A STUDY OF THE EFFECT OF X-RAY RADIATION ON OCCURRENCE OF ABNORMAL INDIVIDUALS, MUTATION RATE, VIABILITY AND FERTILITY OF THE PARASITIC WASP, *HABROBRACON JUGLANDIS* (ASHMEAD)

W. F. DUNNING

Columbia University, New York City

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TABLE OF CONTENTS

INTRODUCTION

The notable discovery by MULLER (1927) that genic mutations could be produced by X-ray radiations in Drosophila led the author to attempt to increase the number of segregating characters in *Habrobracon juglandis* (Ashmead) by the same method, and to study the effect of X-ray radiation on mutation rate, viability and fertility. The experiment was started in November, 1927, and the present publication is a report of the results.

ACKNOWLEDGMENTS

The author gratefully acknowledges indebtedness to Doctor P. W. WHITING at the UNIVERSITY OF PITTSBURGH for material; to the Faculty of Zoology of COLUMBIA UNIVERSITY and Doctor THOMAS HUNT MORGAN, now at the CALIFORNIA INSTITUTE OF TECHNOLOGY, for approval of, and helpful suggestions concerning the problem chosen for investigation; to Doctor JOHN W. GOWEN, ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH, for suggestions and criticisms; to the Director and Staff of the CROCKER INSTITUTE OF CANCER RESEARCH for the use of space and equipment; and to the NATIONAL RESEARCH COUNCIL, Committee on the Effect of Radiation upon Living Organisms, for financial aid.

MATERIAL AND METHODS

The material used consisted of two wild-type strains of Habrobracon, one derived from Lancaster and the other from Iowa City stock, two mutant allelomorphic eye-color stocks, and one mutant reduced wing stock. The eye colors were orange, a recessive to black or type (WHITING 1921), and ivory recessive to type and orange (WHITING and BURTON 1926). The mutant stock reduced wing was a single factor recessive to normal wing (WHITING 1926a).

At the beginning of the experiment the females which were treated were heterozygous for two allelomorphs for eye-color and for normal and reduced wing. That is, the females radiated were either black-eyed normal carrying orange or ivory with reduced wing, or orange-eyed normal heterozygous for ivory and reduced wing. The control females were likewise heterozygous for orange or ivory eye-color and reduced wing. Two independent recessive characters, bar and eyeless, which will be discussed later, arose during the course of the experiment and were added to the genetic constitution of the treated and control females.

The age at which the individuals were treated varied from mature or nearly mature eggs in the body of the adult through all stages of larval development. After the exposure to X-ray the larvae were returned to their vials and allowed to mature at 30 degrees C. The hatched females were observed and allowed to breed as virgins or mated to their recessive brothers and bred. Their progeny were then observed and the females again mated with their recessive brothers or allowed to breed as virgins. This procedure was continued for several generations. The treated adult females were allowed to breed as virgins and their haploid sons were observed for mutations. In the latter case the treatment was effective during the last miotic division of the egg or on the mature egg prior to laying, while in the former it is the egg after it has been laid or the larva during its early development which is exposed to the treatment. The data thus far accumulated are insufficient to show the relationship between the age at which the individual is treated and the appearance of the mutation, and will not be discussed in the present publication.

The X-ray machine used was a Wappler Diex equipped with a Coolidge tube and tungsten target. No metallic filters were used. The efficiency of the machine was repeatedly checked with physical and biological tests by Doctor CHARLES PACKARD. The intensity of the dose varied from 1200 to 8000 Roentgen units in the different experiments.

THE EFFECT OF X-RAY RADIATION ON OCCURRENCE OF ABNORMAL INDIVIDUALS AND MUTATION RATE

The effect of X-ray radiation on the increase in the proportion of individuals in a given population showing somatic abnormalities is presented briefly in table 1. The first four columns show the number of fertile matings, the number of their progeny, the number and percentage of indi-

TABLE 1

The effect of X-ray radiation on the occurrence of abnormal individuals in the directly X-rayed, 1, 2, 3 and later generations from the treatment.

GROUP	NUMBER OF FERTILE MATINGS	TOTAL NUMBER OF PROGENY	NUMBER OF ABNORMALITIES	PERCENT OF ABNORMALITIES	DIFFERENCE FROM CONTROL IN PERCENT ABNORMAL ± THE P.E.	DIFFERENCE ÷ P.E. DIFFERENCE	PERCENT TREATED ABNORMAL ÷ PERCENT CONTROLS ABNORMAL
Controls	928	21504	29	0.135	..	..	..
Directly X-rayed	329	3748	64	1.708	1.573±0.072	21.83	12.65
Generation 1 from X-ray	640	10098	92	0.911	0.776±0.050	15.52	6.75
Generation 2 from X-ray	438	7239	63	0.870	0.735±0.052	14.13	6.44
Generation 3 from X-ray	288	4588	39	0.850	0.715±0.056	12.77	6.30
X-rayed stock	1398	19068	118	0.619	0.484±0.040	12.10	4.59
Total X-rayed	3093	44741	376	0.840	0.705±0.044	16.02	6.22

viduals with somatic abnormalities in the control group, among the directly X-rayed individuals, and the first, second, third and later generations of their descendants. The fifth column represents the difference in the percentage of abnormal individuals between the control and the treated groups with its probable error. The probable error of the difference in the percentages was calculated by the formula:

$$\text{P. E. of } 100p_1 - 100p_2 = 100 \times 0.67449 \times \text{standard error } p_1 - p_2$$
$$= 100 \times 0.67449 \sqrt{p_0 q_0 (1/n_1 + 1/n_2)}$$

where in each case n_2 is the number of individuals in the control group while n_1 is the number of individuals in the group designated in the corresponding line of the table. Similarly p_2 is equal to the proportion of somatic abnormalities in the control group and p_1 the proportion in the respective treated group. The proportions of abnormal and of normal individuals in the progeny of the control and the compared treated group combined are designated respectively by p_0 and q_0. That is, the standard error

of the difference between the two proportions calculated by the method clearly described by YULE (1922) was reduced to the probable error of the difference between the two proportions by multiplying by the constant 0.67449 and was then converted to a percentage basis by multiplying by 100.

The sixth column shows the ratio of the difference between the percentage of abnormalities in each treated group and the control group to the probable error of this difference. This ratio can be considered as a measure of the statistical significance of the difference. PEARL and MINER (1914) prepared from the probability integral tables a table showing for this ratio the expectation in 100 trials of a deviation as great or greater than the one observed if the sole difference between the compared groups was due to sampling. Their table also shows the odds against the observed result being due to pure random sampling chance. This table slightly enlarged by PEARL (1923) extends from Deviation ÷ P. E. Deviation = 1, the point where a deviation as great or greater than the one observed is as likely as not to be due to fluctuations in sampling, to Deviation ÷ P. E. Deviation = 10, where the odds against the observed deviation being due to random sampling are 65,000,000 to 1. The ratios (percent abnormal treated − percent abnormal controls) ÷ (P. E. percent abnormal treated − percent abnormal controls) shown in column six of table 1 vary from 12.10 to 21.83 and therefore the odds against any of the observed differences in proportion of abnormalities in the control and treated groups being due to chance is overwhelming. The final column of the table shows the number of times that the percentage of somatic abnormalities in the treated exceeded the percentage of abnormalities in the controls. The calculations for the table were made to six decimal places and then tabulated to three.

The controls for the experiment consisted of 1023 matings made up from the same stocks with females of the same age and carrying the same genetic characters as the treated individuals. Of these 302 were completed at the time the experiment started and 721 were made up as sister matings and bred contemporaneously with the treated individuals. There were 95 sterile matings and 928 fertile matings which produced 13,787 males, 7,540 females and 5 gynandromorphs or a total of 21,504 progeny. As seen in the first line of table 1 there were 29 or 0.13 percent of abnormal individuals among the 21,504 individuals. The abnormalities were classified as such on the basis of their somatic appearance and those tabulated in the control and treated groups of tables 1 and 2 include all the changes in visible characters which were observed. From the nature of the experiment it is obvious that only certain types of mutations would be detected, that

is, only mutations which were expressed by the third allelomorph among the sons and daughters of females heterozygous for two allelomorphs at the orange eye color locus unless they occurred in mosaic patterns, and at the reduced locus, only the mutations which were expressed as mosaics or affected enough of the germ cells to cause a significant deviation from the expected 1 to 1 ratio, would be observed. The males would show all the new mutations which occurred since they are haploid, but the females would only express the new dominant mutations. This applies equally to the controls and treated groups, and the majority of observed abnormalities were mosaic individuals. Lethal mutations were not observed. This is not

TABLE 2

Showing the comparison between ♂♂ and ♀♀ in percentage of abnormal individuals.

GROUP	TOTAL NUMBER ♂	TOTAL NUMBER ♀	NO. ♂♂ ABNORMAL	NO. ♀♀ ABNORMAL	PERCENT ABNORMAL ♂♂	PERCENT ABNORMAL ♀♀	PERCENT ABNORMAL ♂♂ −PERCENT ABNORMAL ♀♀ ± P.E.	DEVIATION ÷ P.E. DEV.
Controls	13787	7540	15	9	0.11	0.12	−0.01±0.03	0.3
Directly X-rayed	2371	1374	37	24	1.56	1.75	−0.19±0.18	1.0
Generation 1	7838	2258	70	20	0.89	0.88	0.01±0.15	0.7
Generation 2	4808	2431	46	17	0.96	0.70	0.26±0.16	1.6
Generation 3	3255	1329	25	10	0.77	0.75	0.02±0.19	0.1
X-rayed stock	12840	6218	80	28	0.62	0.45	0.17±0.08	2.1
Total X-rayed	31112	13610	258	99	0.83	0.73	0.10±0.06	1.7

surprising since the males are haploid and any bearing lethals would not appear and in females lethals could only be detected in chromosomes which are marked by visible characters. The possibility of discovering lethal changes when only a small number of visible characters are known is slight, and the failure to demonstrate them does not prove that none had occurred.

Individuals from 378 matings were exposed to X-ray radiation, but progeny from only 329 survived. From the 329 fertile matings 2371 males and 1374 females and 3 gynandromorphs or a total of 3748 individuals were hatched. The second line of table 1 shows that of 3748 directly X-rayed individuals 64 or 1.71 percent showed changes in visible characters. Of the 64 abnormal individuals, 37 were males, 24 females and 3 sex mosaics. Disregarding the sex mosaics table 2 shows that the 37 abnormal males occurred among 2371 males or 1.57 percent were abnormal. The 24 abnormal females were equal to 1.75 percent of 1374 females. Of the 29 abnormal individuals in the controls 15 were males, 9 were females and 5 were sex

mosaics. That is, 0.11 percent of the 13,787 males and 0.12 percent of the 7,540 females showed somatic abnormalities. The ratio of the percentage of abnormalities of treated males to that of the control males is 14.46 and of the treated to the control females it is 14.63, or the individuals with somatic abnormalities are equally represented in the male and female population. The difference between the percentage of abnormalities in the totals of the treated and controls was 1.57 ± 0.072 percent which is 21.8 times its probable error. The last figure in the second line of the table shows that the percentage of somatic abnormalities among the directly X-rayed individuals was 12.6 times that of the controls.

That we might at the beginning rule out the possibility that the increased proportion of somatic abnormalities among the X-rayed individuals was due in part to the selection or inclusion of individuals which had an hereditary tendency to produce deficient or abnormal progeny such as the case of deficiency in the posterior part of the digestive tract described by WHITING (1926b), a series of 96 matings was made up and the progeny in each culture while still in the larval stage were divided into two nearly equal groups, one of which was X-rayed and the other allowed to hatch as controls. The result from these divided matings will be found in table 3. From the 96 matings, 1992 progeny including 25 individuals showing

TABLE 3

The effect of X-ray radiation on proportion of abnormal individuals where one-half the individuals from each mating were treated and the other half were the controls.

DIVIDED MATINGS	NUMBER OF ½ MATINGS	TOTAL PROGENY	NUMBER OF ABNORMALITIES	PERCENT ABNORMAL	DIFFERENCE BETWEEN TREATED AND CONTROL ± P.E.	DIFFERENCE ÷ P.E. DIFFERENCE	PERCENT TREATED ABNORMAL ÷ PERCENT CONTROLS ABNORMAL
X-rayed ½	96	924	24	2.597	2.503 ± 0.337	7.43	27.74
Control ½	96	1068	1	0.094	..	..	..
Total	96	1992	25	1.255	..	..	..

somatic abnormalities were hatched. Of these 1992 progeny 1070 were males and 922 females and of the 25 abnormal individuals 13 were observed as males and 12 as females. The first line of the table shows that 924 of these were X-rayed and among them 24 or 2.60 percent somatic abnormalities were observed while in the 1068 individuals of the non-radiated half there was only 1 or 0.09 percent of abnormal individuals. The difference between the percentage of abnormal individuals in the X-rayed half

and the non-treated half of the same matings is 2.50 ± 0.34 percent or 7.43 times its probable error, which means that the expectation of a deviation as great or greater than this if the two groups were random samples of the same population would be 0.0001363 in 100 trials or that the odds against the two being independent random samples of the same population are 642,200 to 1.[1] The final column of the table shows that the percentage of abnormalities among the X-rayed individuals was 27.7 times that of the non-treated half. The fact that the increase in proportion of abnormalities over the controls is greater here than in the group shown in table 1 may in part be due to the selection of dosage used and the age at which the individuals were treated. However, the occurrence of a difference so certainly significant here where all the possibility of a genetic factor difference is ruled out demonstrated beyond any reasonable doubt that it was the treatment that was the effective agent in producing the increased percentage of abnormal individuals. The abnormal individuals recorded here represent quite fairly on a small scale the types of abnormalities observed in the larger group of the directly X-rayed individuals and their descendants. There were 2 black and orange eye-color mosaics, 1 male and 1 female, 4 males and 3 females with defective median veins in the primary wings, 6 males and 7 females with vestigial wings and one of these males was the one observed abnormal individual in the control half, 1 male with fused antennae, 1 male with abnormal head possibly eyeless and 1 female with abnormal abdomen. The progeny from these matings are included in the later generations of the treated individuals and in the controls.

It appears from an examination of tables 1 and 2 that the descendants, male and female, for three successive generations, of the individuals exposed to X-ray and the "X-rayed stock" which includes the fourth generation from the treatment, and all the later generations which were sufficiently tested to be considered showed a significantly higher proportion of abnormal individuals than did the controls. There were 6 times as many individuals with somatic abnormalities in the first, second and third generations of descendants and 4 times as many in the X-rayed stock as there were in the controls.

The occurrence of a substantially increased proportion of abnormal individuals among the progeny of those exposed to X-ray two, three and more generations after the treatment was unexpected and cannot at present be explained. It is quite possible that some of the so called abnormal in-

[1] These values were obtained from PEARL and MINER'S table by interpolation. From the probability integral table, the expectation in 1000 is $p = 0.000,000,556$. The odds are 1,798,560 to 1.

dividuals are the result of non-inherited developmental irregularities such as "shot" described by WHITING (1930), and that their occurrence in later generations is a result of some fundamental injury to the germ-plasm. Some of the mosaic females may have resulted from chromosome deletion or translocations such as those described by MULLER (1928) in Drosophila. Some without doubt represent somatic and germinal mutations, but why they should repeatedly occur two, three and more generations after the treatment is not known. However, in the following tables, 4 to 7 inclusive, are tabulated those abnormal individuals which morphologically resembled a change at the orange-ivory eye-color, reduced wing, bar and eyeless loci, and in the text which accompanies the discussion of each table will be represented the evidence for considering some of them as genic mutations.

Table 4 shows briefly that there were 49 individuals in the experiment which somatically resembled mutations in one of the three known allelo-

TABLE 4

Showing the effect of X-rays on the production of eye-color mutations at the orange locus in the directly X-rayed 1, 2, 3 and later generations from the treatment.

GROUP	NUMBER OF FERTILE MATINGS	TOTAL NUMBER OF PROGENY	NUMBER OF EYE-COLOR MUTATIONS	PERCENT OF EYE-COLOR MUTATIONS	DIFFERENCE FROM CONTROLS IN PERCENT EYE-COLOR MUTATIONS ± P. E.	DIFFERENCE ÷ P.E. DIFFERENCE
Controls	928	21504	1	0.005	..	..
Directly X-rayed	329	3748	7	0.187	0.182±0.021	8.67
Generation 1 from X-ray	640	10098	14	0.139	0.134±0.018	7.44
Generation 2 from X-ray	438	7239	10	0.138	0.133±0.018	7.39
Generation 3 from X-ray	288	4588	3	0.065	0.060±0.014	4.29
X-ray stock	1398	19068	14	0.073	0.068±0.013	5.23
Total X-rayed	3093	44741	48	0.107	0.102±0.015	6.80

morphs for eye-color (that is, orange, ivory and their normal allelomorph black or type). One of these occurred in the controls and 48 in the radiated individuals and their descendants. The directly X-rayed individuals showed an increased percentage of 0.182 ± 0.021 or a difference from the controls which is 8.67 times its probable error. The first and second generations showed a correspondingly significant increase over the controls or a difference 7 times as great as its probable error. Among the third and later generations of descendants the difference is also significant, that is, respectively 4 and 5 times its probable error.

The mutant individuals recorded in table 4 consist of 2 black-eyed sons from orange-ivory heterozygous mothers mated to ivory males, 9 orange

sons from black-ivory heterozygous mothers, 4 ivory sons from black-orange heterozygous mothers, 23 black and orange mosaic individuals from black and ivory heterozygous females mated to ivory males or bred as virgins, 7 black and orange mosaics from black and orange heterozygous females mated to ivory males or bred as virgins, 2 black and ivory mosaic females from ivory females mated to black males, and 2 orange and ivory mosaic sons from orange and ivory heterozygous females.

It is impossible to say whether the 7 black and orange mosaics from black and orange heterozygous females, and the 2 orange and ivory mosaic sons from orange-ivory heterozygous mothers were genic mutations or arose from binucleate eggs. That is, the female parent in each case was heterozygous for the two colors represented in the eyes of the mosaic. However, these female parents were also heterozygous for reduced and normal wing but in no case did the eye-color mosaics have mosaic wings.

The two black and ivory mosaic females from ivory females mated to black reduced males may represent genic mutations or abnormal fertilization. One of these females produced both black and ivory sons and the other was sterile. The two black males from orange-ivory heterozygous females mated to ivory males, and the four ivory males from black-orange heterozygous virgin females could not have resulted from either abnormal fertilization or binucleate eggs.

The 9 orange, and 23 black and orange mosaic individuals from black-ivory heterozygous females express a color, orange, which was not present in the germ-plasm of the parents. These mosaics appear to be quite similar to those previously described by WHITING (1927). The amount of each color represented varied from individuals with one eye orange and the other black (figure 1), or individuals with orange eyes each containing black sectors (figure 2) or bars, to individuals with the orange eye-color represented only by a few ommatidia or the ocelli (figure 3). There were also 4 individuals which phenotypically expressed all three allelomorphs (figures 4 and 5). The ratio of mutant to non-mutant color in the eyes bore no direct relation to the behavior of the germ-plasm.

WHITING (1928b) suggested that the occasional occurrence of black-orange mosaic sons from black-ivory heterozygous mothers might be due to an orange appearance in a black-ivory mosaic caused by the proximity of the black and ivory facets. If these mosaics are truly black and ivory and not black and orange they may have arisen either as genic mutations or from binucleate eggs. There is no evidence that any of these were black-ivory mosaics but there is proof that several of them were not. Three of the orange mutants were tested and 2 proved to be genetically orange; the

LEGEND FOR PLATE 1

Magnifications are × 20 for figures 1–8 inclusive, × 15 for figures 9–15 inclusive.

FIGURES 1–3.—Dorsal view of head of eye-color mosaics showing distribution of black and orange color. (Mosaics $192A_1$, $4463A_1$, $5289A_1$.)

FIGURES 4, 5.—Dorsal and sinistral view of tri-color mosaic showing distribution of black, orange and ivory color. (Mosaic $1195A_1$.)

FIGURES 6, 7.—Dorsal and sinistral view of head of a bar-eyed male.

FIGURE 8.—Dorsal view of head of an eyeless male.

FIGURE 9.—Normal right primary wing.

FIGURE 10.—Defective wing showing the absence of a part of the median vein.

FIGURES 11, 12.—Normal right male and female antenna.

FIGURE 13.—Antennae of a fused antennal male.

FIGURE 14.—Dextral view of thorax showing vestigial wing.

FIGURE 15.—Dextral view of thorax of wingless male.

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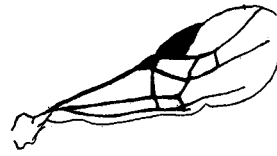

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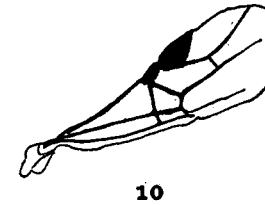

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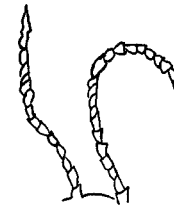

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other bred as black and might therefore have been a black-ivory mosaic, but both of its eyes were orange and did not show any black tissue. Seven of the black-orange mosaics were tested, 4 bred as black, 1 as orange and 2 as black and orange. Since five of the orange mutants, 2 orange, and 3 mosaics of orange, proved to be genetically orange and none proved to be ivory, we are not justified in considering those which did not breed as orange as black-ivory mosaics. Furthermore, the female parent was in each case heterozygous for reduced and non-reduced wing, and it would be expected that if these mosaics arose as a result of the fusion of an ivory and a black bearing nucleus thereby making them black-ivory mosaics, that some of them would also be mosaic for reduced and normal wing, but this was not the case.

WHITING (1928b) also suggests a "genetic" hypothesis which might account for the occurrence of orange eye-color among the progeny of the black-ivory heterozygous females. That is, ivory may be caused to mutate to orange in association with black. The evidence relating to this hypothesis is not very satisfactory. But if ivory is more likely to mutate to orange in association with black than with orange or shows a higher mutation rate than the orange and black allelomorphs, the progeny of the black-ivory heterozygous females in the treated group should be compared separately with the progeny of the black-ivory heterozygous females in the control group. When this is done, it appears that the only mutation observed in the control group was a black-orange mosaic from a black-ivory heterozygous female. In the control group there were 127 black-ivory heterozygous females which produced 1969 progeny including 1 or 0.05 percent of orange mutants. This mosaic was sterile. There were 60 matings in the directly X-rayed group with 706 progeny including 3 or 0.42 percent of orange individuals. The difference is 0.37 ± 0.11 percent or a difference 3.3 times its probable error. Generation 1 included 264 fertile black-ivory heterozygous females which produced 4172 progeny with 8 or 0.19 percent of orange mutants. The difference in this case is 0.14 ± 0.07 percent or 2.0 times its probable error. In generation 2 there were 146 black-ivory matings with 2438 progeny including 9 or 0.37 percent of orange individuals. This difference or 0.32 ± 0.10 percent is 3.2 times its probable error. In generation 3 there were 115 black-ivory females with 1913 progeny and 2 or 0.11 percent of orange mutants and in the X-rayed stock 9 or 0.17 percent among 5387 progeny. In neither of these cases was the difference statistically significant although the percent of orange mutants in the treated was 2 and 3 times respectively that in the controls.

When the progeny of the black-ivory heterozygous females in the treated group are compared separately with the progeny of the black-ivory females in the control group as is shown above, the increased percentage of orange mosaics is statistically significant for the directly X-rayed and for the second generation of descendants. The first, third, and later generations all show an increase in the same direction which is not statistically significant. This is the most vigorous selection of a control group since it includes the only mutation observed in untreated material in the smallest possible group. The decrease in the number of progeny in this selected control increases the percentage which the one observed mutation makes and at the same time increases the size of the probable error of difference. Yet it is conceivable that ivory is more likely to mutate in association with black than either of the other two allelomorphs in any combination, and that the apparent increase in eye-color mosaics among the treated individuals is due to a disproportionate percentage of these black-ivory heterozygous females in the treated and control groups. If this were the case when the progeny of such females are eliminated from both treated and controls, we would not expect a significant difference in the percentage of mutations in the two groups. In the treated group of 21,831 individuals not derived from black-ivory heterozygous females 18 or 0.082 percent showed changes in eye-color. There were no eye-color mutants among the 19,534 control individuals descended from black-orange and orange-ivory females. The difference between zero in the control and 0.082 ± 0.14 percent in the treated group is 6.0 times the probable error of the difference and therefore certainly significant. If we consider as mutations only the 2 black males from orange-ivory heterozygous females mated to ivory males, the 4 ivory males from black-orange heterozygous virgin females, the 2 orange and 3 black and orange mosaic individuals from black and ivory heterozygous mothers which were fertile and produced offspring showing the mutant eye-color, and compare them with the progeny of the black-orange, black-ivory and orange-ivory females in the control group, the increase in percent of mutations among the treated and their descendants is 0.034 ± 0.017 percent or only twice its probable error. There were also 6 sterile orange males and 17 sterile black and orange mosaics from black-ivory heterozygous females. It is of course quite impossible to prove that they were mutations, but the fact that they were sterile does not constitute evidence for or against an assumption that they were mutations. The 1 orange male and 3 black and orange mosaics from black-ivory heterozygous females which bred as black may have been so-

matic mutations to orange which failed to affect the germ-plasm or affected such a small fraction of it that it was not represented in their progeny.

In table 5 are included those individuals which morphologically resembled changes at the "reduced" locus. There were 3 in the controls and 3 among the directly X-rayed which gave an increase in the treated of

TABLE 5

Showing the effect of X-rays on the production of reduced wing mutations in directly X-rayed 1, 2, 3 and later generations from the treatment.

GROUP	NUMBER OF FERTILE MATINGS	TOTAL NUMBER OF PROGENY	NUMBER OF REDUCED WING MUTATIONS	PERCENT OF REDUCED WING MUTATIONS	DIFFERENCE FROM CONTROLS IN PERCENT OF REDUCED WING MUTATIONS ± P. E.	DIFFERENCE ÷ P. E. DIFFERENCE
Controls	928	21504	3	0.014	..	..
Directly X-rayed	329	3748	3	0.080	0.066±0.018	3.67
Generation 1 from X-ray	640	10098	0	0	−0.014±0.008	1.75
Generation 2 from X-ray	438	7239	0	0	−0.014±0.009	1.56
Generation 3 from X-ray	288	4588	0	0	−0.014±0.012	1.17
X-rayed stock	1398	19068	2	0.010	−0.003±0.007	0.43
Total X-rayed	3093	44741	5	0.011	−0.003±0.006	0.50

0.066 ± 0.018 percent or an increase 3.67 times its probable error. There were none in the first, second, or third generation of descendants and only 2 occurred among the X-rayed stock. In the total X-rayed group there were 5 or 0.01 percent of reduced wing mosaics with a difference from the controls of −0.003 ± 0.01 percent or an insignificant difference in favor of the controls. The failure to show a higher proportion of individuals resembling reduced mutations among the descendants of the treated group is probably influenced by the fact that only obvious mosaics could be detected. Since all of the females would produce both reduced and normal sons and daughters mutations affecting the entire individual or producing only small patches of the mutant tissue would not be easily observed.

Table 6 shows the effect of X-ray radiation on the occurrence of bar-eye (figures 6 and 7) mutations. This character is represented in the controls by one mosaic male in which one eye resembled the character as it occurred among the treated individuals. This control mosaic was sterile. Of the 29 which occurred among the progeny of the treated and their descendants, 17 showed the character in both eyes and 12 were mosaics. Eight of the first group were tested and 7 or 87.5 percent proved to be genetically bar. Six of the mosaics were tested and only 1 or 16.7 percent bred as bar. The occurrence of mutations at this locus in contrast to the reduced shows an insignificant difference between the controls and directly X-rayed indi-

viduals, but the first, second, third and later generations all show a significant increase over the controls.

The control and treated females represented in this table were not all heterozygous for the bar gene. The females therefore could not all have

TABLE 6

Showing the effect of X-rays on the production of bar eye mutations in the directly X-rayed, 1, 2, 3 and later generations from the treatment.

GROUP	NUMBER OF FERTILE MATINGS	TOTAL NUMBER OF PROGENY	NUMBER OF BAR-EYE MUTATIONS	PERCENT OF BAR-EYE MUTATIONS	DIFFERENCE FROM CONTROLS IN PERCENT OF BAR-EYE MUTATIONS± P. E.	DIFFERENCE ÷ P. E. DIFFERENCE
Controls	928	21504	1	0.005	..	..
Directly X-rayed	329	3748	1	0.027	0.022±0.011	2.00
Generation 1 from X-ray	640	10098	7	0.069	0.064±0.013	4.92
Generation 2 from X-ray	438	7239	10	0.138	0.133±0.018	7.39
Generation 3 from X-ray	288	4588	3	0.065	0.060±0.014	4.29
X-rayed stock	1398	19068	8	0.042	0.037±0.010	3.70
Total X-rayed	3093	44741	29	0.065	0.060±0.012	5.00

shown the mutation had it occurred. If we consider only the male progeny from pure non-bar females of non-bar stock which have never been mated with bar stock, the percentage of bar mutation among them could not have arisen either from binucleate eggs or any form of chromosome duplication

TABLE 7

Showing the effect of X-rays on the production of eyeless mutations in the directly X-rayed, 1, 2, 3 and later generations from the treatment.

GROUP	NUMBER OF FERTILE MATINGS	TOTAL NUMBER OF PROGENY	NUMBER OF EYELESS MUTATIONS	PERCENT OF EYELESS MUTATIONS	DIFFERENCE FROM CONTROLS IN PERCENT EYELESS MUTATIONS± P. E.	DIFFERENCE ÷ P. E. DIFFERENCE
Controls	928	21504	0	0	..	..
Directly X-rayed	329	3748	1	0.027	0.027±0.008	3.38
Generation 1 from X-ray	640	10098	1	0.010	0.010±0.005	2.00
Generation 2 from X-ray	438	7239	1	0.014	0.014±0.005	2.80
Generation 3 from X-ray	288	4588	0	0	..	..
X-rayed stock	1398	19068	8	0.042	0.042±0.009	4.67
Total X-rayed	3093	44741	11	0.025	0.025±0.007	3.57

There were 8 mosaics, 7 females and 1 male which occurred among the progeny of the females heterozygous for the bar gene. The seven mosaic females since they were heterozygous for bar and non-bar may owe their appearance to either the loss of the non-bar chromosome on one side of the

individual, partial fertilization, or to a somatic mutation from the normal gene to bar, but it would be impossible to discover which was the case without a cytological examination. The one male mosaic from a heterozygous bar mother may have arisen either as a somatic mutation or from a binucleate egg. There were 21 male bar mutants from pure non-bar females; 8 of these were tested and 7 or 87.59 percent behaved as true genic mutations; the other a mosaic of bar and normal bred as normal, as might be expected if the mutation had not affected the germ-plasm or only a small part of it. The remaining 13 were sterile. The one or 0.008 percent of bar mutants observed for the control group occurred among the 13,208 males from 871 pure non-bar females. One bar mutant among 2,145 directly X-rayed males from 298 non-bar females increased the percentage by 0.039 ± 0.017 percent. In generation 1, 7381 males including 3 or 0.41 percent of bar mutations were derived from 586 pure non-bar females. The difference in percent of bar mutations is 0.033 ± 0.014 or 2.5 times its probable error. The 405 pure non-bar females of the second generation produced 9 or 0.20 percent of bar mutants among their 4,396 sons. The difference in this case is 0.197 ± 0.028 percent or 7.0 times its probable error. In generation 3, 2 bar mutants occurred among 2,920 males from 246 pure non-bar females giving an increase over the controls of 0.061 ± 0.018 percent of bar mutants or an increase 3.2 times its probable error. The X-rayed stock likewise shows an increase of 0.061 ± 0.017 percent of bar mutants which is a significant increase over the controls. The 21 male bar mutants among the 25,600 sons of pure non-bar females in the sum of the treated individuals and their descendants show an increase over the controls of 0.074 ± 0.017 percent or an increase 4.4 times its probable error. If we consider as mutations only the 7 bar individuals which were fertile and bred as bar, the increased percentage over the controls is 0.027 ± 0.010 percent or 2.7 times its probable error. However, the character appeared the same in the sterile males as it did in the fertile ones, and that the former as well as the latter were genic mutations is obviously quite possible.

Table 8 shows the independent segregation of bar with three known independent characters, namely, black and orange eye-color, defective and non-defective wing venation, a factor for the presence or absence of vein r_4 (WHITING 1924) and reduced and normal wing. From 203 F_1 females from black bar males crossed with orange-eyed normal females, 433 black-eyed bar and 484 orange-eyed bar sons were obtained. Among the normal-eyed sons 49.5 percent were black and 50.4 percent orange. The deviation in the former case from the expected 50 percent is 2.78 ± 1.11 percent or not statistically significant. Thus eye-color is seen to segregate independently

with bar eye, but here bar is seen to lose out in competition with non-bar for as many bar as normal sons would be expected from these females.

The following two lines of the table show the progeny of 35 F_1 females from defective bar-eyed males crossed to normal females. There were 288 double dominant, 295 defective non-bar, 158 bar-eyed non-defective and

TABLE 8

Showing the independent segregation of the factor for bar eye and other known factors.

CHARACTERS TESTED	NUMBER OF HETEROZYGOUS F_1 ♀♀	TOTAL PROGENY	NUMBER DOMINANTS	NUMBER RECESSIVES	PERCENT DOMINANTS	PERCENT RECESSIVES	DEVIATION FROM EXPECTED 50 PERCENT ± P. E. DEV.	DEVIATION ÷ P. E. DEV.
Non-bar eye and eye-color	203	1467	727	740	49.557	50.443	0.443±0.881	0.50
Bar eye and eye-color	203	917	433	484	47.219	52.781	2.781±1.114	2.50
Non-bar eye and wing venation	35	583	288	295	49.400	50.600	0.600±1.397	0.43
Bar eye and wing venation	35	346	158	188	45.665	54.335	4.335±1.813	2.39
Non-bar eye and reduced wing	34	224	124	100	55.357	44.643	5.357±2.253	2.38
Bar eye and reduced wing	34	158	93	65	58.861	41.139	8.861±2.683	3.30
Non-bar eye and eyeless	32	202	132	70	65.347	34.653	15.347±2.373	6.47
Bar eye and eyeless	32	86	86	0	100.000	0	50.000±3.637	13.75
Non-bar eye and bar eye	518	4759	2939	1820	61.757	38.243	11.757±0.489	24.04

188 bar-eyed defective sons. Non-defective appears in 45.66 percent of the bar-eyed males and defective appears in 54.34 percent. The deviation from the expected 50 percent is 4.34± 1.81 percent or less than three times its probable error, and bar can be said to assort independently of the factor for the defect in r_4 vein of the wing.

The fifth and sixth lines of table 8 show the segregation of the bar-eye factor with the factor for reduced wing. From 34 F_1 females heterozygous for bar and non-bar and reduced and normal wing, 124 normal eye non-reduced, 100 normal eye reduced, 93 bar-eye, normal and 65 bar-eye reduced sons were obtained. Reduced wing appeared in 41.14 percent of the bar-eyed sons with a deviation from the expected 50 percent of 8.86± 2.68 percent which appears to be significant. However, this difference is probably due to the failure of some of the reduced wing individuals to come

through and not to any linkage between bar and reduced. The last line of table 9 shows the progeny from 110 females heterozygous for reduced and

TABLE 9

Showing the independent segregation of the factor for eyeless and other known factors.

CHARACTERS TESTED	NUMBER OF HETEROZYGOUS F_1 ♀♀	TOTAL PROGENY	NUMBER DOMINANTS	NUMBER RECESSIVES	PERCENT DOMINANTS	PERCENT RECESSIVES	DEVIATION FROM EXPECTED 50 PERCENT ± P. E. DEV.	DEVIATION ÷ P. E. DEV.
Normal eye and eye-color	110	991	502	489	50.656	49.344	0.656±1.071	0.61
Eyeless and eye-color	110	397	206	191	51.889	48.111	1.889±1.693	1.12
Normal eye, and wing venation	65	664	327	337	49.247	50.753	0.753±1.309	0.58
Eyeless and wing venation	65	257	130	127	50.584	49.416	0.584±2.104	0.28
Normal eye, and reduced wing	76	700	393	307	56.143	43.857	6.143±1.275	4.82
Eyeless and reduced wing	76	300	172	128	57.333	42.667	7.333±1.947	3.77
Normal eye and eyeless	266	2557	1741	816	68.088	31.912	18.088±0.667	27.12
Normal and reduced wing	110	924	517	407	55.952	44.048	5.952±1.109	5.37

TABLE 10

Showing the difference in viability between bar eye, eyeless and reduced wing and their normal allelomorphs.

CHARACTERS TESTED	2× NORMAL =N	EXPECTED NUMBER WITH MUTANT CHARACTER, THAT IS, NUMBER NORMAL	OBSERVED NUMBER WITH MUTANT CHARACTER	100×OBSERVED NUMBER ÷ N	DEVIATION FROM EXPECTED 50 PERCENT ± P. E.	DEV. ÷ P. E. DEV.	RATIO OF OBSERVED TO EXPECTED	DEV. FROM 100 PERCENT
Non-bar eye and bar eye	5878	2939	1820	30.963	19.037±0.440	43.27	61.926	38.074
Normal eye and eyeless	3482	1741	816	23.435	26.565±0.572	46.44	46.870	53.130
Normal and reduced wing	1034	517	407	39.362	10.638±1.049	10.14	78.724	21.276

normal and the deviation from the expected 50 percent of reduced wing individuals to be 5.95 ± 1.11 percent. Table 10 shows that in a theoretical population of 1034 or twice the number of normal-winged individuals only 78.7 percent of the expected reduced winged individuals were observed or that 21.3 percent failed to come through. This difference may account for the deviation observed betweeen the number of bar-eyed reduced and normal individuals.

All the tests for bar-eye were made from crosses of bar-eyed males to normal females because a female homozygous for bar is sterile. During the course of the experiment 122 bar-eyed females were tested. Of these, 121 were sterile and 1 has a record of 15 normal sons. This is either a mistake in the record which could not be detected, or this female was genetically non-bar although she had the somatic appearance of a homozygous bar individual. The cause of sterility[2] of the bar-eyed females is not known.

In table 7 are recorded all the mutants which morphologically resembled eyeless (figure 8). There were 11, all of which occurred among the treated individuals and their descendants. All of the eyeless mutants were males, 7 showed the character in both eyes, and 4 were mosaics. The 7 non-mosaic males were tested and all proved to be genetically eyeless. There was one each in the directly X-rayed, first and second generations, and 8 in the X-rayed stock. Each of these groups shows an increase over the controls in percent of eyeless mutations. Now if we exclude from each treated group and from the controls the progeny of the heterozygous eyeless females, and consider only the male progeny of the pure non-eyeless females, here, as in the case of bar, the results are not materially affected. There were 893 pure normal-eyed matings in the control group which produced 13,292 normal males. There were 2,297 males from 313 non-eyeless matings in the directly X-rayed group including 1 eyeless mutant. Therefore, the increase over the controls is 0.044 ± 0.012 percent or an increase 3.7 times its probable error. In the first generation 1 eyeless mutant occurred among 7,325 males from 591 normal females and the increase over controls is 0.014 ± 0.007 percent or twice its probable error. In the second generation, 1 eyeless mutant occurred among 4,426 from 402 pure non-eyeless females. The increase over the controls in this case is 0.023 ± 0.009 percent or an increased percentage of eyeless mutants 2.6 times its probable error. In the third generation, there were 2,837 normal males from 257 non-eyeless females. In the X-rayed stock 2 eyeless mosaic males occurred among 2,477 progeny from 173 heterozygous eyeless females. These might,

[2] Recently another new character small and its allelomorph extreme small has been produced which morphologically resembles bar in which the homozygous female is fertile.

therefore, have arisen either from binucleate eggs or as somatic mutations. The 1225 pure non-eyeless females of the X-rayed stock produced 11,453 males including 6 eyeless mutants. The increase in percentage of these eyeless mutants over the controls is 0.052 ± 0.013 percent or 4.0 times its probable error. If the treated individuals and their descendants are totaled and compared with the controls, the 9 eyeless mutants from pure non-eyeless females occurred among 28,338 males and none occurred among 13,292 males in the similar control group. The increase in percentage of eyeless mutations is 0.032 ± 0.010 percent or 3.2 times its probable error, and if we consider only those which were fertile and bred as eyeless, the increase over the controls is 0.025 ± 0.009 percent or 2.8 times its probable error. The latter figure involves the unsupported assumption that the sterile eyeless individuals from non-eyeless mothers were genetically normal.

The independent segregation of eyeless with other known characters is shown in table 9. In the first two lines of the table are the progeny of 110 F_1 females heterozygous for the factors for normal eye and eyeless and black and orange eye-color. In the case of the eyeless the eye-color can be classified by the color of the ocelli which in all of these stocks corresponds to the color of the compound eyes. The normal-eyed sons show a ratio of 50.7 percent black and 49.3 percent orange and the eyeless 51.9 percent black and 48.1 percent orange with an insignificant deviation of 1.89 ± 1.69 percent. Eye-color and the factor for eyeless segregate independently, but here as in the case of bar-eye, the eyeless sons are not represented in the same proportion as the normal eyed sons as would be the expectation. The second line of table 10 shows that if we assume the theoretical population to be twice the number of normal eyed sons only 46.87 percent of the expected eyeless individuals were observed, or that 53.13 percent fail to come to maturity.

The third and fourth lines of the table show the independent segregation of eyeless and the factor for defective wing venation. The next two lines, however, seem to show a significant deviation from the expected 50 percent in both the normal and eyeless sons for reduced and normal wing. In this group are the F_1 males from 76 females obtained from crossing normal eyed reduced males with eyeless normal winged females, and the difference between the expected and observed number of reduced individuals in both classes, here, as in the case of bar, is probably due to the failure of some of the reduced individuals to compete successfully with the normals during development.

Bar-eyed males mated to eyeless females produced normal daughters.

The progeny of 32 of these normal appearing F_1 females are shown in table 8. There were 132 normal sons, 70 eyeless, 86 bar-eye, and none which could be classed as bar-eyeless. These normal males each mated to a heterozygous bar and a homozygous eyeless female produced only normal daughters. Furthermore, if the fact that less than 50 percent of the expected eyeless individuals hatch and only 62 percent of the bar-eyed males, is taken into account, these three groups are seen to be represented in a 1 to 1 to 1 ratio as would be the expectation if bar and eyeless were independent and the fourth group bar eyeless was lethal.

There were, in addition, 58 individuals observed among the treated group and their descendants which were characterized by the absence of a part of the median vein of both primary wings (figure 10). It bore no direct relationship to the presence or absence of the factor for defect in the r_4 vein of the wing. Six of the eleven tested produced descendants with a similar defect in the median vein of the wing. The progeny like the original defective-winged individuals were highly sterile, not very vigorous, and too few in number to tell how the character was inherited. Some of the original defective-winged individuals were females so it seems very improbable that they represent recessive mutations although the F_1 females from some of the males produced a few sons with a similar defect in the median vein. The same is true of the 47 individuals with fused antennae (figure 13) and 121 vestigial wing (figure 14) individuals. Whether these are genetic changes or non-inherited somatic irregularities induced by the treatment, it is of interest to note that the directly X-rayed and each generation of their descendants showed a statistically significant excess over the controls of fused antennae, defective median vein, and vestigial winged individuals. A miscellaneous group included 23 individuals with abnormal abdomens, 23 with abnormal heads, 2 with abnormal thoraces, 3 wingless (figure 15) which may have been extreme vestigial wings, 4 with notched wings, 1 male with red eyes and 2 males with cream-colored eyes, all of which were sterile or too weak to mate.

There were also 23 sex mosaics similar in somatic appearance to the gynandromorphs reported by WHITING (1928c). Five were predominantly female, 2 predominantly male, 6 in which one-half the head and abdomen were male and the other half of the head was female, 8 in which the head was female and the abdomen male, 1 with half the head male, the rest of the head and abdomen being female and 1 with a male head and female abdomen. The individual with a half a male head and the abdomen and other half of the head female had 17 normal sons and 9 normal daughters. All of the other sex mosaics were sterile. The directly X-rayed individuals showed

an increase over the controls in percent of sex mosaics of 0.057 ± 0.021 percent or a difference 2.7 times its probable error. The descendants did not show an excess over the controls except in the third generation.

There was a general increase in effectiveness of the treatment from 1200 Roentgen units to 8000. Those treated with 1200 R units showed an increase over the controls of individuals showing somatic abnormalities of 1.54 ± 0.12 percent, those treated with 2400 R units showed an increase of 2.28 ± 0.16 percent of abnormal individuals and those treated with 8000 R units an increased percentage of 10.35 ± 0.27 percent. The percent treated $\div$ percent control for the first group is $1.67 \div 0.14$ or 12.4, for the second group $2.41 \div 0.14$ or 17.9, and for the third $10.48 \div 0.14$ or 77.7 times that for the controls.

THE EFFECT OF VARIOUS DOSES OF X-RAY RADIATION ON THE FERTILITY OF THE DIRECTLY X-RAYED INDIVIDUALS AND THE FIRST, SECOND, THIRD AND LATER GENERATIONS OF THEIR DESCENDANTS

A comparison of the number of progeny per fertile mating for the control and the respective treated group is found in table 11. From 832 fertile control matings the mean number of progeny was 25.11 ± 0.61. The mean number of progeny from 198 directly X-rayed matings is 12.12 ± 0.50. The difference between the mean for the controls and the directly X-rayed is 12.99 ± 0.79 and less than half as many progeny are seen to hatch from the directly X-rayed matings as from the untreated. The first

TABLE 11

Showing the effect of X-ray radiation on number of progeny per fertile mating in directly X-rayed, 1, 2, 3 and later generations from the treatment.

GROUP	NUMBER OF FERTILE MATINGS	MEAN NUMBER OF PROGENY PER MATING ± P.E.	DIFFERENCE BETWEEN TREATED AND CONTROLS IN MEAN NUMBER PROGENY	DIFFERENCE ÷ P.E. DIFFERENCE
Controls	832	25.114 ± 0.610	..	..
Directly X-rayed	198	12.121 ± 0.498	12.993 ± 0.787	16.51
Generation 1 from X-ray	640	16.109 ± 0.404	9.005 ± 0.731	12.32
Generation 2 from X-ray	438	16.826 ± 0.434	8.288 ± 0.748	11.08
Generation 3 from X-ray	288	16.233 ± 0.521	8.881 ± 0.802	11.08
X-rayed stock	1398	14.145 ± 0.228	10.969 ± 0.651	16.85

generation of descendants from the treatment showed a difference from the controls in mean number of progeny of 9.00 ± 0.73. The mean for the second generation differs from the mean for the controls by 8.29 ± 0.75 and

that for the third generation by 8.88 ± 0.80 or essentially the same difference. The 1398 fertile females from all the later generations show a difference from the mean of 10.97 ± 0.65 which is even slightly greater than that for the three preceding generations.

All of the factors which contribute to this reduced fertility in the treated females and their descendants are not known. Some of the reduction in fertility may be due to the occurrence of lethal mutations in chromosomes which are not yet marked by any genetic character. The number of progeny for each female is based on the number which actually come to maturity, and some of the decrease in number is due to the failure of some of the progeny to come through after it has reached the pupal stage as will be discussed in the following section. No record has been kept of the number of eggs laid by these treated females so that what part of the reduction in number of hatched progeny is due to the failure of eggs which have been laid to reach the pupal stage and what part is due to a reduction in number of eggs laid is not known.

All of the treatments over 1600 Roentgen units significantly decreased the percentage of fertile matings among the directly X-rayed and their descendants. There were 95 sterile out of a total of 927 control matings or 89.8 percent were fertile. Of those treated with 1694 R units 161 out of 208 or 77.4 percent were fertile. The difference -12.35 ± 1.71 percent is 7.2 times its probable error. Of those treated with 3850 R units 167 or 58.6 percent of 285 matings were fertile. This difference is -31.16 ± 1.74 percent or 17.9 times its probable error. Of those treated with 8000 R units only 14 out of 32 or 43.8 percent were fertile. The difference or -46.0 ± 3.9 percent is 11.8 times its probable error.

THE EFFECT OF VARIOUS DOSES OF X-RAY ON THE VIABILITY OF THE TREATED INDIVIDUALS AND THEIR DESCENDANTS

The effect of the various doses of X-ray employed on the viability of the treated individuals and their descendants was measured by the proportion of the pupae which completed their metamorphosis. These data were obtained by counting the hatched individuals (checked by count of the empty pupae cases) and the pupae cases containing dried incompletely metamorphosed pupae. From the 832 fertile control matings 23,054 pupae were observed of which 20,436 or 88.6 percent were hatched. This percentage of viable pupae was significantly higher than that observed for any of the treated groups. The percentage of pupae hatched varied from 82.1 percent for those treated with 1440 R units to 65.6 percent for those treated with 2400 R units. Seventy-six and three tenths of those treated with

4000 R units hatched. This group includes the progeny of the 50 adults which were treated. Seventy-four and nine tenths of those treated with 4800 R units, 71.6 percent of those treated with 6000, and 68.7 percent of those treated with 8000 R units hatched.

A part of the effect on the viability of the directly X-rayed individuals is undoubtedly due to mechanical injury imposed on the larvae during preparation for the treatment. It is difficult to avoid such injury when it is necessary to handle these larvae during the early stages of their development. That this mechanical injury is a factor of the decreased viability of the directly X-rayed group is indicated when the results for the 96 divided matings are compared with those for the general controls. The larvae from these 96 matings were separated into a treated and control group, the latter differing from the general controls only in the fact that they had been handled exactly the same way as their treated sibs. Table 12 shows that the

TABLE 12

Showing for the divided matings the effect on viability of mechanical injury and of X-ray radiation.

GROUP	NUMBER OF FERTILE MATINGS	TOTAL PROGENY WHICH PUPATED	NUMBER HATCHED	PERCENT HATCHED	DIFFERENCE FROM CONTROL IN PERCENT HATCHED ± P.E.	DIFFERENCE ÷ P.E.	DIFFERENCE FROM CONTROL ½ MATINGS	DIFFERENCE ÷ P. E. DIFFERENCE
Controls	832	23054	20435	88.644	..	..	..	..
Control ½ divided matings	96	1351	1068	79.052	−9.592±0.611	15.70	..	..
Treated ½ divided matings	96	1462	924	63.201	−25.443±0.609	41.78	−15.851 ±1.157	13.70

control half matings had a significantly lower proportion of viable pupae than the group of general controls and that the treated half matings differed from the general controls by 25.4 percent, and from their untreated sibs by -15.85 ± 1.16 percent or a difference which was 13.7 times its probable error. A decreased viability of the descendants of treated individuals was a relatively constant feature. It was observed in the matings where all the hatched progeny appeared normal and is characteristic for the strains derived from the mutants produced by X-ray as has been observed for bar and eyeless in table 10.

DISCUSSION

The effectiveness of X-rays for modifying germ-plasm discovered by MULLER (1927) in Drosophila has been confirmed by WEINSTEIN (1928)

and others, for producing somatic mutations by PATTERSON (1927), demonstrated for radium in the same species by HANSON (1928), and independently demonstrated by STADLER (1928) for barley and maize. The WHITINGS (WHITING 1928a, 1929, WHITING and WHITING 1929, and WHITING 1930) have found X-rays effective in producing visible mutations, somatic irregularities, and reduced fertility in Habrobracon.

From the present experiment, it appears that mutations occur at a significantly higher rate among the directly X-rayed individuals than among the controls, at the orange-ivory eye-color, reduced wing, and eyeless loci, and that the bar locus shows an increase in percent of mutations in the same direction. All these mutations were observed as mosaics which is in keeping with the results of PATTERSON (1929) and GOWEN (1929) from treating larval stages of Drosophila with X-rays except that 5 of the 7 eye-color mosaics were observed in haploid males.

The descendants of the treated individuals also show an increased proportion of eye-color mosaics, bar, and eyeless mutations. In the case of the eye-color mosaics and the bar mutations the increase over the controls is statistically significant for the first, second, third and later generations. Some of the eye-color mosaics may have resulted from the fusion of two oocytes or a refusion of the polar body with the egg nucleus (WHITING and WHITING 1927). If we consider only the "orange" appearing mutants from black-ivory heterozygous females the increase in percentage over the controls is statistically significant only for the directly X-rayed and the second generation of descendants, but the first and third generations also show an increase in the same direction. If these are truly mosaic for black and ivory as has been suggested by WHITING for similar mosaics from black-ivory heterozygous females, they may have resulted from binucleate eggs. It would be of interest if it could be demonstrated that the treatment was effective in promoting the fusion of the oocytes in the descendants of the treated individuals, but, as pointed out in the text, there is at present no evidence that any of these individuals were mosaics of black and ivory, and some proved to be true orange mutations. In the case of the bar and eyeless mutations the majority proved to be true genic mutations and could not have arisen from binucleate eggs since the parents in neither case were heterozygous for these recessive factors.

Therefore, it seems probable that the appearance of orange ivory eye-color, bar and eyeless mutants among the descendants of the treated individuals is due to a process of somatic mutation stimulated by the original treatment, or the gene is a compound of elements, a part of which may be affected by the X-rays, and that these mutants, many of which are mosaic

either phenotypically or genotypically, are the result of the sorting out of mutant and non-mutant elements in the soma and germ cells of the later generations. MULLER (1928) on the other hand finds that mutations do not appear at a significantly higher rate in the descendants of the treated Drosophila than in the controls.

A significantly higher proportion of individuals showing defective and vestigial wings, and fused antennae and other somatic abnormalities which could not be tested was observed among the directly X-rayed and their descendants. These may represent either genetic changes or non-inherited somatic irregularities, but the stimulus to produce them was maintained by the descendants of the treated individuals for several successive generations as was a reduced fertility and a decreased viability.

SUMMARY

1. X-ray radiation is shown to be effective in increasing the mutation rate at the orange-ivory eye-color, reduced wing, bar and eyeless loci in the directly X-rayed individuals of the Parasitic Wasp *Habrobracon juglandis* (Ashmead).

2. The descendants of the directly X-rayed individuals for several successive generations show a statistically significant increase over the controls of individuals mosaic for eye-color.

3. The descendants of the treated individuals also show an increase in percent of bar and eyeless mutations.

4. There was a statistically significant increase over the controls in percent of individuals with fused antennae, a defect in the median vein of the wing, vestigial wing and other somatic abnormalities among the directly X-rayed and their descendants.

5. With an increase of intensity from 1200 to 8000 Roentgen units there was a progressive increase in the proportion of individuals showing somatic abnormalities, and a general reduction in fertility and decrease in viability.

6. The maximum treatment or 8000 Roentgen units increased the production of visible character changes including genic mutations and somatic abnormalities to 77.7 times that for the controls; reduced the percentage of fertile matings 46.0 percent, and decreased the proportion of pupae which hatched to 68.7 percent.

7. A significant decrease in fertility and reduction in viability was observed for the descendants of the treated individuals.

8. Two new recessive factors, bar, a factor causing a reduction in size of the compound eyes, and eyeless, causing the complete absence of the

compound eyes, were produced by X-ray and appeared to be independent of each other and of the factors for orange-ivory eye-color, defective, and reduced wing.

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