OBSERVATIONS ON CROSSING OVER INDUCED BY X-RAYS IN THE MALES OF DROSOPHILA

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THE induction of crossing over in the male of *Drosophila melanogaster* has been reported by PATTERSON and SUCHE (1934), who concluded that it occured in the growth stages of the spermatocytes. This conclusion was based on the assumption that, were the crossing over taking place in pre-spermatocyte stages (that is, spermatogonia or stem cells), its rate should be highest in the youngest age irradiated, since a larger proportion of the gametes to be sampled would be represented by such stages. They found the rate in 40–52 hour larvae to be some six times greater than the rate in 24–36 hour larvae.

FRIESEN (1934) has likewise reported the production of crossing over in the male by irradiation. In a later paper (1936), on the basis of the occurrence of clusters of similar crossovers in the progeny of individual males, he concluded that crossing over must be occurring in a stage earlier than the spermatocyte; he assumed it to be spermatogonial crossing over. If FRIESEN is correct in assuming that crossing over in one cell results in all of the crossovers recovered in one cluster, then in some cases the large size of the clusters makes it likely that crossing over is being induced in one of the apical stem cells. An attempt will be made in this paper to explain the occurrence of clusters without assuming spermatogonial (or stem cell) crossing over.

Both PATTERSON and SUCHE, and FRIESEN (1937) describe the crossover chromosomes as being normal, with few exceptions. The few lethals found were not associated with the point of break. FRIESEN'S studies included examination of the crossover chromosomes in the salivary glands. On the basis of the absence of lethal position effects, PATTERSON and SUCHE concluded that induced crossing over could not be the effect of chromosome breakage by X-rays, but rather is due to a possible alteration of the rate of maturation. However, if the crossover chromatids are broken at identical loci, the absence of lethal position effects might be expected. Thus if the breakage is produced by irradiation in the same manner as in aberrations, the absence of lethal position effects would show such effects to be the result of rearrangement and not of breakage.

If induced crossing over is due to orthodox chromosome breakage, and if the majority of the crossover chromosomes recovered are essentially normal as reported, then the possibility suggests itself that the breakage of the two chromatids involved is accomplished by a single ionizing particle. The primary purpose of this paper is to open up the question of the relationship between dosage and frequency of induced crossing over to see if this relationship indicates breakage by a single particle.

EXPERIMENTAL PROCEDURE

Larvae heterozygous for the third chromosome multiple recessive stock "*3ple*" (*ru h st* p^p *ss* e^s) were irradiated when 40–52 hours old, the age range where PATTERSON and SUCHE found the highest incidence of crossing over. On emerging, each of the males was mated individually to four homozygous "*3ple*" virgin females. The F_2 offspring emerging during the first three days were counted.

To reduce the errors of dosage measurement, the sample to receive the half dose in each case was divided into two halves, one being substituted for the other mid-way through the treatment. The peak voltage employed was 56 kv; the radiation was filtered through 1 mm of aluminum. The dose rate was approximately 100 r/minute, as determined by the use of a Victoreen r-meter.

EXPERIMENTAL RESULTS

The results are summarized in the accompanying tables, with table 1 showing the frequencies of all crossovers at each dose employed. While the lower

TABLE 1

Frequency of crossing over.

SERIES	DOSE	NUMBER MALES TESTED	NUMBER PRODUCING CROSS-OVERS	NON-CROSS-OVERS	TOTAL SINGLES	TOTAL DOUBLES	TOTAL COUNTED	PERCENT CROSSING OVER	LIMITS OF ERROR*	
1	500 r	354	58	18,744	80	3	18,827	.46	.37	.56
	1000 r	180	50	9,030	59	4	9,093	.74	.54	.88
2	1000 r	198	47	11,451	70	1	11,527	.63	.48	.86
	2000 r	22	6	891	11	—	902	1.22	.62	2.20
	Control	128	1†	9,937	1†	—	9,938			

* Calculated by the method of STEVENS (1942) with a significance of .05.

† One *ru* male which when crossed with homozygous "*3ple*" females produced only wild and "*3ple*" offspring.

doses had little apparent effect on the viability and fertility of the imagos from the irradiated larvae, adults failed to emerge in a very large proportion of those receiving 2000 r. Many of the latter which did emerge died shortly thereafter, or at best failed to produce offspring. Approximately five percent of the survivors produced offspring, producing almost as many per male as did those receiving the lower doses. In the controls and in the series receiving 500 r and 1000 r, approximately 90 percent of the males tested were fertile. The calculations of limits of error in table 1 follow the method of STEVENS (1942).

Table 2 shows the distribution of crossovers in the samples. Over one half of the crossovers recovered were in clusters of from two to five crossovers each. In practically all of these cases the crossovers in each cluster were similar—

TABLE 2

Distribution of crossovers. Vertical columns show total number of males producing 0, 1, 2, 3, 4, 5 crossovers.

DOSE	NUMBER OF CROSSOVERS FROM A SINGLE MALE					
	0	1	2	3	4	5
500 r	296	40	13	4	—	1
1000 r	281	70	19	6	1	1
2000 r	16	2	3	1	—	—
Totals	593	112	35	11	1	2

TABLE 3

Composition of clusters with dissimilar crossovers.

NO. OF CLUSTERS	CROSSOVERS
1	*p^p, p^p ss e^s*
1	*ru h, ru h st*
1	*ru h, p^p ss e^s*
1	*p^p ss e^s, ru h st p^p e^s*
1	*ru, ru h st*
2	*ru, p^p ss e^s*
1	*p^p ss e^s, e^s*
1	*st, ru h st, p^p ss e^s*
1	*st p^p ss e^s, ru h st, p^p ss e^s*
1	*ru h st, p^p ss e^s, e^s*
1	*ru h st, p^p ss e^s, 2 ru h st p^p*
1	*3 ru h, 2 p^p ss e^s*

produced by crossing over in the same region with both classes being freely represented. The exceptional cases are listed in detail in table 3.

Of the 35 clusters of two crossovers each, 27 were similar pairs. One cluster consisted of a double and a related single. Two clusters consisted of single crossovers in which the regions of exchange were adjacent. One was made up of a double involving two adjoining regions and a single involving a third adjoining region. Four of the pairs were single crossovers involving non-adjacent regions.

Of the 11 clusters of three crossovers each, eight consisted of three similar

crossovers. One consisted of a double with two related singles. One consisted of two similar singles, with the third single involving an adjacent region. One was made up of a pair of similar singles, with a third single involving a non-adjacent region.

The one cluster of four singles involved two adjacent regions of crossing over, each being represented twice. One of the clusters of five was made up of five similar singles, while the other involved two adjacent regions, one being represented twice the other thrice.

Table 4 shows the types of doubles recovered, and compares them with the doubles reported by PATTERSON and SUCHE. Eight double crossovers were recovered in this experiment, in all cases involving two adjacent regions of exchange. The 12 doubles reported by PATTERSON and SUCHE are quite similar in this respect, with the exception of four cases, three of which involved the distal

TABLE 4

Types of double crossovers recovered.

PRESENT DATA:		DATA OF PATTERSON AND SUCHE:	
REGIONS	NUMBER	REGIONS	NUMBER
		ru-h, h-th	1
		h-th, th-st	1
h-st, st-p^{p}	2	*th-st, st-cu*	2
st-p^{p}, *p*^{p}*-ss*	4	*st-cu, cu-sr*	3
p^{p}*-ss, ss-e*^{s}	2	*cu-sr, sr-e*^{s}	1
		ru-h, e^{s}*-ca*	3
		h-th, st-cu	1
Total	8	Total	12

regions of the two arms of the third chromosome. One of their doubles involved two regions of exchange which were neither adjacent nor in opposite arms of the chromosome approximately equidistant from the centromere.

Thus, both the doubles and the clusters of dissimilar singles indicate considerable restrictions on crossing over, making adjoining regions the most likely possibility if two regions of crossing over are to be represented in the progeny of an individual male. The relative frequencies with which any two exchanges involving different regions are recovered are not dependent simply on the frequencies of crossing over, as in normal crossing over, since on this basis many of the possibilities which are not realized would be equally likely to occur as those which actually do.

The restrictions on the occurrence of crossing over are further indicated by the failure of doubles to increase at higher doses much more rapidly than do the singles. If crossing over can occur at the time of irradiation of a given cell in all parts of the chromosome, with increasing dosage (therefore with increasing frequency of crossing over) the probability of two simultaneous exchanges would be increased, and the ratio of singles to doubles should be decreased.

Table 1 shows 28 singles/double at 500 r and 25.8 singles/double at 1000 r—essentially the same.

Table 5 shows the distribution of crossing over by regions. The results for each dose show a similar distribution of crossing over, with approximately 60 percent of the observed crossing over taking place in the *st-p*^{p} region.

DISCUSSION

While the question of time of crossing over in the male is of no great significance to the main question of relationship of frequency to dosage, the facts at present available concerning X-ray induced crossing over can be explained

TABLE 5

Percentage of total crossing over for each marked region.

DOSE	REGIONS				
	ru-h	*h-st*	*st-p*^{p}	*p*^{p}*-ss*	*ss-e*^{s}
500 r	3.5	15.1	60.5	12.8	8.1
1000 r	9.3	10.8	63.3	9.3	7.2
2000 r	27.3	—	72.7	—	—

without assuming spermatogonial crossing over. The objections given to spermatogonial crossing over do not rule it out entirely; at the same time they certainly add no support to the idea. I propose the following explanation of crossing over occurring in the growth stage of the spermatocyte, based on the parallels between cytological observations and the characteristics of clusters described above.

LEAGUE (1931) states that some larvae will show growth stages, while others of similar age will show none, the space being occupied by second spermatocytes and spermatids. This corresponds to the production of several crossovers by some males, none by others.

The crossovers making up one cluster are most often alike, but when they differ they frequently involve adjacent regions of crossing over, indicating the restriction of crossing over at any time to a very short region. If crossing over is taking place in the spermatocyte, then crossing over of essentially the same type must be taking place simultaneously in several cells to produce these clusters. Both HUETTNER (1930) and LEAGUE agree in their descriptions of spermatogenesis that the Drosophila testis shows cyst formation, although the cysts are described as being weak, and walls are lacking. All of the cells in one cyst develop more or less synchronously, which would indicate that they would be subject to similar restrictions. If crossing over were then induced in several cells, several similar crossovers might be recovered.

Thus we can think of a short region in the chromosome which is competent to have crossing over induced in it, with synapsis presumably being the primary condition necessary for such competence. Possibly such competence is

gained first in the vicinity of the centromere, extending distally as it is lost proximally. This would account for the three doubles of PATTERSON and SUCHE involving the most distal regions. On this interpretation, the frequency of induced crossing over in a region is a function of the duration of competence in the region rather than of the absolute length of the region. The similarity of induced crossing over in male and female suggests that somewhat similar conditions might exist in the female.

Since only a short region of the chromosome is competent at any time, the production of two exchanges during any short interval usually would be undetectable, since they would seldom involve two separate marked regions. Thus a doubling of the actual rate of exchange would not result in a doubling of the observed rate of crossing over, due to the increased probability of getting two exchanges in a single marked region. If the two breaks resulting in crossing over are due to the passage of a single ionizing particle, then the frequency of exchanges should show a straight line relationship to dosage. However, the observed rate of crossing over should show such a straight line relationship only at very low doses, levelling off as an upper limit is approached. The upper limit would depend upon the percentage of sperm in the sample which arose from cells which were competent at the time of irradiation.

On the basis of the observed rates at doses of 500 r and 1000 r, we may calculate such an upper limit to be approximately 1.2 per cent. The expected frequency of crossing over at 2000 r would be about 1.0 percent; the value actually obtained, 1.22 percent, lies fairly close but is obviously none too reliable due to the small numbers involved. It would be of some value to see how well the frequencies at lower doses fit such a theoretical curve.

None of the results here discussed are inconsistent with the interpretation that crossing over induced by X-rays is the result of direct breakage of the chromosomes. The frequency/dose relationship is that which would be expected on the basis of breakage of two chromatids by a single particle. This is in agreement with observations on isochromatid breakage in Tradescantia (LEA 1947).

The interpretation of crossing over as a result of chromosome breakage is further reenforced by the recent report of LEFEVRE (1947) which indicates that, since fast neutrons are "relatively much more effective per ionization than gamma rays" in inducing somatic crossing over, such induced somatic crossing over must be a "direct" effect of the radiations, resulting in chromosome breakage. LEFEVRE concludes that the two breaks are produced independently, since the frequency is dependent on the dose rate. If this is the case, crossover chromosomes would be produced with small duplications and deficiencies, which might account for the statement, "Evidence was found that mosaic size and age at exposure are not simply related. Twin mosaics in eosin-white females showed great inequality in size." If this interpretation should prove to be appropriate, somatic and germinal induced crossing over are not identical. It is not appropriate at this time to make any extensive comparisons between the two.

SUMMARY

Induced crossing over in the male is quite restricted in that only a short region of the chromosome can be involved at any time. This is indicated by the preponderance of doubles involving adjacent regions of crossing over, as well as by the occurrence of clusters of crossovers involving adjacent regions.

The frequency of double crossing over failed to increase greatly with increased dose. This is to be expected if crossing over is restricted to a region shorter than one marked region.

The occurrence of several crossovers in the progeny of some males and absence from the progeny of others is correlated with the presence of growth stages in some testes and absence from the testes of other males of similar age.

The cluster effect may be correlated with the similarity of stage of maturation of all cells in a cyst. All cells in one cyst might be similar in their crossing over restrictions at any time.

It is therefore suggested that the assumption that crossing over is being induced in the spermatogonia is unnecessary.

Induced crossing over may be interpreted as a result of chromosome breakage by X-rays, with the frequency/dose relationship indicating that both breaks involved in one exchange are produced by a single particle.

The absence of lethal position effects does not rule out the explanation of direct breakage by X-rays if both chromatids are broken at the same locus.

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