DOES SISTER-STRAND CROSSING OVER OCCUR IN DROSOPHILA MELANOGASTER?[1]

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The problem of whether sister-strand crossing over does or does not occur is of some importance for general theories of crossing over. In most genetic experiments, it is not possible to decide whether sister-strand exchange has or has not occurred because the results of both alternatives are indistinguishable. But with the use of the closed-X chromosome, described by L. V. MORGAN (1933), a solution of this question seems possible.

In heterozygous condition, the closed-X chromosome gives no offspring coming from single or triple crossing over, and there is a corresponding and fairly large proportion of eggs that fail to hatch. When total crossing over is reduced by the use of an inversion-bearing chromosome opposite the closed-X, there is observed a reduction in the frequency of inviable eggs. It was inferred (loc. cit.) that in meiosis, single and triple exchanges between a closed-X and a normal X result in chromosome attachments which give these inviable eggs. Hence, in the presence of an inversion which reduces the frequency of crossing over, fewer single and triple crossings over occur to give inviable eggs. In this case, especially if the suppression of crossing over is almost complete, the chief causes of inviable eggs would be (a) the standard inviability of the stocks, and (b) sister-strand crossing over between closed-X chromosomes. With adequate precautions and controls, the standard residual inviability could be estimated, and a direct measure obtained of the frequency of sister-strand crossing over.

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METHOD

All the stocks from which the parental flies were obtained had been closely inbred for several generations prior to the beginning of the experiment.

Females for the P_1 cross were derived from seven stocks. These stocks included three inversions, (1) *ClB*, (2) delta-49, and (3) scute-8, for the

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three experimental crosses, and for the four controls the stocks (4) attached-X forked, (5) vermilion, (6) yellow-2 crossveinless vermilion forked and (7) wild-type (Columbia University stock). Virginity of each P_1 female was assured by a test period of egg-laying prior to mating. Sister females from each of the seven stocks were crossed singly to three types of males—to closed-X yellow for the experimental crosses and to vermilion and to yellow-2 crossveinless vermilion forked for the control crosses. One of each of the seven types of females was mated in turn to the selfsame male, and only those F_1 cultures were used in which all the females of the set of seven were fertilized by one male. In table 1, the genotypes of the ♀ and ♂ parents are given, together with the number of F_1 females of each type, whose egg mortality was tested. The F_1 females were mated individually to wild (C.U.) males and the eggs counted and observed for viability. In all, egg counts for 25 types on a total of 215 individual females were recorded.

TABLE 1

Crosses made and number of F_1 females used for egg counts.

♂ ♀	$X^c\ y$	v	$y^2\ cv\ v\ f$
ClB/f fu	10	7	15
	12	8	6
$N^8/y\ Hw\ dl$-49	6	4	7
	6	8	5
$sc^8\ w^a$	18	4	14
$\underline{f}$	4	6	6
v	17	4	18
$y^2\ cv\ v\ f$	12	10	—
+	6	2	—

The eggs, whose mortality was to be tested, were deposited on strips of colored blotting paper which had been soaked in fermenting banana juice and then overlaid with a thin layer of banana agar and mounted each on a glass slide. Blotters were changed daily, and the 1×4″ vials used were kept free from condensed moisture. Counts were made of total eggs laid and of those that had failed to hatch after 2–3 days.

The progeny of one or more days' egg-laying of 55 females were subsequently examined to make sure that the frequency of the various classes that occurred was that expected for the particular cross. Every P_1 culture bottle was represented at least once among these 55, although every one of the 215 females was not tested in this way. Since no errors were found as a result of this check, and because of the precautions taken in making up the original crosses, it may be assumed that each F_1 female used for the egg counts had the chromosomal constitution indicated.

RESULTS

The frequency of inviable eggs for each type of cross is given in table 2, and the frequencies are summarized in table 3. The relative inviability of

TABLE 2

Frequency of inviable eggs.

NO.	CONSTITUTION OF F_1 FEMALE	EGGS LAID	NUMBER INVIABLE	PERCENT DIED AND STANDARD ERROR
1	$ClB/X^c\,y$	1828	147	8.0±9.63
2	ClB/v	604	45	7.5±1.07
3	$ClB/y^2\,cv\,v\,f$	2105	229	10.9±0.69
4	$y\,Hw\,dl\text{-}49/X^c\,y$	471	19	4.0±0.90
5	$y\,Hw\,dl\text{-}49/v$	1137	38	3.3±0.53
6	$y\,Hw\,dl\text{-}49/y^2\,cv\,v\,f$	1374	112	8.2±0.74
7	$sc^8\,w^a/X^c\,y$	3301	419	12.7±0.58
8	$sc^8\,w^a/v$	244	31	12.7±2.13
9	$sc^8\,w^a/y^2\,cv\,v\,f$	1762	193	11.0±0.75
10	$\underline{f}$ ♀ × $X^c\,y$ ♂	229	84	36.7±3.23
11	$\underline{f}$ ♀ × v ♂	181	44	24.3±3.10
12	$\underline{f}$ ♀ × $y^2\,cv\,v\,f$ ♂	306	103	33.7±2.70
13	$N^8/X^c\,y$	355	127	35.8±2.54
14	N^8/v	310	116	37.4±2.75
15	$N^8/y^2\,cv\,v\,f$	308	81	26.3±2.50
16	$f\,fu/X^c\,y$	765	170	22.2±1.50
17	$f\,fu/v$	461	49	10.6±1.45
18	$f\,fu/y^2\,cv\,v\,f$	393	17	4.3±1.02
19	$v/X^c\,y$	136	27	20.0±3.44
20	v/v	278	54	19.4±2.37
21	$v/y^2\,cv\,v\,f$	2277	208	9.1±0.60
22	$y^2 cv\,v\,f/X^c\,y$	2225	722	32.4±0.90
23	$y^2 cv\,v\,f/v$	655	23	3.5±0.72
24	$+/X^c\,y$	1236	196	15.9±1.04
25	$+/v$	181	3	1.7±0.96

the closed-X chromosome as compared with that of the normal chromosome may be estimated from the crosses to attached-X forked females ($\underline{f}$). Table 2 shows 36.7 percent ± 3.23 for the closed-X as comparedwith 33.7 ± 2.70 for y^2 *cv v f*, and 24.3 ± 3.19 for *v* (25 percent egg inviability is due in each case to *YY* eggs.) The residual inviability for X^c *y* is therefore about that of a multiple mutant stock like y^2 *cv v f*. The tests with females carrying *ClB* show that the male lethal effect associated with this inversion does not cause the death of *ClB* males during the egg stage.

TABLE 3

Egg mortality summarized from table 2.

CONSTITUTION OF F_1 FEMALE	CLASSES OF TABLE 2	TOTAL EGGS	PERCENT DIED AND STANDARD ERROR
$\underline{f}$ ♀ × X^c ♂	10	229	36.7±3.23
$\underline{f}$ ♀ × X ♂	11+12	487	30.2±2.06
ClB/X^c	1	1828	8.0±0.63
ClB/*X*	2+3	2709	10.1±0.58
dl-49/X^c	4	471	4.0±0.90
dl-49/*X*	5+6	2511	6.0±0.47
sc^8/X^c	7	3301	12.7±0.58
sc^8/X	8+9	2006	11.2±0.68
X/X^c	16+19+22+24	4361	25.6±0.66
X/X	17+18+20+21+23+25	4245	8.2±0.43

The frequency of inviable eggs for all three inversions was not different from that of their respective controls. For the *ClB* and *dl*-49, the closed-X inversion was slightly lower than normal/inversion; and for scute-8, slightly in excess; but in no case was the difference as great as twice the standard error.

The absence of extra inviable eggs in any given inversion stock heterozygous for closed-X as compared with the same inversion heterozygous for normal X, can only be taken as proof that sister-strand crossing over does not occur in the absence of homologous strand crossing over. But in the absence of increased egg mortality for all three inversions, which are of different sizes and include different loci, and at least one of which, *dl*-49, allows a considerable amount of normal crossing over to occur, it may be concluded that the possibility of sister-strand crossing over is excluded for Drosophila. This conclusion is consistent with that drawn from other considerations by WEINSTEIN, MULLER and WEINSTEIN, STURTEVANT, and L. V. MORGAN.

The data given here, however, do not prove the assumed direct relation between the occurrence of single and triple crossing over in closed-X heterozygotes and the frequency of inviable eggs. To some extent, the data presented may even argue against this simple relation. In table 2, Nos. 16, 19, and 24, the frequency of inviable eggs is lower than the expected occurrence of singles. In the absence of more definite information on this relation, it therefore seems inadvisable to give an extended discussion of the questions raised.

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