Radiation of *Drosophila melanogaster* with Low-Intensity Ultra-Violet Light for One Complete Generation. I. Effect on Crossing-Over in the Second Chromosome

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Introduction

Considerable work has been done concerning the effects on Drosophila of treatment with X-rays, heat, and radium. This includes production of mutations and modifications of the rates of non-disjunction and crossing-over.

In recent years the effect of ultra-violet radiation has been investigated in some degree. Altenburg studied the production of mutations by unfiltered ultra-violet radiation. Promptov induced mutations by monochromatic ultra-violet radiation. Rifenburgh modified crossover rates by treatment with monochromatic ultra-violet light. All these investigators used relatively heavy doses for short periods.

Since no work had been published on the effect of radiating Drosophila with ultra-violet light for long periods, this work was undertaken to study the effect of unfiltered radiation for a complete generation on crossing-over in the second chromosome of Drosophila melanogaster

Review of Literature

I. **Effect of X-radiation.**—Mavor (1923) reported from a series of experiments on X-radiation that X-rays caused a decrease in crossover values between White and Miniature either by inhibiting crossing-over or by increasing double crossovers.

Later Mavor and Svenson (1924) investigated the effect of X-rays on crossing-over in the second chromosome. Treated females heterozygous for Black-Purple-Curved were backcrossed to recessive males with the following results: (1) crossover value between Black and Purple 3.77% in controls and 16.67% in the experimentals, (2) crossover value between Purple and Curved 17.38% in controls and 30.58% in the experimentals. This was a very significant increase in crossing-over in the case of the X-rayed mothers.

Muller (1925) published results of work with X-radiated autosomes. He found in both long autosomes a regionally differentiated susceptibility to X-rays, with the maximum susceptibility in the morphologically differentiated portion at or near the spindle-fiber attachment. The second and third chromosomes reacted similarly—that is, with an increase of crossing-over in this region.

II. Effect of Radium.—Plough (1924), working with treated females heterozygous for Black-Purple-Curved backcrossed to pure recessive males, found that the beta and gamma rays of radium caused an increase in crossing-over.

III. Effect of Heat.—Plough (1917) reported results from an experiment on the effects of temperature and environment. Extreme heat and cold, acting upon females up to the time of hatching, caused a decided increase of crossover percentage in the second chromosome for the first ten day broods. He found no effect of high and low temperature on crossing-over in the first and third chromosomes.

He (Plough 1921) studied the effect of heat on flies heterozygous for autosomal genes. The following results were obtained: (1) there was no significant increase in the percentage of crossing-over in the second chromosome as a result of the exposure of developing eggs to high temperature; (2) in the third chromosome there was an increase in the region of the spindle-fiber attachment only; (3) crossing-over varied with the age of the female but in those regions only which showed a reaction to temperature.

Stern (1926) raised experimental females at 30° C. and controls at 25° C. He obtained an increase in percentage to 16 in the experimentals against 11½ in the controls. This was in the Bar-Bobbed region of the first chromosome, which is near the attachment of the spindle-fiber.

Schwab (1935) made a further study of effect of temperature on crossing-over. Under high temperature there was increased recombination in the third chromosome which was found to be concentrated between Scarlet and Pink (the area of the spindle-fiber attachment) and spreading with high decrement to the adjacent regions. This result agrees with those reported by Muller for X-rays and by Bridges for age.

IV. Effect of Ultra-Violet Light.—In this laboratory Rifenburgh (1935) treated virgin females, heterozygous for Black-Vestigial-Brown, with monochromatic ultra-violet light and backcrossed them to Black-Vestigial-Brown males. With radiated adults, he obtained a slight increase in crossover percentage near the spindle-fiber attachment of the second chromosome. In the case of treated eggs and young larvae, there was a decrease in crossover percentage.

General Methods

The source of ultra-violet light for the following experiments was a General Electric Sun Lamp (S_2 Model K). In this lamp, the ultra-violet rays are produced by gaseous mercury heated to incandescence by a tungsten filament within a bulb of special glass. A silver-colored shield placed below the bulb reflects and diffuses the light so that the radiation is nearly uniform over a fairly wide area.

The radiated generation was reared in 100 cc. beakers filled to within an inch of the top with Purdue medium (Rifenburgh, 1933). A thin film of yeast was allowed to grow on the surface of the cultured medium before the flies were placed in the beakers. Since the medium tended to shrink from the sides of the containers, water was added at frequent intervals. This prevented the females from laying their eggs in the crevices around the edge of the medium. Thus, the eggs were laid on the exposed surface. This assured radiation for every egg. Cellophane, held in place by rubber bands, was used for a covering. In the controls, a glass Petri plate in addition to the cellophane was used to shield the flies from the ultra-violet rays.

Since the temperature was increased by the lamp, a small fan was used which cooled the air around the beakers and kept the temperature down to approximately 27° C., which was 2° C. higher than room temperature.

Parents treated under the lamp and those used for controls were taken from the same stock bottles so that they would be of the same age, parentage, and physiological condition.

The female parents were pure Black-Vestigial-Brown virgins, whereas the males were taken from the Wild stock. The flies were mated, one pair to each beaker, were allowed to recover from etherization for a few hours, and then were placed under the lamp. They remained in the beakers until pupae appeared, then they were removed.

The offspring were collected at eight-hour intervals in order to insure virginity of the females. These females (wild in phenotype and heterozygous for Black-Vestigial-Brown) were backcrossed to males from pure stock and pairs were placed in regular wide-mouth mating bottles containing yeast-treated medium. These parents were allowed to remain in the bottles for four days after which they were removed.

The offspring of this backcross were examined, classified, and counted until eighteen days after mating.

From the data so collected, crossover percentages were computed.

Results

1. **Experiment 8.**—Virgin females homozygous for Black-Vestigial-Brown were mated, in beakers as described above, to males from the pure Wild stock and allowed to recover from etherization for a few hours. These beakers then were placed under the ultra-violet lamp at a distance of 37 inches. The parents were removed as soon as pupae appeared. When offspring emerged, the heterozygous Wild females were backcrossed to homozygous untreated Black-Vestigial-Brown males. The results of these mating are shown in Table I. From these results were calculated the crossover value which are shown in Table II and Table III.

2. **Experiment 9.**—This differed from Experiment 8 in two respects: first, the distance was increased to 47 inches; second, the room temperature during the second generation was about 27° C. instead of 25° C. as in Experiment 8.

The observed results are shown in Table I and the computed results in Table II and Table III.

Combined results of experiments 8 and 9 are shown in the same tables with the uncombined results.

TABLE I.—Emergence Record of Backcrosses by Phenotypes

	Experimentals			Controls		
No. of Experiment	8	9	8+9	8	9	8+9
Wild	3838	751	4589	4073	751	4824
Black-Vestigial-Brown	3558	633	10191	3706	655	4361
Black	691	164	855	530	135	655
Vestigial-Brown	608	224	720	454	101	555
Black-Vestigial	1541	112	1765	1387	260	1647
Brown	1728	338	2066	1548	293	1841
Black-Brown	177	34	211	87	20	107
Vestigial	168	31	199	88	11	99
Totals	12309	2287	14596	11880	2226	14106

TABLE II.—Crossover Percentage Between Black and Vestigial Loci. (Region of Spindle-fiber Attachment)

Exp. No.	Experimentals			Controls			Difference	D
	No. ♀♀ tested	Population	Crossover % B to V	No. ♀♀ tested	Population	Crossover % B to V		P.E. (diff.)
8	58	12309	13.36± .207	70	11880	9.76± .272	3.60± .28	13
9	16	2287	14.91± .445	12	2226	11.99± .46	2.92± .65	4.1
8+9	74	14596	Increase 13.59± .196	82	14106	Increase 10.10± .057	3.49± .20	17.1

Map Distance=18.5 (Morgan 1932).

TABLE III.—Crossover Percentage Between Vestigial and Brown Loci. (Remote Region)

Exp. No.	Experimentals			Controls			Difference	D
	No. ♀♀ tested	Population	Crossover % V to Bw	No. ♀♀ tested	Population	Crossover % V to Bw		P.E. (diff.)
8	58	12309	29.36± .278	70	11880	26.18± .270	3.18± .39	8.22
9	16	2287	27.42± .629	12	2226	26.24± .629	1.18± .89	.132
8+9	74	14596	29.05± .241 Increase	82	14106	26.18± .244 Increase	2.87± .34	8.36

Map Distance=37.5 (Morgan 1932).

Discussion

In nearly every published record there has been reported an increase in crossing-over produced by treatment with X-rays, radium, heat, and ultra-violet light. The increase has occurred largely in the region of the spindle-fiber attachment (the region which is most susceptible to the effects of radiation).

In the sex chromosome, Mavor found that X-rays caused a decrease in the remote region, but he studied no near region in this chromosome. For the second chromosome he agrees with the others. Plough, Schwab, and Stern found that heat caused an increase in the region of the spindle-fiber attachment. In the Purdue Laboratory, an increase in crossover percentage was produced by ultra-violet radiation between the Miniature and Bar loci which is near the region of the spindle-fiber attachment. An increase also occurred in the remote region in treated individuals in this laboratory (unpublished work).

In the third chromosome some X-radiation and heat caused an increase in the near region (the region of greatest susceptibility).

In the second chromosome heat, radium, and X-rays caused an increase of crossing-over in the region of the spindle-fiber attachment. In the Purdue Laboratory experiments have shown a marked effect of ultra-violet radiation on increase in crossing-over.

Data obtained in this experiment indicate a definite increase in crossing-over percentage due to ultra-violet radiation in the second chromosome, the most striking increase being in the region of the spindle-fiber attachment. Statistical analysis made by dividing the difference by its probable error indicates that there is no question of these results being significant, factors of 17 and 8 being obtained whereas most statistical consciences require but 3.

Probably, in regard to modifying crossover values, ultra-violet radiation should be classed in importance with X-rays, radium, and heat.

Summary

The purpose of the experiments was to determine the effects of unfiltered ultra-violet radiation for a complete generation on crossing-over in the second chromosome of *Drosophila melanogaster* and specifically in the two regions limited by the Black, Vestigial, and Brown loci.

1. Definitely greater crossover values were found in the treated individuals than in the controls.

2. There is no doubt of the difference being significant, since it was more than 17 times its probable error in the Black-Vestigial region and over eight times its probable error in the Vestigial-Brown region.

3. These results are in agreement with those observed by investigators working with other kinds of radiation, who found an increase in crossing-over in treated flies, this being greater in regions near the spindle-fiber attachment than in remote regions.

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