flies. Four triploid females and 1 male-type intersex arose during the course of the experiment.

SUMMARY

1. Among the progeny from attached-X females heterozygous for the 5 genes, y, s_c, w^a, e_c, r_b, situated in the left end of the X chromosome of *Drosophila melanogaster*, there was a significant excess over the expected 16.7 percent of flies equational for these genes, that is, the segregation of the left ends of the four strands is not at random with respect to the fiber attachment. Counts were made on approximately 42,000 flies.

2. The data as obtained still show some effects of differential viability, although the most viable available mutant allelomorphs were used in each case.

3. Five reversions of forked to its wild-type allelomorph occurred in attached-X females, making it possible to determine the frequency of homozygosis for this gene. It was found to be 4.9 percent.

4. There were 39 regular offspring among the exceptional flies. They arose through detachments of the attached-X's. Four triploid females and 1 male-type intersex were found.

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TABLE 2

Exceptional daughters from attached-X mothers of

$\dfrac{y \qquad\qquad f}{s_c\ w^a \quad e_c\ r_b \quad f}$ *constitution.*

f	y f	s_c w^a e_c r_b f	s_c w^a e_c f	s_c w^a f	s_c f	w^a e_c r_b f	e_c r_b f	r_b f	s_c r_b f	s_c e_c r_b f	y r_b f	w^a e_c f	s_c w^a e_c r_b	Total
8477	2793	1903	97	189	171	148	192	82	1	2	1	2	1	14,059

TABLE 3

Exceptional daughters from attached-X mothers of

$\dfrac{y\ w^a \quad e_c\ r_b \quad f}{s_c \qquad\qquad\quad f}$ *constitution.*

f	y w^a e_c r_b f	s_c f	y w^a e_c f	y w^a f	y f	w^a e_c r_b f	e_c r_b f	r_b f	s_c r_b f	Total
2704	607	825	26	73	47	36	59	21	1	4399

TABLE 4

Exceptional daughters from attached-X mothers of

$\dfrac{y\ w^a \qquad f}{s_c \quad e_c\ r_b \quad f}$ *constitution.*

f	y w^a f	s_c e_c r_b f	y f	w^a f	s_c e_c f	s_c f	e_c r_b f	r_b f	e_c f	Total
215	74	59	4	7	2	20	16	1	1	399

TABLE 5

Exceptional daughters from attached-X mothers of

$\dfrac{y\ w^a \quad e_c \quad f}{s_c \qquad r_b \quad f}$ *constitution.*

f	y w^a e_c	s_c r_b f	y w^a f	y f	w^a e_c f	e_c f	s_c f	r_b f	Total
646	173	195	20	19	15	15	45	36	1164

TABLE 6

Exceptional daughters from attached-X mothers of

$\dfrac{y \qquad r_b \quad f}{s_c\ w^a \quad e_c \quad f}$ *constitution.*

f	y r_b f	s_c w^a e_c f	y f	r_b f	s_c w^a f	s_c f	w^a e_c f	e_c f	w^a f	+	Total
4607	1291	1237	332	308	107	136	115	113	2	2	8250

TABLE 7

Exceptional daughters from attached-X mothers of

$\dfrac{y \quad e_c\ r_b \quad f}{s_c\ w^a \qquad f}$ *constitution.*

f	y e_c r_b f	s_c w^a f	y e_c f	y f	e_c r_b f	r_b f	s_c f	w^a f	e_c f	+	Total
1888	451	536	17	109	78	20	39	41	1	1	3181

TABLE 8

Exceptional daughters from attached-X mothers of

$$\frac{y \quad e_c \quad f}{s_c\, w^a \quad r_b \quad f}\ \textit{constitution.}$$

f	y e_c f	s_c w^a r_b f	y f	e_c f	s_c w^a f	s_c f	w^a r_b f	r_b f	Total
2098	596	547	135	109	107	53	56	90	3791

TABLE 9

Exceptional daughters from attached-X mothers of

$$\frac{y\, w^a \quad r_b \quad f}{s_c \quad e_c \quad f}\ \textit{constitution.}$$

f	y w^a r_b f	s_c e_c f	y w^a f	y f	w^a r_b f	r_b f	s_c f	e_c f	Total
45	8	14	0	2	1	0	0	1	71

TABLE 10

Exceptional daughters from attached-X females of

$$\frac{y \qquad f}{s_c \qquad f}\ \textit{constitution.}$$

f	y f	s_c f	Total
2172	668	627	3467

TABLE 11

Exceptional daughters from attached-X females of

$$\frac{y \quad e_c\, r_b \quad +}{s_c\, w^a \qquad f}\ \textit{constitution.*}$$

+	y e_c r_b	s_c w^a	y e_c	y	e_c r_b	r_b	s_c	w^a	f	y e_c r_b f	e_c r_b f	s_c w^a f	s_c f	w^a f	Total
508	129	195	1	31	25	10	10	12	34	9	4	4	1	3	976

* It is impossible to tell which chromosome contains the mutant factor forked (f) and which contains the normal allelomorph.

TABLE 12

Exceptional daughters from attached-X females of

$\dfrac{y \quad r_b \quad +}{s_c\ w^a\ e_c \quad f}$ *constitution.*

+	y r_b	s_c w^a e_c	y	r_b	s_c w^a	s_c	w^a e_c	e_c	w^a	f	y r_b f	r_b f	s_n w^a e_c f	s_c w^a f	s_c f	w^a e_c f	e_c f	Total
1018	287	278	54	54	35	28	17	31	1	46	12	8	8	2	1	2	1	1883

TABLE 13

Exceptional daughters from attached-X females of

$\dfrac{y\ w^a \quad e_c \quad +}{s_c \quad r_b \quad f}$ *constitution.*

+	y w^a e_c	s_c r_b	y w^a	y	w^a e_c	e_c	s_c	r_b	f	y w^a e_c f	e_c ff	s_c r_b f	s_c f	r_b f	Total
273	87	53	10	4	10	10	17	17	15	2	1	1	2	3	505

TABLE 14

Exceptional daughters from attached-X females of

$\dfrac{y \quad +}{s_c\ w^a \quad e_c\ r_b \quad f}$ *constitution.*

+	y	s_c w^a e_c r_b	s_c w^a	s_c	w^a e_c r_b	e_c r_b	r_b	f	r_b f	s_c f	s_c w^a e_c r_b f	Total
74	30	11	3	2	1	2	3	8	3	1	1	139

TABLE 15

Exceptional daughters from attached-X females of

$\dfrac{y \quad e_c \quad +}{s_c\ w^a \quad r_b \quad f}$ *constitution.*

+	y e_c	s_c w^a r_b	y	e_c	s_c w^a	s_c	w^a r_b	r_b	f	r_b f	Total
86	32	21	2	2	4	2	2	4	6	1	162