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The Distribution of Interchanges in Eradiated Sperm of Drosophila Melanogaster

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THE DISTRIBUTION OF INTERCHANGES IN IRRADIATED SPERM OF DROSOPHILA MELANOGASTER

BY

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A C K N O W L E D G M E N T

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INTRODUCTION

Muller (1927) found that X-rays are able to induce mutations and rearrangements which are indistinguishable from those appearing spontaneously in Drosophila, although the X-ray induced mutation rate was many times greater than that of spontaneous mutation. The earliest explanation of the mechanism of induction of rearrangements was the contact theory, which states that for two breaks, the chromosomes must be together, that both breaks are caused by the same event; this event is presumably a "hit" by an electron (Serebrovsky, 1929).

If the contact theory were correct, one would expect a linear relationship between rearrangement frequency and dose. Muller (1940) found the relationship to be exponential and formulated the "breakage first" theory, that each break is independently produced, each break being the result of a separate "hit".

Adapting the target theory of radiation inactivation to genetic changes, Lea (1945) suggested that breakage is immediate and simultaneous with the passage through a chromosome of an ionizing particle, producing seventeen or more ionizations in the chromosome. He assumed a single ionization in a gene to be sufficient to induce mutation. Thus, according to Lea, the action of radiations is physical; the effect is a direct effect on the gene or on the chromosome. The ionizing particles are distributed at random in the irradiated material, therefore mutations and breaks should be similarly distributed.

In recent years, certain evidence not wholly consistent with the target theory has been accumulated. Stone, Wyss, and Haas (1947) worked with Escherichia coli irradiating the culture media. Bacteria grown on these irradiated media showed a higher frequency of mutation to penicillin resistance than the control; this has been related to peroxide production. The mutation rate in Neurospora is significantly higher than the normal rate with hydrogen peroxide. These mutations are of the types that have been obtained by direct radiation (Wagner et. al. 1950). Darlington and Koller found that nitrogen mustards act like chronic X-rays (1947).

King (1947) found that lower temperatures increased the mutation rate. Mickey (1938) found the same to be true for rearrangements. Sax (1947) found that lower temperature increases aberrations if the temperature is reduced during or very shortly after radiation. This indicates the effects of radiation to be chemical rather than physical.

If the target theory is correct, breaks should be distributed in a random fashion among similar irradiated cells (e.g. mature sperm), therefore, rearrangements should be similarly distributed. Other modes of action of radiations likewise might be expected to yield a random distribution. For example, if the effects of radiation are indirect, and due to very localized production of hydrogen peroxide, we would also expect random distribution.

One reason for considering the possibility of a non-random distribution is the report by Sparrow and Christensen (1949), who found in unirradiated Trillium that the frequency of aberrations is about 4%, a rate equivalent to that produced by a dose of about 100 r units at a highly sensitive stage. The distribution of this break frequency differed significantly from that of a random distribution. Giles (1940) reported that certain individuals of Tradescantia show a high frequency of spontaneous breaks, while other individuals show none.

If rearrangements occur at random in sperm, they would be as likely to occur in the sperm of one male as in another. Therefore, it was decided to test a certain number of sperm from similar males, mated individually to see if a random distribution might be obtained.

EXPERIMENTAL PROCEDURE

Young adult wild type (Stephenville) males of *Drosophila melanogaster* were treated with a dose of 3000 r units of 60 K.V. X-ray filtered with 1 mm of aluminum. The dose rate was approximately 100 r/minute in all experiments. The males were mated individually to four virgin S/Cy; D/C3X females each, immediately after treatment. The mating period was limited to not more than twenty-four hours, after which the adult males were removed to prevent the use of sperm that were not mature at the time of the X-ray treatment. This mating period was well within the time limit indicated by Demerec and Kaufmann (1941). The inseminated females were allowed to remain in the vials for an additional twenty-four hour period to increase the number of F_2 individuals.

If possible, twenty F_1 Curly Dichaete male offspring of each irradiated P_1 male were mated individually to wild type virgin females; if fewer than twenty F_1 males emerged, they were mated the same way. The presence of a translocation between the second and third chromosomes would be indicated by failure of Curly to segregate from Dichaete. If less than ninety-six F_2 adults emerged in a culture, it was discarded. The results include only those trials in which more than ten F_1 males produced a sufficient number of F_2 individuals for the results to be considered reliable.

R E S U L T S

The numerical data obtained in this experiment are presented in Table 1. The number of translocations per sample of twenty or fewer irradiated sperm varied from zero to eight. The columns numbered "0" to "8" give the number of trials (e. g. the number of P_1 males that were irradiated) which yielded the number of translocations indicated by the figure at the head of the column.

The number of P_1 males found to be sterile was ten; 280 P_1 cultures were made, giving a percentage of P_1 sterile males of 3.57. The number of F_1 males found to be sterile was twenty-

Table 1

Distribution of Interchanges

No. of F_2 cultures counted per trial	Number translocations per F_2 culture								
	0	1	2	3	4	5	6	7	8
20 actual	117	23	20	21	18	7	3	1	1
20 expected	115.6	24.6	22.6	20.3	19.9	6.68	1.99	.38	.21
19 actual	14	4	1	1		3	1		
19 expected	13.5	4.12	2.94	1.23	.35	1.64	.09		
18 actual	5	3		1					
18 expected	4.74	2.02	1.65	.51					
17 actual	2	1			1				
17 expected	1.45	1.2	.74	.23	.15				
16 actual	2	1		1					
16 expected	1.16	1.14	.52	.15					
15 actual	4	1	1						
15 expected	3.58	1.3	1.08						
14 actual	3			1.					
14 expected	2.7	1.49	.59	.15					
13 actual	None	found							
13 expected									
12 actual	3	2	1						
12 expected	2.79	1.85	.67						
11 actual	3	1							
11 expected	2.38	1.4							
Total actual	153	36	22	25	19	10	4	1	1
Total Exp.	147.8	39.1	30.8	22.5	20.4	8.3	2.08	.38	.21
Deviation	+5.2	-3.1	-8.8	+2.5	-1.4	+1.7	+1.9	+.62	+.79
Deviation^2	27.04	9.61	77.44	6.25	1.96	2.89	3.69	.38	.79
d^2/e	.18	.76	2.51	.28	.09	.04	1.77	1.0	2.95

$\Sigma = 9.578$

eight; 5,225 cultures were mated, giving an F_1 male sterility percentage of 0.54. The tabulation of F_1 male sterility is presented in Table 2. The total number of F_2 cultures observed and recorded was 5,197, 320 translocations being found, giving a frequency of 6.16%. Table 2 also gives the percentage of survivals found in each class group, and the percentage of translocations found in survivals.

Table 2

Record of F_1 Trials

No. of F_1 cultures per trial of 20	No. of trials	Percent of total trials	Percent translocation found	No. of sterile per trial
20	211	77.86	6.30	all fertile
19	23	8.49	6.41	10
18	9	3.32	4.23	8
17	4	1.47	7.35	4
16	4	1.47	8.33	3
15	6	2.23	3.33	2
14	4	1.47	5.36	
13				
12	6	2.23	5.56	1
11	4	1.47	2.27	

DISCUSSION OF RESULTS

If distribution is random, expected values may be calculated by the binomial expansion:

$$(1-p)^N,\ Np(1-p)^{N-1},\ \frac{N(N-1)}{2!}\,p^2\ (1-p)^{N-2},\ \text{etc.}$$

Where N = number of F2 cultures counted per trial, and

p = frequency of translocations in the experiment, 0.0616

Probability was calculated by the "Chi-square" method for Goodness of Fit. "Chi-square" was found to be 9.578, giving a probability of 0.299616 deviations as great as, or greater than, actually found. We may conclude that in this experiment, a random distribution has been found.

The deviation for a random distribution should be of such a nature that there would be an increase in the zero and three or more translocation classes, with a decrease in the one and possibly in the two class. The characteristic of the deviation in this experiment was not of that nature.

The distribution of the breaks found was of a random nature; the events which caused the breaks also would be of a random distribution. Since the "columns" of ionizations are randomnly distributed, it seems likely that the effects of such ionization are extremely localized.

SUMMARY

1. The frequency of translocations between the second and third chromosomes was found to be 6.16% in males of *Drosophila melanogaster* in which mature sperm were treated with 3000 r units.

2. The distribution of these translocations was studied by mating the irradiated males individually.

3. Distribution expected on the basis of random occurrence was calculated by the binomial expansion.

4. The probability of finding deviations from expectation as great as, or greater than those actually found, as calculated by the "Chi-square" method, is 0.299616.

5. It was concluded that distribution is random.

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