THE EFFECTS OF INFRA-RED RADIATION AND DESICCATION ON CROSSINGOVER IN DROSOPHILA MELANOGASTER

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Communicated by A. F. Blakeslee, September 24, 1953

Previous studies have shown that following treatment with infra-red radiation, chromosomes of both Drosophila and Tradescantia are broken more frequently by subsequent treatment with x-rays.[1-4] The effects of the infra-red treatment are most pronounced in Tradescantia at a temperature of about 13°C., which is also the temperature that produces a large increase in crossingover compared to that which occurs at 25°C.[5] McElroy and Swanson[6] have advanced the view that the infra-red treatment releases an amount of energy in the chromosome insufficient to break it but which is sufficient to keep the chromosome for some time in a condition in which it is more easily broken by energy from other sources.

Crossingover has long been assumed by many geneticists to be associated normally with chromosome breakage. If this is true then somehow more chromosome breakage of this type occurs in *D. melanogaster* at 13°C. than at 25°C. One might expect, then, that treatment with infra-red radiation at 13°C. would result in a still larger amount of crossingover than occurs normally at that temperature.

This paper reports an attempt to test the above hypothesis. In the course of the experimental work unexpected evidence was obtained suggesting effects of drying on crossingover. This led to a direct test of desiccation effects, using $CaCl_2$ as a drying agent.

Materials and Methods.—The stocks used in these experiments were an Oregon-R wild-type and a second chromosome marker stock, *net b cn bw* (*net* wings, *black* body, *cinnabar* eye color, *brown* eye color). The wild-type stock had been inbred for 100 generations by single pair brother-sister matings. The marker stock was synthesized in this laboratory about ten years ago and had been maintained in close-bred vial cultures with about ten pairs of parents in each generation. At the beginning of the present study both stocks were shifted to half-pint bottle cultures and were maintained thereafter with about 100 pairs of parents per generation.

The females tested for crossingover in these studies were heterozygous for the wild-type and mutant marker stocks. They were backcrossed to *net b cn bw* immediately after experimental treatment. Individual females were mated to two or more males in shell vials and were shaken to new vials generally every third day until from three to six transfers had been made. The longer series constituted practically the entire productive period of the females.

Crossingover test vials were kept at 25°C. Flies which eclosed during the first 18 days after the parents had been admitted were scored for crossingover. They constituted practically the entire potential yields of flies. Cultures were tabulated individually for the 16 possible types of offspring, excepting in the $CaCl_2$ series where scoring was confined to crossovers in the *b-cn* region. The procedure followed made possible a statistical check of variation in crossingover and the elimination of occasional cases of aberrant results from whatever cause—non-virginity of the tested female, a chance inversion in a chromosome being tested, contamination, and so forth.

The infra-red radiation source and the method of exposing flies were similar to those reported earlier.[7] Pupae were exposed by allowing larvae to pupate on strips of towelling inserted in the medium against the vial so that the larvae pupated chiefly on one side of the paper. About 12 hours later the strip was taken out. The wet end and all larvae were removed. Then the strip was cut into two pieces each containing 20 or more pupae. The pieces were suspended each in an empty shell vial by means of the cotton stopper used to plug culture vials. One vial was placed in line for radiation in the usual manner, with the broad side of the paper containing the pupae facing the radiation source. The other vial was wrapped in a rubber cloth and placed elsewhere in the same water bath, shielded from the radiation. Other pupae developing in the original culture served as a 25°C. control for those in the 13°C. water bath. This led to three sets of heterozygous females for testing, all of whom had spent their larval period in one culture vial. In the series of tests using adult flies for treatment there were also three sets of females which had developed completely in one culture vial. Both pupae and adults were radiated for 24 hours, the pupae during the period 0 to 36 hours after the onset of the pupal period and the adult females during the period 0 to 36 hours after eclosion.

Two series were run testing females which were radiated in the pupal stage and two testing radiated adults. One series was run testing the effects of $CaCl_2$ at 25°C. only, but with six sets of females representing different lengths of time over the drying agent. This adds to a total of 18 sets of females, 8 to 12 in each set, with each female ovipositing in from 4 to 7 vials. Some 51,000 flies were scored for crossingover. Variation between females in each group proved to be random, nor was there significant variation between the two pupal or the two adult radiation series. Accordingly, all comparable data have been lumped for consideration in this report.

Experimental Results.—Table 1 shows the amount of crossingover observed in the *b-cn* region in heterozygous females raised under the indicated temperature conditions. The first series is the 25°C. control of the other two series. The second, the pupal stage series, consisted of females which spent their larval period at 25°C. and their pupal stage at 13°C. In the

third series, the young adult, the females developed at 25°C. and were kept for three days at 13°C. beginning from 0 to 12 hours after eclosion.

In the control series there is no significant variation in the amount of crossingover in the different laying periods. This is shown in the P value of 0.68 obtained by the use of chi-square. Whatever variation may have been caused by the increasing age of the females was statistically un-

TABLE 1

CROSSINGOVER IN SUCCESSIVE CLUTCHES OF EGGS IN THE *black-cinnabar* REGION. FEMALES KEPT IN PART AT 13°C.; AT 25°C. OTHERWISE

	LAYING PERIOD (3 DAYS EACH)						
	1	2	3	4	5	6	7
	Control (at 25°C.)						
Flies	584	829	712	672	651	610	447
Crossovers	33	33	35	24	27	23	21
%	5.7	4.0	4.9	3.6	4.1	3.8	4.7
	Pupal Stage at 13°C.						
Flies	924	856	607	650	324	664	63
Crossovers	91	72	39	33	17	30	2
%	9.8	8.4	6.4	5.1	5.2	4.5	3.2
	Young Adult (for 3 days) at 13°C.						
Flies	585	811	759	725	637	579	363
Crossovers	28	40	54	50	25	24	15
%	4.8	4.9	7.1	6.9	3.9	4.1	4.1

TABLE 2

CROSSINGOVER IN YOUNG ADULT FEMALES EXPOSED TO INFRA-RED RADIATION FOR 24 HOURS AT 13°C.

	LAYING PERIOD IN DAYS AFTER ECLOSION OF THE FEMALES									
	2-4	5-7	8	9	10	11	12	13	14-17	18-20
	Untreated									
Flies	709	807	251	636	724	462	430	613	783	652
Crossovers	26	33	12	32	39	35	20	31	38	36
%	3.7	4.1	4.8	5.0	5.4	7.6	4.7	5.1	4.9	5.5
	Radiated									
Flies	709	748	465	731	704	449	420	572	655	508
Crossovers	25	24	19	36	43	26	25	33	37	27
%	3.5	3.2	4.1	4.9	6.1	5.8	6.0	5.8	5.6	5.3

noticeable in this and all other control series of this study. All told, including data not reported in this table, there were 12,102 control flies scored, among which were 541 crossovers in the *b-cn* region. This gives a crossover percentage of 4.5, which is only one-half of the amount expected according to the standard genetic map.[8] Since there was at no time significant variation from 4.5 per cent of crossingover in our control series, we have used that figure for "normal" throughout this report.

According to the findings of Plough it is expected that in the pupal stage series in table 1 there will be higher than normal crossingover in the first week's eggs and a reduced amount thereafter, approaching normal in later periods. It can be seen that this expectation is fully realized. The combination of highly inbred Oregon-R and long closebred *net b cn bw* is thus proved to be sensitive to the effects of low temperature on crossingover. The amount of crossingover was significantly above normal (P is less than 0.01) in the first three laying periods and then dropped to the normal level.

In the young adult series it was expected that the first eggs laid would show normal crossingover and that the subsequent period of above normal crossingover would be less broad than in the pupal stage series. An inspection of the data shows that again this expectation is realized. Only in the third and fourth laying periods was crossingover above the normal level (P for those two periods together is less than 0.01).

Table 2 shows the effects of infra-red radiation at 13°C. on crossingover in treated adult flies, the untreated series in this case receiving the 24 hours of low temperature but being shielded from the radiation. Because of the shortened period of exposure to cold the females were shaken to new vials each day from the 8th through the 13th day, since it was expected that the period of enhanced crossingover might be confined to one or two of those days. It can be seen that in the untreated (non-irradiated) series only on the 11th day was there a striking increase in crossingover. It looks as though part of the eggs laid on the 10th day were also affected. The value of P for those two periods together compared to the normal (4.5 based on 12,105 flies) is less than 0.01. In the radiated series the amount of crossingover was never as high as on the 11th day of the untreated series but it was more than 1.0 unit above the normal level from the 10th through the 17th days. Taken together these five laying periods have a P value of 0.0015 when compared to normal. Considering the five periods in two groups, the first one including the 10th and 11th days and the other the three later periods, the values of P compared to normal are 0.020 and 0.018, respectively, both significant differences. Because of the smaller number of flies involved, the comparison of days 12 through 17 in the two series of table 2 shows no significant difference between them, P being 0.22 in this case. However, the consistently high level of crossingover in the radiated series in the full eight-day period, 10–17, makes it seem to us that the comparison with normal is the more important one here. Therefore, we interpret this as suggesting that infra-red radiation prolongs the effectiveness of the cold treatment. Admittedly, the difference is not as clear-cut as desired and more data are needed to establish this point beyond reasonable doubt. Certainly in the untreated series crossingover is not above the control level in the 12th through 17th days, for P is up to 0.43 in that comparison.

Table 3 shows the effects of treating young pupae with infra-red radiation in a semidry condition at 13°C. The pupae were on strips of paper in vials containing no medium or liquid. At the end of the 24-hour period the strips were replaced in vials containing food and were moistened with water. Due to the influence of outdoor winter temperature on indoor relative humidity the paper strips had become very dry during the exposure period.

Comparing the results in table 3 with those of the pupal stage series in table 1 it is at once apparent that they differ remarkably in the amount of

TABLE 3

CROSSINGOVER IN FEMALES TREATED WHILE YOUNG PUPAE WITH INFRA-RED RADIATION FOR 24 HOURS AT 13°C. IN DRY CONDITION

	LAYING PERIOD IN DAYS AFTER ECLOSION OF THE FEMALES						
	2–4	5–7	8–10	11–13	14–17	18–20	21–23
			Untreated				
Flies	741	556	781	768	834	793	604
Crossovers	24	16	30	42	31	40	32
%	3.2	2.9	3.8	5.5	3.7	5.0	5.3
			Treated				
Flies	675	525	679	601	659	664	593
Crossovers	19	16	31	28	33	26	31
%	2.8	3.0	4.6	4.7	5.0	3.9	5.2

TABLE 4

CROSSINGOVER AT 25°C. IN FEMALES PARTIALLY DESICCATED BY MEANS OF $CaCl_2$ WHILE IN EARLY PUPAL DEVELOPMENT

LAYING PERIOD IN DAYS AFTER ECLOSION		NUMBER OF HOURS OVER $CaCl_2$					
		0	24	48	72	96	108
2 – 7	Flies	1855	1352	1201	760	660	1370
	Crossovers	73	38	39	20	18	39
	%	3.9	2.8	3.2	2.6	2.7	2.8
8–13	Flies	1387	745	946	714	662	1099
	Crossovers	62	32	49	36	23	45
	%	4.5	4.3	5.2	5.0	3.5	4.1

crossingover in the early laying periods. What is more, they are consistently lower than the 4.3 per cent observed in similar laying periods of all controls together (200 crossovers in 4623 flies). The value of P in this comparison is less than 0.01 when the four sets of data in the first two periods in table 3 are lumped. Crossingover is lower in the two earliest periods than in the two subsequent laying periods in the series of table 3; the value of P is 0.002 for that comparison. There is obviously no difference between the two series of table 3 in amount of crossingover.

It may be concluded, therefore, that under the conditions existing in the

experimental series reported in table 3 infra-red radiation had no detectable effect on crossingover, but that something, possibly excessive drying of the pupae, did depress the amount of crossingover in the first week's eggs to a level even below that normally observed in individuals kept continuously at 25°C.

Table 4 shows the results of a direct test of the effects of severe drying of the pupae on crossingover at 25°C. In this case the dry shell vial containing pupae on a paper strip was left open and was put into a mason jar over $CaCl_2$, the jar then being closed. At the indicated number of hours the jar was opened and part of the strip of pupae was cut off and placed in a vial containing food and moistened. The rest of the pupae were returned to the $CaCl_2$ jar for more dry treatment. Thus five treated series were obtained.

It was observed that very young pupae, probably less than two hours of age, were unable to withstand the drying over $CaCl_2$. But pupae older than that apparently did not suffer; indeed, some had already eclosed in the jar at 108 hours. These findings are in substantial agreement with those of an earlier study with *D. pseudoobscura*.[9]

The females of these six series were carried through six laying periods, only four of which were classed when it became obvious that they would give the desired information. Because the first two periods were the only ones to show effects and because low counts made broader variation in some sets than had previously been encountered, the data of the first two periods and of the third and fourth periods have been grouped together in two longer laying periods for ease of presentation in table 4.

It can be seen at once that in all $CaCl_2$-treated series crossingover was below normal in the first week and in the normal range in the second week Comparison of these five series, first week's laying vs. second, and first week. vs. normal, gave P less than 0.01. Neither the intensity nor the extent of the effectiveness of the desiccation seems to have been changed by increasing the length of the desiccation period. The level of crossingover in the affected period is very similar to that observed in the first two laying periods of the "naturally" dried pupae reported in table 3.

Discussion.—The results of these experiments suggest that infra-red radiation is capable of influencing the amount of crossingover which occurs in the heterochromatic regions (not necessarily in the heterochromatin) of Drosophila chromosomes. While no effects were noticeable on crossingover in the long euchromatic regions, *net-b* and *cn-bw*, both of these regions are so long to begin with that any increase in crossingover in them would be detectable only by the use of more marker genes in each region.

In this particular instance the effect of infra-red radiation appears to be not an increase in the rate of crossingover but the extending of the effective period of low temperature. At the time when low temperature by itself

normally increases crossingover there was no evidence whatever of further increase as a result of added infra-red radiation. When pupae in semidry condition were exposed to infra-red radiation at low temperature, the amount of crossingover was not affected by the radiation. While the effects of the low temperature were also suppressed in this last test, some crossingover, at least half as much as occurs at 25°C., did nevertheless occur. Were the effects of infra-red radiation such as to increase the effectiveness of the force responsible for "spontaneous" crossover breakages, one would expect to observe such effects even in this series. It may well be that the effects of both low temperature and infra-red radiation are on some other part of the still unknown process of genetic crossingover. Indeed, we see no compelling reason at present to offer these results as evidence favoring a breakage hypothesis of crossingover.

The observations regarding the effects of pupal drying on subsequent crossingover are of particular interest because of its implications in population genetics. They do not show nor suggest what happens to the chromosomes during such treatment. They suggest that something does happen which depresses crossingover below the normal level at 25°C. and which inhibits the effects of low temperature on crossingover during the drying treatment. In the pupal stage a developing fly is immobile and is accordingly at the mercy of its environment. Presumably pupation occurs in a place most satisfactory to the physiological needs of the organism at that time. In habitats with a climate similar to much of the U. S. A. a sharp change in relative humidity might occur within a few hours thereafter, either in the form of a continuously wet or an exceptionally dry period. The inference from the present observations is that such a change could determine to a significant extent the amount of genetic recombination which occurs in the germ cells during the first week of reproductive life. Genetic recombination is one of the major means by which new phenotypes are produced in natural populations. It has already been shown that naturally occurring inversions[10-13] as well as temperature, age of the female[14] and larval nutrition[15] affect the amount of genetic recombination which may be expected to occur in the heterochromatic regions in flies of natural populations. It has not previously been supposed necessary to assume that crossingover is also under the control of atmospheric relative humidity. The control of crossingover in a natural population is obviously very complex.

One other point of genetic interest remains in the data reported here. That concerns the map distance between the genes *b* and *cn*. The standard distance is 9.0 crossover units; in the present study it was only half as long. One possible explanation is that both of the stocks used here are probably free of inversions in all chromosomes. The standard map was determined largely by using Florida wild stocks. Such stocks very often carry a high

proportion of Payne inversions in the third chromosome[16, 17] which can increase crossingover in other chromosomes.[10] It should be possible to combine one or both of the Payne inversions with one of the stocks used here and see if the amount of crossingover between *b* and *cn* then approaches 9.0 per cent. But it will never be possible to ascertain how much this may have been the cause of Bridges' finding the longer map distance between the two genes.

Summary.—Experiments were carried out to test the effects of 24 hours of infra-red radiation at 13°C. on crossingover in the *black-cinnabar* (and heterochromatic) region of the second chromosome in *Drosophila melanogaster*. Within the limits of the experimental technique used, infra-red radiation increased the duration of the effects of low temperature on crossingover but did not increase the actual rate of crossingover at the time when the low temperature itself was most effective in increasing crossingover. In tests with young pupae in a semidry condition at 13°C. there was no apparent effect of infra-red radiation, and the effects of the low temperature were suppressed. Crossingover was in fact lowered beneath the normal 25°C. level and a subsequent direct test at 25°C., using $CaCl_2$ as a drying agent, gave similar crossover values. The effects of that treatment on crossingover were no more intensive or extensive at 108 hours of drying than at 24 hours of drying. Some implications of these results are discussed.

* The experiments reported here were organized and supervised by the senior author who has also prepared the manuscript. The technical work was done by Fenton. Yost directed the infra-red radiation and Levine supervised the dessication procedure. The general problem was suggested by Yost, and all have shared in its development. The data on the effects of temperature and of infra red radiation are taken from Fenton's thesis for a Degree with Honors, submitted to the Faculty of Amherst College in 1953.

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