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

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Introduction

Crossing-over in the male of *Drosophila melanogaster* formerly was supposed never to occur, but recent investigations have shown that it occasionally happens, particularly upon treatment with x-rays and heat. As yet, however, no literature has been found concerning the effect of ultra-violet light on this process.

The experiments here reported were undertaken to determine the effects upon crossing-over in the male of prolonged radiation with low-intensity ultra-violet light.

Review of Literature

Morgan (1912) found no crossing-over in the males of Drosophila between the black and vestigial loci. He (Morgan 1914) also pointed out that the F_2 generation contains no double recessives when one recessive enters from each P_1 parent. This he considered additional evidence of no crossing-over in the male.

Muller (1916) reported a case of spontaneous crossing-over in which all of the mature germ cells of a single male Drosophila were affected. Thus, all of the backcross offspring showed crossing-over.

Bridges and Morgan (1919) found a single crossover among the offspring of Star speck/purple speck males.

From the backcross of a single male *D. simulans* Sturtevant (1929) found one crossover between scarlet and peach among the 184 offspring.

In the control cultures of a Drosophila experiment by Patterson and Suche (1934) the frequency of crossovers was one to 8,239.

Kikkawa (1935) studied crossing-over in the male of *Drosophila virilis*. He performed an experiment continuous through ten generations and found three exceptional flies in a population of 38,598. He concluded that spontaneous crossing-over does occur in the male of *D. virilis*, though the frequency is very low.

Philip (1935) found the rate of double crossing-over and inverted crossing-over between the X- and Y-chromosome in the male Drosophila to be about one in 3,000.

These cases of crossing-over are the seven exceptions in which crossing-over of a spontaneous nature has been reported in the literature of Drosophila males (Whittinghill, 1937).

Friesen (1933) X-radiated males which were heterozygous for second or third chromosome characters. These were backcrossed, and 22 crossovers were obtained among a progeny of 561. He established the fact that crossing-over in males occurred in the central region of the

chromosome—in the same region where crossing-over is increased by X-radiation of females. He believed that there is an identical mechanism of crossing-over in both sexes.

Patterson and Suche (1934) found 77 crossovers in a population of 8,371 flies after exposure of heterozygous male parents in the larval stage to X-radiation. They concluded that X-radiation induced crossing-over in the male when applied to immature germ cells containing the diploid number of chromosomes.

Shull and Whittinghill (1934), working with heat, produced crossing-over in the male of Drosophila. They found three crossovers in the third chromosome of treated males among a population of 182 flies.

Philip (1934) investigated crossing-over between X- and Y-chromosomes during normal spermatogenesis and obtained three exceptional flies among a population of 997. The probability of mutations in accounting for these exceptions was eliminated by their frequency.

Friesen (1937) in further research on second and third chromosomes of X-radiated males found no effect of stock on crossing-over in males. In studying the effect of suppressors of the inversion type on crossing-over, he concluded that inversions were more feeble suppressors of crossing-over in males than in females.

Whittinghill (1937) induced crossing-over in Drosophila males by a temperature of 35° C. applied during the larval period.

Rifenburgh (1935) obtained crossing-over in the male between the black and vestigial loci following ultra-violet radiation of young larvae.

Methods

The source of the ultra-violet light for these experiments was a General Electric sun lamp (S2 Model K). Matings to be irradiated were made in 100 cc. beakers which were filled nearly to the top with Purdue culture medium (Rifenburgh, 1933). To prevent the medium from shrinking away from the sides of the beakers, water was added each day. Thus, the flies could not lay eggs below the surface of the medium where they might be shielded from the radiation. Both control cultures and experimental cultures were covered with cellophane. In addition, each control culture was shielded with a glass Petri plate presumably impervious to ultra-violet rays. A small electric fan was used to prevent an increase of temperature under the lamp. Room temperature was maintained at approximately 25° C. throughout the experiments.

In the first three experiments, wild-type males were mated to virgin b vg bw females. In the fourth and fifth experiments, wild-type males were mated to al b vg bw females. Parents for both treated matings and control matings were taken from the same stock cultures in order to insure identical heredity as nearly as possible. As soon as pupae appeared, the parents were removed from the beakers. The offspring were irradiated until their emergence from the pupa cases. Emergent flies $\left(\frac{\text{b vg bw}}{+} \text{ or } \frac{\text{al b vg bw}}{+}\right)$ were removed daily from the radiation, and the males backcrossed in ordinary culture bottles (in the room away from the lamp) to females of the recessive type. Each male was

TABLE I.—Emergence Record of Backcrosses (Second and Third Regions)

	Experimentals			Controls Under Lamp					Controls Not Under Lamp		
Number of Experiment.....	II	IV	Total II & IV	I	II	IV	VA	Totals I, II, IV & VA	III	VB	Total III & VB
Parental Types.....	16,010	4,293	20,303	11,514	9,403	5,470	9,228	35,615	23,712	9,179	32,891
Cross-overs b to vg.....	88	58	146	12	31	22	18	83	76	.....	76
Cross-overs vg to bw.....	20	.....	20	1	.....	10	.....	11	.....	.....	.....
Totals.....	16,118	4,351	20,469	11,527	9,434	5,502	9,247	35,710	23,788	9,179	32,967

mated to a group (3 or 4) of virgin females. On the following day each male was removed from the females and transferred to a new group of virgin females. This process was repeated every day with other groups until each male had been mated successively to five groups of females. Each group of females was placed successively in several (3 to 5) culture bottles and allowed to remain three days in each bottle. This procedure resulted in a relatively large number of cultures and an enormous progeny from each tested male.

In Experiment I a distance of 47 inches from the lower end of the bulb to the top of the medium was maintained. Twelve treated cultures and twelve control cultures were used. In starting these cultures, the flies were etherized for convenience in handling, placed in the beakers, and immediately subjected to the ultra-violet rays. Eight emergent F_1 treated males and eight emergent F_1 control males were backcrossed individually to b vg bw females with a total of 400 backcross cultures. However, the experimental cultures were ruined by accident; therefore, only the 200 control cultures are included in the report.

In Experiment II the distance was 52 inches. Eighteen treated cultures and six control cultures were used. Males and females were placed in the beakers and allowed to recover from etherization for eight hours before exposure to radiation. Of the emergent F_1 progeny, 25 treated males and 11 control males were used for back-crossing. There was a total of 430 backcross cultures.

In order to investigate crossing-over of a spontaneous nature, a third series of controls was run with the same procedure and technique as in the controls of Experiment II, except that controls III were not placed under the ultra-violet lamp and were not subject to any radiation from it.

In Experiment IV the character aristaless was added to black vestigial brown, giving more complete coverage of the chromosome. The procedure and technique was the same as in the preceding experiments, except that the distance from bulb to medium was increased to 52½ inches. A total of 256 backcross cultures was obtained by back-crossing thirteen emergent F_1 treated males and ten emergent F_1 control males (heterozygous for aristaless black vestigial brown) to virgin al b vg bw females.

In Experiment V two control populations were obtained. One set (VA) was run under the lamp; the second set (VB) was placed in the room away from the influence of the radiation. Twelve emergent F_1 males were tested from each set, giving a total of 263 backcross cultures.

In all the experiments reported in this paper, a grand total of 105 males were tested in 1,217 backcross cultures. These males produced a population of 89.146 offspring.

Results

Emergence tabulations for the backcross are shown in Table I. Parental and single crossover classes are represented, but there is no instance of double crossing-over.

Cross-over between the aristaless and black loci occurred but once, a single exceptional fly (a black vestigial brown female) being

TABLE II.—The Percentage of Recombination Between Black and Vestigial Loci

Experiment Number	Experimentals			Controls			Difference in %	D / P. E. (diff)
	No. ♂♂ Tested	Population	Recombination % b to vg	No. ♂♂ Tested	Population	Recombination % b to vg		
I				8	11,527	.104 ±.0199		
II	25	16,118	.546±.039	11	9,434	.3286±.0398	.2174±.0557	3.9
III				14	23,788	.3195±.0236		
IV	13	4,351	1.333±.117	10	5,502	.3998±.0483	.9302±.1265	7.35
VA				12	9,247	.195 ±.03095		
VB				12	9,179			
II & IV	38	20,460	.713±.0397					
I, II, IV & VA				41	35,710	.232±.0171	.001±.025	.04
III & VB				26	32,967	.231±.01779		

found among the VA (under the lamp) controls. This one recombination occurred among a total radiated and control population of 28,459.

Percentages of recombination between the black and vestigial *loci* and also between the vestigial and brown loci are indicated in Tables II and III, respectively. Although crossing-over occurred in both regions among controls, there is a significantly higher percentage of recombination between these loci in the radiated cultures. Also, crossing-over is more frequent in the region of the spindle-fiber (between the black and vestigial loci than in either remote region.

Of the 38 treated males, nine (23.7%) showed crossing-over. Five (17%) of the 53 control males showed crossing-over. The total percentage of recombination is given in Table IV. The number of crossovers for each region produced by each of these males is shown in Table V. It is significant that crossovers did not appear in all cultures. Usually, several crossovers were found among the offspring of any one male or else crossing-over was completely absent (only four males of the 24 showing crossing-over produced less than three crossovers each.)

In Table VI the number of crossovers is given according to the age of the male at the time of mating. In the treated cultures and in controls as well, the number of crossovers produced varied with the age of the male, the greatest number appearing as a result of matings when the males were from two to seven days of age.

A total of 337 crossovers were found among the 89,146 backcross offspring. These were distributed as follows: 166 from experimental cultures, 95 from controls under the lamp, and 76 from controls not under the lamp.

Discussion

Crossing-over occurred in both radiated and control males. Treated males produced .811 ± .0423% of recombination in comparison with .246 ± .0127% from the control males. This gives a difference of .565 ± .0442%, which is certainly significant, it being 12.8 times its probable error.

The rate of recombination, not only in the experimental cultures but also in the controls, is high compared with records in the literature. Spontaneous crossing-over in males has been reported before in chance findings (Muller, 1916, Bridges and Morgan, 1919, Sturtevant, 1929) and also in researches directed at this possibility. The frequency was one in 3,000 (Philip, 1935), one in 8,239 (Patterson and Suche, 1934), and in another species, *D. virilis*, three in 38,598 (Kikkawa, 1933). These records are much lower than the frequency found in the work reported in this paper, which was 337 in 89,146 or about one in 264. The controls gave one in 404, and the radiated males produced one crossover for each 123 offspring.

Crossing-over in the left end of the second chromosome is so rare (one in 28,279) that it almost never occurs; nevertheless, the one instance shows that it is not impossible here. It is not known, however, just how far from the central region it happened since there was no marker between aristaless and black.

TABLE III.—The Percentage of Recombination Between Vestigial and Brown Loci

Experiment No.	Experimentals			Controls			Difference in %	D / P. E. (diff)
	No ♂♂ Tested	Population	Recombination % vg to bw	No. ♂♂ Tested	Population	Recombination % vg to bw		
I				8	11,527	.0087 ± .00586		
II	25	16,118	.1241 ± .0187	11	9,434			
III				14	23,788			
IV	13	4,351		10	5,502	.182 ± .03876		
VA				12	9,247			
VB				12	9,179			
II & IV	38	20,469	.0977 ± .01473					
I, II, IV & VA				41	35,710	.0308 ± .00623	.0308 ± .0062	4.9
III & VB				26	32,967			

TABLE IV.—Total Percentage of Recombination

	Experimentals	Controls	Difference	$\frac{D}{P.\ E.\ (diff)}$
Number of Males Tested	38	86	.....	.....
Population	20,469	69,030	.....	.....
Recombination % al to b	.....	.0108 ± .00728	.....	.....
Recombination % b to vg	.713 ± .0397	.2303 ± .01228	.4827 ± .0416	11.6
Recombination % vg to bw	.0977 ± .01473	.0159 ± .00318	.0818 ± .0151	5.42
Total % of Recombination	.811 ± .0423	.246 ± .0127	.565 ± .0442	12.8

Grand Total % of Recombination, Experimentals and Controls Combined, .378 ± .0138

TABLE V.—Crossovers by Males According to Region of Chromosome

al to b—1 b to vg—2 vg to bw—3

Male Number	Experimentals										Controls Under Lamp									Controls Not Under Lamp							
	Exp. II								Exp. IV	Total Exp.	Exp. I			Exp. II		Exp. IV		Exp. VA		Total	Exp. III						Total
Region Number	2X	4X	7X	10X	14X	15X	16X	18X	10X		4C	6C	10C	2C	5C	5C	10C	2XC	12XC		1C	2C	7C	11C	12C	13C	
1	....	....	....	....	....	....	....	....	.....	.....	....	....	....	....	....	....	....	....	1	1	....	....	....	....	....	....	.....
2	1	3	16	6	22	11	20	9	58	146	3	3	6	11	20	....	22	18	.....	83	7	1	10	16	41	1	76
3	....	7	6	....	....	2	4	1	.....	20	....	....	1	....	....	10	....	....	.....	11	....	....	....	....	....	....	.....
Totals	1	10	22	6	22	13	24	10	58	166	3	3	7	11	20	10	22	18	1	95	7	1	10	16	41	1	76

Total Population Involved.....20,469.....35,710.....32,967

Grand Total of All Crossovers..... 337

Grand Total Population.....89,146

Crossing-over occurred most frequently in the control region between the black and vestigial loci which includes the spindle-fiber attachment. More than nine-tenths of the crossing-over occurred in this region, and less than one-tenth in the region toward the right end of the chromosome (vestigial to brown), which covers a map distance about twice as great as the inclusive region.

In several males, crossing-over was not limited to a single region. With one exception (male 12XC), it never occurred in a remote region unless it also occurred in the inclusive region. Among males showing crossing-over in two regions, the frequency was higher for each male in the inclusive region with one exception (male 4X). These results agree with those of Whittinghill (1937) and Friesen (1933), who found most of the crossing-over in the inclusive region. However, occurrence in several males of crossing-over in two regions differs from the results of these two investigators who found it to be localized in one region only. It seems that Whittinghill was not justified in concluding that crossing-over is limited to only one region of the chromosome in any male. Perhaps he would not have reached such a conclusion had not the population in his experiments been so small (some 3,000 flies.)

The region most susceptible to modification of frequency rate (due to ultra-violet radiation) was a region (vg to bw) remote from the spindle-fiber attachment. The rate of crossing-over was increased more than six times in this region after treatment with ultra-violet light, whereas the rate was only slightly more than tripled in the inclusive region.

In regard to the two types of controls—*i.e.*, under the lamp or not under the lamp—the rate for the first region was almost identical (.232 ± .071% and .231 ± .0178%), but for the right limb of the chromosome, it was significantly different (.0308 ± .0062% and zero). However, in view of the fact that there was no significant change in the inclusive region, it is suggested that perhaps the cause of the difference may not be the permeability of the glass cover to radiation but some other factor such as genes, or effect of stock, as it is sometimes called.

Effect of stock is suggested also by the fact that only certain males showed crossing-over (24 out of 105 tested), by the variation in number of exceptional offspring from such males (1 to 58), and by the rather large average number of such exceptional offspring per male (14).

The results of Friesen's work (1937) show no effect of stock, but here again the data seem insufficient owing to small populations.

Crossing-over in the male may be much the same process as in the female since ultra-violet radiation increases its rate in both sexes (Rifenburgh, 1935, as well as experiments here reported). However, its relative rate in the various parts of the chromosome differs in the sexes since in the male it is grouped around the spindle-fiber attachment—at least in the second chromosome.

Summary

1. More crossing-over occurred in radiated individuals than among controls. The difference was statistically significant, it being nearly 13 times its probable error.

TABLE VI.—Crossovers by Males According to Age at Mating

Male Number	Experimentals										Controls Under Lamp										Controls Not Under Lamp						
	Exp. II								Exp. IV	Total	Exp. I			Exp. II		Exp. IV		Exp. VA		Total	Exp. III						Total
Age at Mating	2X	4X	7X	10X	14X	15X	16X	18X	10X		4C	6C	10C	2C	5C	5C	10C	2XC	12XC		1C	2C	7C	11C	12C	13C	
1 Day							4			4	1	2			2			2		7							
2 Days		1	11		2	4	6	9		33	1	1	4	11	4		20	1		42				4	5		9
3 Days	1	8	9	6	16	1	14	1		56	1		1		9	1		1	1	14				12	13		25
4 Days		1	2		4				10	17			1		5	2				8	1				17	1	19
5 Days						8			8	16			1					4		5	3						3
6 Days									19	19							2			2	3	1	1				5
7 Days									12	12						4		5		9							
8 Days									9	9								5		5							
9 Days																											
10 Days																3				3					6		6
11 Days																							9				9
Totals	1	10	22	6	22	13	24	10	58	166	3	3	7	11	20	10	22	18	1	95	7	1	10	16	41	1	76

Grand Total of All Crossovers 337

2. In most males, crossing-over was limited to the region including the spindle-fiber attachment.
3. In several males, crossing-over occurred in two regions.
4. Modification of crossover frequency was highest in a region of the chromosome remote from the spindle-fiber attachment.
5. Crossing-over in the second chromosome of the male differs from that in the female in respect to relative rates in different regions.
6. These results agree with those of certain other investigators who, working with various types of radiation, found crossing-over in the male concentrated in the region of the spindle-fiber attachment.
7. These results do not agree with those of certain other investigators who found crossing-over in a given male to occur in one region only.
8. These results disagree with those which led certain investigators to believe that crossover frequency in the male is more easily modified in the region including the spindle-fiber attachment than in remote regions.
9. Effect of stock is suggested as a probable factor in frequency of crossing-over.

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