CROSSING OVER INDUCED BY X-RAYS IN DROSOPHILA MALES

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INTRODUCTION

It has long been known that crossing over does not take place in the male of *Drosophila*. In attempts to explain this fact several different suggestions have been made, among them the failure of synapsis to occur in the male germ cell. Dr. BESSIE LEAGUE (unpublished paper) has since shown cytologically that synapsis does occur in the male, but the process takes place rapidly and is easily overlooked. Although generally crossing over does not take place in the heterozygous sex, yet it does occur in both sexes in several different species, notably in the locust, a crustacean, and the mouse. More than a year ago the senior author obtained, in a preliminary experiment, evidence that crossing over could be induced in the male germ cell of *Drosophila melanogaster* by means of X-radiation. Since then the experiments have been extended, and it is the object of this paper to give the evidence upon which this conclusion was based. The junior author carried out all of the linkage tests on the stocks thus derived from induced crossing over. We wish to acknowledge our indebtedness to Mr. WILSON STONE for valuable help in connection with the linkage studies.

In order to produce an exchange of segments between homologous chromosomes, it is necessary to treat male larval stages which are known to contain immature germ cells. If the larvae are made heterozygous for a series of recessive mutant genes in any one of the pairs of autosomes, an induced exchange of segments between the homologues of this pair could be detected among the offspring arising from the backcross. In case the exchange involved segments of equal length of the two homologous elements, and produced viable zygotes, the result would be equivalent to *induced crossing over*.

The long III chromosome was chosen for the test. The experiments were initiated by crossing normal females to rucuca/Moiré-claret males, and then mating individually the F₁ non-Moiré males from the treated larvae to rucuca/Moiré females. The rucuca stock carries eight recessive genes in the III chromosome (*ru h th st cu sr e^s ca*). On account of the poor viability of homozygous flies, the rucuca stock is carried in heterozygous form, balanced to a III chromosome, known as Moiré, which has a gene rearrangement that prevents crossing over except at the extreme right end,

or the region of claret, where a slight amount of crossing over occurs. Flies carrying the Moiré chromosome, except when homozygous for claret, exhibit the dominant variegated eye-color (GLASS 1932), and can thus be distinguished from those not having this chromosome.

A few normal crossovers (between sooty and claret) will occur in the females used in the backcross, giving claret and *ru h th st cu sr e*^s. The F₂ claret flies can readily be tested to determine whether the presence of claret has been the result of a normal crossover or of an induced change. If a claret fly, when mated to wild-type, gives Moiré individuals among its offspring, this would indicate that it arose from a normal crossover, because in that event the fly must have received from its mother a III chromosome carrying the gene for variegated eye-color, which lies in the left arm of the Moiré chromosome. If, however, the tested F₂ claret fly yielded no Moiré progeny we would then know that the presence of claret was due to some effect produced by the X-radiation—an induced crossover, a point mutation, or, possibly, a deficiency. We have tested all of the F₂ claret flies obtained in the control and experimental series by this method.

In conducting the experiments, the flies were allowed to lay eggs for twelve hours on food contained in paper spoons placed in one by four inch culture vials. At the end of each twelve-hour period the spoons were removed and new ones with fresh food were introduced. The cultures were then allowed to develop until the larvae had reached a stage at which it was desired to give the X-ray treatments. Each culture was divided, one half being treated and the other half being used as a control. The flies and developing cultures were kept in an air-cooled room in which a temperature of 76 degrees Fahrenheit was maintained.

EXPERIMENTAL RESULTS

The exposures were given at four different stages of development namely, 24 to 36 hours, 40 to 52 hours, 60 to 72 hours, and 72 to 84 hours. All treated cultures were given a dosage of 1325 r units. We have included the results obtained from all the experiments and their controls in table 1. Only the F₂ flies in which the induced variation could be detected are listed in the table. The Moiré flies were disregarded.

The figures show that twenty-three classes of flies were obtained, and that the total number of flies in the experimental series was 8371 while the control series gave 8329 flies. The first and second classes constitute the expected non-variant wild-type and rucuca flies, respectively. The third class includes the normal crossover arising from the rucuca-Moiré females used in the backcross, while the fourth class includes the reciprocal crossovers arising from the same type of female. The flies belonging to this class were determined by testing all claret flies to wild-type, as was suggested

above. The remaining classes, fifth to twenty-third, include all other variants, and, with one exception (one *ru h th st* fly of the control series), represent variations induced by the X-radiation. The number of variants for each of the four stages at which the exposures were given is as follows: five for the I-stage, twenty-six for the II-stage, twenty-seven for the III-stage, and nineteen for the IV stage. This gives percentages of 0.27, 1.65, 1.03, and 0.81, respectively, for the four stages.

The highest rate occurred in larvae treated at forty to fifty-two hours, while the lowest occurred in larvae treated at twenty-four to thirty-six hours, the other two stages showing intermediate rates. We take this to mean that X-radiation is most effective in cells that are in the growth stages of the germ-cell cycle. Dr. LEAGUE has shown that at twenty-four hours old the larval testis contains chiefly spermatogonia, with only slight indications of growth stages. In the testes of thirty-six hour larvae growth stages are more noticeable. In older larvae mid-growth stages are found, and, finally, in larvae as much as eighty-four hours old well-developed primary spermatocytes are present. We thus see that the highest rate produced by the X-rays takes place in larvae containing the largest number of germ cells in the early growth stage. This corresponds to the stage in the female germ cell in which normal crossing over is supposed to take place (PLOUGH 1917). The fact that a few variants were produced in the youngest treated stages (twenty-four to thirty-six hours) probably means nothing more than that such variations were induced in the oldest larvae in the culture, that is, in larvae that had reached, or nearly reached, the thirty-six hour stage. The continuous development of spermatozoa in *Drosophila* will account for the fact that treating larvae that had passed the peak of early growth stages, results in the production of fewer variants of the type with which this paper is concerned.

Genetic studies show that many of the induced variations listed in the table were the result of an exchange of segments between the homologues of the III chromosome. The evidence further shows that in some of the cases the two chromosomes must have been matched gene by gene at the time the exchange was induced. In this sense the exchange so closely simulated normal crossing over in the female germ cell as to warrant its designation by the term induced crossing over.

Table 1 likewise shows that the majority of crossovers occurred between the loci of *st* and *cu* and in the two adjacent regions. This is in accordance with experimental data indicating that the middle of the III chromosome, or the spindle fiber region, is most susceptible to X-ray treatment (MULLER 1925). It also corresponds to the region most susceptible to heat (PLOUGH 1921), and to triploidy (REDFIELD 1930).

Of the 77 variants caused by X-radiation, 18 were either sterile or died

before breeding tests were completed. Forty-six of the 59 fertile cases were bred long enough to determine that 37 of these were viable in homozygous form and 9 non-viable. The location of the lethal was determined in six cases, and will be discussed later. Seven of the non-viable cases represent variants that involve a single marked locus. These are similar to cases ob-

TABLE 1

CLASSES	AGE IN HOURS AT WHICH TREATMENTS WERE GIVEN				CONTROLS
	I 24 TO 36	II 40 TO 52	III 60 TO 72	IV 72 TO 74	
1. Wild-type	1386	1217	1906	1926	6321
2. rucua	446	330	681	374	1993
3. <i>ru h th st cu sr e*</i>	4	1	1	2	7
4. <i>ca</i> (normal crossovers)	10	0	7	3	7
5. <i>ca</i> (induced change)	2	1	8	1	0
6. <i>ru th st cu sr e* ca</i>	0	0	1	0	0
7. <i>ru h th st sr e* ca</i>	0	0	1	1	0
8. <i>ru</i>	0	1	1	2	0
9. <i>th</i>	0	0	1	0	0
10. <i>st</i>	0	1	1	0	0
11. <i>cu</i>	0	1	0	0	0
12. <i>sr</i>	0	1	0	0	0
13. <i>ru ca</i>	0	2	1	0	0
14. <i>th st</i>	0	0	0	1	0
15. <i>ru h</i>	0	1	0	2	0
16. <i>ru h th</i>	0	0	2	0	0
17. <i>ru h th st</i>	1	10	10	5	1
18. <i>st cu sr e* ca</i>	0	0	0	1	0
19. <i>cu sr e* ca</i>	0	3	1	2	0
20. <i>ru h th st cu</i>	1	0	0	1	0
21. <i>sr e* ca</i>	1	3	0	3	0
22. <i>e* ca</i>	0	1	0	0	0
23. <i>ru h th st cu sr</i>	0	1	0	0	0
Totals	1851	1574	2622	2324	8329

tained by X-raying the X chromosome, and like them, probably represent small deficiencies, except in cases where the lethal proved to be away from the marked region (PATTERSON 1932). It is significant that in the majority of cases from variants showing two or more of the mutant characters, flies homozygous for the affected region are viable. This shows that the induced variation was not the result of a deficiency caused by loss of a piece of the treated chromosome.

The stocks established from variant flies were kept in heterozygous form, balanced to *Mo ca* or *ru h Dcx*. Thirty-seven such stocks were developed. In case the variant fly was a male, a single stock was established; if the variant was a female, three lines of the same stock were established

from her sons. This was done as a precaution against the possibility that normal crossing over in the female might produce a son in which the affected region had been crossed out.

It is of the greatest importance to know whether or not the point, or points, at which the induced exchange of segments occurred involved a demonstrable chromosome abnormality (duplication or deficiency). This can be determined by crossover tests, which are described in the next section.

CROSSOVER TESTS

Of the thirty-seven stocks which were built up from variant flies, twenty were tested to determine if crossing over was normal. These stocks represented crossovers in each region of the rucuca chromosome. With the exception of region 6, each region was represented by two or more stocks. Since the object of the crossover experiments was to determine if the break in the chromosome which had been responsible for a variant fly was accompanied by any abnormality, crossovers in the backcross were counted only in the region of the break. However, a preliminary count was made in every stock to determine if crossing over occurred in each marked region.

The procedure in each case was the same. Stock virgins were crossed to males of a suitable composition to produce heterozygous females with the two mutants adjacent to the break in opposite chromosomes. For stocks having *ru* only, or *ru* and *ca*, "hes" (*h th st p cu sr e**) was used in the backcross. For the other cases special stocks were built up from males having two or more mutant genes adjacent to the break in the stock being tested. These males were crossover flies obtained from the control crossover experiment, and were balanced to *Mo ca*/rucuca, or *ru h Dcx*/rucuca. Because of an inversion, the Dichaete chromosome crosses over only occasionally in the *ru h* region, and even less in the region of *ca*. Like Moiré, homozygous Dichaete is lethal. Homozygous males and females of all stocks were used so far as possible to decrease the number of *Mo* and *D* flies, which had to be discarded. The phenotypically normal F_1 females were selected and kept 3 to 5 days to make sure all were virgin. They were then backcrossed to *Mo ca*/rucuca or *ru h Dcx*/rucuca males with ten pairs to a yeast bottle. At the end of six days the flies were transferred to a second yeast bottle. The *Mo* and *ru h Dcx* F_2 flies were discarded, and the remainder classified. The reciprocal classes of crossover flies were counted in separate columns in all cases. In approximately half the cases the reciprocal classes of non-crossovers were likewise counted separately. In a number of cases, the flies hatching during the second six-day period were kept separate from those of the first period to determine the difference in crossing over, if any.

Six of the stocks tested did not live homozygous. To locate the lethals, males with all possible combinations of mutant genes were selected during

the crossover experiments and crossed to *Mo ca*/rucuca or *ru h Dcx*/rucuca females. The males carrying the lethal chromosome over *Mo ca* or *ru h Dcx* were then backcrossed to females of the original stock, as will be described later in more detail.

For the control, wild females were crossed to *Mo ca*/rucuca males and the non *Mo* F_1 females backcrossed to *ru h Dcx*/rucuca males. To reduce the possibility of error in the *ca* region and to eliminate the Dichaete flies, an attempt was made to backcross the heterozygous females to homozygous rucuca males. No larvae were produced in this cross, even though a trial bottle of homozygous rucuca males and females showed larvae which, however, died on the fourth day.

When it became apparent that crossing over values in the middle of the chromosome were unusually low because of viability differences, a new control was set up with females having *ru h th st* in one chromosome and *cu sr e* in the other. The set up of this control was more nearly like that of the experiments. The F_1 females were crossed to *ru h Dcx*/rucuca as before. The results given in table 2 are from this control, with the exception of the *e⁺ ca* region, the data for which are from the first control. The crossing over percentages were essentially the same at the ends of the chromosome in both controls; in the middle, around *th*, *st* and *cu*, the second control gave a more accurate percentage of crossing over, while the intermediate regions varied only slightly.

Crossing over in region 1, ru-h

Region 1 was represented by three single crossover stocks, 513 and 1141, developed from males, and 1346 developed from a female; and by two double crossover stocks, both derived from females. All stocks were *ru*. Virgin females were crossed to "hes" males and the F_1 females back crossed to *Mo ca*/rucuca.

For stock 513, 841 crossover flies were obtained in a total of 3129. The percent of crossing over was 26.6, which is 0.6 percent more than the control. The variation is less than the error of difference, which is 0.75 percent. In stock 1141, the total number of flies was 3656. Of these, 879 were crossovers, giving a crossover percent of 24.0. The 2.0 percent variation from the control is less than three times the error of difference, which is 0.71 percent. The total number of flies obtained from stock 1346 was 3687. The crossovers numbered 923, giving a crossover percent of 25.0. The variation from the control is 1.0 percent, which is less than twice the 0.71 percent error of difference. These figures are the combined data for two separate stocks of 1346 derived from sons of the variant female. The total number of flies counted for stock 539 was 4784. The number of crossovers in region 1 was 1194, giving a crossover percent of 25.0. The variation from

the control is 1.0 percent, which is less than twice the 0.67 percent error of difference. The percent of crossing over for the two stocks of 539, which when combined gave the results shown here, differed only 0.1 percent from each other. For stock 588, 7628 flies were counted. Of these, 1893 were crossovers in region 1. The percent of crossing over is 24.8, which is 1.2 percent less than the control. This variation is less than twice the error of

TABLE 2
Crossing over in the different regions.

STOCK	REGION	TOTAL	CROSSEOVERS	PERCENT	CONTROL PERCENT	E.D.	DIFFERENCE
513, <i>ru</i>	1, <i>ru-h</i>	3129	841	26.6		.75	0.6
1141 "	"	3656	879	24.0	26.0	.71	2.0
1346 "	"	3687	923	25.0		.715	1.0
1309, <i>ru h</i>	2, <i>h-th</i>	3339	547	16.4		.63	1.5
1239 "	"	2767	525	18.9	17.9	.68	1.0
294 "	"	2498	491	19.7		.699	1.8
94, <i>ru h th</i>	3, <i>th-st</i>	3434	17	0.496		.107	.116
127 "	"	2041	12	0.575	0.38	.130	.155
1182 <i>st cu sr e⁺ ca</i>	"	7456	32	0.428		.077	.048
578, <i>ru h th st</i>	4, <i>st-cu</i>	2937	103	3.51		.339	0.25
103 "	"	2479	151	6.10		.333	2.84
545 "	"	2624	87	3.31	3.26	.399	0.05
274, <i>cu sr e⁺ ca</i>	"	4351	200	4.60		.296	1.34
1388 "	"	6867	256	3.78		.270	0.52
521, <i>sr e⁺ ca</i>	5, <i>cu-sr</i>	1676	186	11.1		.630	0.94
1199 "	"	2988	356	11.9	10.2	.540	1.70
1449 <i>ru h th st cu</i>	"	2105	256	12.1		.600	1.94
283, <i>e⁺ ca</i>	6, <i>sr-e⁺</i>	5096	436	8.6	9.16	.44	.56
539, <i>ru-ca</i>	1 and 7	4784	R 1,1194	25.0	26.0	.670	1.0
	<i>ru-h-e⁺ca</i>		R 7,1648	34.4	33.6	.634	0.8
588 "	"	7628	R 1,1893	24.8	26.0	.625	1.2
			R 7,2617	34.2	33.6	.565	0.6

difference, which is 0.625 percent. This represents the combined data for the three stocks of 588 which were derived from sons of the original variant female and tested separately. Only one case, which gave a crossover percent of 23.1, differed significantly from the control, and even this case is no longer significant when the data for the three stocks of 588 are combined. Crossing over in region 1 is thus normal in all experimental stocks which were tested.

Crossing over in region 2, h-th

Region 2, between *h* and *th*, was represented by three stocks, one derived from a male and two from females. All stocks were *ru h*, and were crossed to *th st cu* males.

For stock 1309, 3339 flies were counted. The total number of crossovers was 547, giving a crossover percent of 16.4. This is 1.5 percent less than the control, but the variation is less than three times the error of difference, which is 0.63 percent. The total number of flies counted for stock 1239 was 2767. The crossover percent calculated from 525 crossover flies was 18.9. This is 1.0 percent higher than the control. The variation is less than twice the error of difference, which is 0.68 percent. These data are the combined results of two separate stocks derived from sons of the original variant female. For stock 294, 2498 flies were obtained. Of these, 491 were crossovers, giving a crossover percent of 19.7. This is 1.8 percent more than the control, but the variation is less than three times the 0.69 percent error of difference. Although the values for the stocks tested for region 2 differ considerably and are all higher than the standard, they do not vary significantly from each other nor from the control. It is possible that the original crossover from which each of these stocks was derived occurred at a different locus between *h* and *th*.

Crossing over in region 3, th-st

All stocks tested for region 3 were derived from females. Two, stocks 94 and 127, were *ru h th* and were crossed to *st cu* males. Stock 1182 was *st cu sr e^s ca* and was crossed to *h th* males. F_1 females of both classes were backcrossed to *ru h Dcx/rucu*.

For stock 94, 3434 flies were counted. Of these, seventeen were crossovers giving a crossover percent of 0.496. This is 0.116 percent higher than the control, but the variation is less than twice the error of difference, which is 0.107 percent. For stock 127 the total number of flies was 2041. Twelve crossovers gave a crossover percent of 0.575, which is 0.155 percent higher than the control. The variation is less than twice the error of difference, which is 0.13 percent. The total number of flies obtained from stock 1182 was 7456. Thirty-two crossovers gave a crossover percent of 0.428, which is 0.048 percent higher than the control. This variation is less than the error of difference, which is 0.077 percent. The figures for stock 1182 represent the results obtained from three separate stocks derived from sons of the variant female. All stocks tested for crossing over in region 3 gave values higher than the control but none varied significantly.

Crossing over in Region 4, st-cu

Both contrary classes were represented in the stocks tested for region 4.

Three were *ru h th st*, and two were *cu sr e⁺ ca*. All except stock 274 were derived from females.

For stock 578, *ru h th st*, 2937 flies were counted. Of these, 103 were crossovers, giving a crossover percent of 3.51. The 0.25 percent variation from the control is less than the error of difference, which is 0.339 percent. The total number of flies counted for stock 103, *ru h th st*, was 2479. The percent of crossing over calculated from 151 crossover flies was 6.1, which is 2.84 percent more than the control. Since this is more than three times the error of difference, which is 0.333 percent, the variation is significant. For stock 545, *ru h th st*, 2624 flies were counted. Eighty-seven of these were crossovers, giving a crossover percent of 3.31. The 0.05 percent variation from the control is less than the error of difference, which is 0.399 percent. For stock 274, *cu sr e⁺ ca*, 4351 flies were counted. The number of crossovers obtained was 200, giving a crossover percent of 4.6. The variation from the control is 1.34 percent, which is more than three times the error of difference, which is 0.296 percent. A total of 6867 flies was counted for stock 1388, *cu sr e⁺ ca*. The number of crossovers was 256, giving a crossover percent of 3.78. The 0.52 percent actual difference from the control is less than twice the error of difference, which is 0.27 percent. These data represent the combined figures of three stocks of 1388. Two of the above cases proved to be significantly higher than the control. It is possible that these stocks, 103 and 274, were not derived from true crossovers but were the result of an exchange of segments which were not wholly homologous. Stock 103 is the only case in which crossing over for region 4 was as high as the standard. The 4.6 percent crossover value for stock 274 shows that crossing over in this stock is still subject to the influence which reduces crossing over in the middle of the rucuca chromosome.

Crossing over in region 5, cu-sr

Region 5 was represented by both contrary classes of a crossover between *cu* and *sr*. Stocks 521 and 1449 were derived from males and stock 1199 from a female. The *sr e⁺ ca* stocks were crossed to *st cu* males and the *ru h th st cu* stock to *sr e⁺ ca* males. The F₁ females of both classes were backcrossed to *ru h Dcx/rucuca*.

For stock 521, *sr e⁺ ca*, 1676 flies were counted. Of these, 186 were crossovers, giving a crossover percent of 11.1. This is 0.94 percent higher than the control. The variation is less than twice the error of difference, which is 0.63 percent. The total number of flies obtained from the two stocks of 1199 was 2988. Of these, 356 were crossovers, giving a crossover percent of 11.9. This is 1.7 percent higher than the control, and varies slightly more than three times the error of difference, which is 0.54 percent. For stock 1449, *ru h th st cu*, 2105 flies were counted. The number of crossovers was

256, giving a crossover percent of 12.14. This is 1.94 percent more than the control, and varies more than three times the error of difference, which is 0.6 percent.

All three cases had higher rates of crossing over than the control, the crossover percent of two of them proving to be significantly higher. Crossing over in region 5 of the control may be slightly reduced because of the same factor which reduces crossing over in regions 3 and 4. In this case the inhibiting effect may have been eliminated in these crossover stocks, thus permitting normal crossing over. Or, owing to the difficulty of distinguishing *sr*, *sr e^s*, or *e^s* alone in an unselected rucuca stock, the crossover values for regions 5 and 6 of the control may not represent the true crossover ratio between the two regions. It is possible, judging from the close agreement of the three experimental stocks with each other and with the standard, that the results of these experiments are more accurate than those of the control. On this assumption crossing over in these stocks may be considered as perfectly normal.

Crossing over in region 6, sr-e^s

Region 6 was represented by only one stock which was derived from a female. Homozygous *e^s ca* flies were crossed to *cu sr* males and the F₁ females backcrossed to *ru h Dcx/rucuca*. The total number of flies counted was 5096. The crossovers numbered 436, giving a crossover percent of 8.6. The variation from the control is 0.56 percent, which is less than twice the 0.44 percent error of difference. These data are the combined results obtained from three separate stocks derived from the original female. All stocks gave practically the same amount of crossing over. Although the percent of crossing over does not vary significantly from the control, it is higher and agrees with the standard. The sooty in this stock was very dark and non-crossovers and crossovers between *sr* and *e^s* were easily distinguished. For this reason, the experimental figures for region 6, as suggested above for region 5, may be less subject to personal error than the same region in the control.

Crossing over in region 7

Region 7 was represented by two double crossover stocks, both derived from female. Stock females, *ru-ca*, were crossed to "hes" males and the F₁ females backcrossed to *ru h Dcx/rucuca*. The data for crossing over between *ru* and *h* have been given above in the results for region 1.

In stock 539, 4784 flies were obtained. Of these, 1648 were crossovers in region 7. This gave a crossover percent of 34.4, which is 0.8 percent higher than the control. The variation is less than twice the error of difference which is 0.634 percent. For stock 588, 7628 flies were counted. The cross-

overs in region 7 numbered 2617, giving 34.2 percent of crossing over. This is 0.6 percent higher than the control. The variation is less than twice the error of difference, which is 0.565 percent.

The data for region 7 are the combined results of two stocks of 539, and of three stocks of 588. There was no appreciable difference in crossing over in any of the five stocks, which were tested separately. The percent of crossing over for all was slightly higher than the control, but not significantly so. The fact that crossing over in both region 1 and region 7 was normal is evidence that the stocks were derived from true crossovers.

Location of Lethals

Of the twenty stocks which were tested for linkage of characters, six failed to live in the homozygous state. The locus of the lethal was determined by breeding males obtained from the linkage experiments. Males produced by crossing over in one or more of the marked regions were crossed to *Mo ca*/rucuca or *ru h Dcx*/rucuca. F_1 males carrying the lethal chromosome over *Mo ca* or *ruh Dcx* were then backcrossed to females of the original stock. The appearance among the offspring of the backcross of phenotypically normal flies, or of flies showing one or more characters of the lethal-bearing stock, indicated that the lethal had been crossed out. The production of *Mo* or *D* flies exclusively showed that the lethal was still present.

Tests of crossover males obtained from stock 1141, *ru*, showed that flies carrying any part of the original stock chromosome except the region of *sr e*^a produced viable zygotes when backcrossed. The lethal is therefore to the right of *sr*. The lethal in stock 1346, *ru*, was located between *cu* and *sr* in both stocks derived from the variant female. The lethal in stock 294, *ru h*, was located between the loci of *h* and *th*, perhaps at the point of breakage or crossing over. The lethal in stock 94, *ru h th*, was located between *th* and *st*, likewise at or near the point of crossing over. The lethal in the three stocks of 588 was located at the right of *th* and left of *cu* in the region of *st*. It was therefore associated with neither point of crossing over. The lethal in stock 1449, *ru h th st cu*, proved to be in the region of claret. These tests show that in four cases the lethal was not at the point of crossing over in the chromosome which determined the composition of the variant fly.¹ The agreement of the location of the lethals in both stocks of 1346 and in the three stocks of 588 shows that the sons from which the stocks were established carried identical chromosomes and that crossing over data can well be combined.

¹ Even in the two cases where the lethal was associated with the point of breakage, normal crossing over in the experiments indicates that the lethal effect was not due to a pronounced chromosomal abnormality.

The unexpected variant fly found in the control is of interest, because the case seems to represent a normal crossover occurring in the male germ cell. This variant was a *ru h th st* female that appeared in one of the F_2 control cultures (table 1). Tests were made and it was found that stocks developed from her sons were viable in the homozygous condition, exactly like those from similar variants obtained in the experimental series. No stocks of the composition of this female were present in the laboratory at this time, and the care with which the experiments were conducted would exclude the possibility of a "slip" in technique. It must, therefore, represent a very rare case of normal crossing over in the male.

DISCUSSION

The important point with reference to the experimental results described above is how the X-radiation has brought about the evident exchange of segments between the homologous chromosomes. There are two possible ways by which this may have been accomplished: (1) It is conceivable that X-radiation in some way modifies the inhibitory mechanism which prevents crossing over in the male germ cell; or (2) the exchange of segments between the homologues is brought about in the same manner as those producing mutual translocations between non-homologous chromosomes when mature germ cells are treated.

In this connection, it may be well to point out that the work on segmental interchange in *Oenothera* and *Datura* (CLELAND 1923, BELLING and BLAKESLEE 1926, DARLINGTON 1929) and on induced translocations in *Drosophila* (MULLER 1930, STURTEVANT and DOBZHANSKY 1930, BOLEN 1931, GLASS 1932, OLIVER and VAN ATTA 1933) has indicated that most translocations consist of an exchange of segments between two non-homologous chromosomes. Most of the reported cases in *Drosophila* have been obtained by treating mature germ cells, which contain the haploid number of chromosomes. Any exchange of segments would, therefore, have to occur between non-homologous elements; there would be no opportunity for the exchange of segments to take place between homologous chromosomes. In treating the male larval stages the opportunity for an exchange to occur between homologues is present, and such exchanges might be of the same general nature as those occurring in the case of mutual translocations.

It has been found, however, that a large majority of translocations in *Drosophila* are lethal in the homozygous condition. Miss SARAH C. BEDICHEK of this laboratory has recently completed a study of translocations between the II and III chromosomes in stocks developed by Mr. WILSON STONE. She found that out of 120 tested stocks, only nineteen, or less than sixteen percent, were viable when homozygous for the transloca-

tion. The lethal character of so many of these translocations when homozygous is not fully understood, but in most cases it is in some way related to the break in the chromosomes.

In contrast to these conditions found in translocations, the induced crossover stocks show a relatively high percentage of viability in homozygous; about eighty percent. Furthermore, genetic studies showed that in only two out of the six tested stocks that were non-viable in homozygous, was the lethal factor located at or near the point of breakage. It is evident, therefore, that a large majority of the induced crossover cases represent a phenomenon entirely different from that underlying ordinary mutual translocations.

This conclusion is strongly supported by the facts brought out in the crossover tests made by the junior author. A large majority of the tested stocks showed normal crossing over in the regions where the induced exchange of segments occurred. With two exceptions, such variations from the control and standard crossover values as were observed could be explained on the basis of differential viability. The two exceptions (Nos. 103, 274) gave values significantly higher than those of the control, suggesting that they were the result of an exchange of segments in homologues that were not entirely matched. In no case was crossing over significantly lower for an affected region. All of the evidence shows that the majority of variants produced by X-radiation must have arisen through an exchange of segments while the homologous chromosomes were matched gene by gene, and this would be equivalent to normal crossing over.

Exactly how X-radiation brings about this effect is not known. But, if it is assumed that one effect of X-rays is to retard mitosis, a suggested explanation of induced crossing over in *Drosophila* males can be offered. Dr. LEAGUE's observations show that synapsis actually occurs during the growth period of the primary spermatocyte, but that it represents only a fleeting phase of the maturation process, too rapid, perhaps, to allow for crossing over to take place. It may well be that the retarding effect of X-radiation on this process furnishes the necessary opportunity for crossing over to occur.

SUMMARY

1. The paper describes a series of experiments designed to induce by X-radiation crossing over in *Drosophila* males. To accomplish this, male larvae, heterozygous for a series of eight mutant genes in the III chromosome, were X-rayed and crossed to females having this same type of chromosome. In all, seventy-seven variants that could be assigned to the effects of X-rays were obtained in the F_1 cultures out of 8371 flies. Only one such fly was found among 8329 control flies.

2. Genetic tests showed that a majority of these cases were due to an

exchange of segments between the homologous III chromosomes. Such tests further showed that eighty percent of the cases were viable in the homozygous condition. Crossover tests demonstrated that a majority of the cases gave approximately normal crossover values for the regions affected by the induced crossovers.

3. The conclusion is reached that X-radiation induces crossing over in the male when applied to immature germ cells which contain the diploid number of chromosomes. The effect is much more marked when treatment is given during the growth stages of the spermatocyte that correspond to the stages in the female germ cell when normal crossing over is supposed to occur.

LITERATURE CITED

- BEADLE, G. W., 1932 A possible influence of the spindle fiber on crossing over in *Drosophila*. *Proc. Nat. Acad. Sci., Washington* 18: 160-165.
- BELLING, JOHN and BLAKESLEE, A. F., 1925 On the attachments of non-homologous chromosomes at the reduction division in certain 25-chromosome *Daturas*. *Proc. Nat. Acad. Sci., Washington* 12: 7-11.
- BOLEN, HOMER R., 1931 A mutual translocation involving the fourth and the X chromosomes of *Drosophila*. *Amer. Nat.* 65: 417-422.
- CLELAND, R. E., 1925 Chromosome arrangements during meiosis in certain *Oenotheras*. *Amer. Nat.* 57: 562-566.
- DARLINGTON, C. D., 1929 Ring formation in *Oenothera* and other genera. *J. Genet.* 20: 345-363.
- GLASS, H. B., 1932 A study of dominant mosaic eye color mutants in *Drosophila*. *Proc. Sixth Int. Congress Genetics* 2: 62-63.
- MULLER, H. J., 1925 Regionally differential effect of X-rays on crossing over in autosomes of *Drosophila*. *Genetics* 10: 470-507.
- 1930 *Oenothera*-like linkage of chromosomes in *Drosophila*. *J. Genet.* 32: 335-355.
- OLIVER, C. P. and VAN ATTA, E. W., 1932 Genetic and cytological correlations of chromosomal aberrations of *Drosophila*. *Proc. Sixth Int. Congress Genetics* 2: 145-147.
- PATTERSON, J. T., 1932 Lethal mutation and deficiencies produced in the X chromosome of *Drosophila melanogaster* by X-radiation. *Amer. Nat.* 64: 193-206.
- PLOUGH, H. H., 1917 The effect of temperature on crossing over in *Drosophila*. *J. Exp. Zool.* 24: 147-193.
- 1921 Further studies on the effect of temperature on crossing over. *J. Exp. Zool.* 32: 187-201.
- REDFIELD, HELEN, 1930 Crossing over in the third chromosomes of triploids of *Drosophila melanogaster*. *Genetics* 15: 206-251.
- STURTEVANT, A. H., and DOBZHANSKY, T., 1930 Reciprocal translocations in *Drosophila* and their bearing on *Oenothera* cytology and genetics. *Proc. Nat. Acad. Sci., Washington* 16: 533-536.