The results herein recorded indicate the extreme capriciousness of the method recommended by Guilliermond. The success of the method seems to be a matter largely of chance or depends upon factors which are as yet unknown.

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SPECIAL ARTICLES

THE EFFECT OF X-RAYS IN PRODUCING RETURN GENE MUTATIONS[1]

MOST of the natural mutations known in Drosophila are to the recessive condition. With the exceptions of the reversions from bar eye to full eye and possible return mutations at the white-eye locus there is but scant evidence that this is a reversible reaction.

Muller found that gene mutations produced by X-rays are, in general, in the same direction and of the same nature as those occurring spontaneously in the fruit fly. In the numerous mutations arising in his recent experiments as a result of irradiation Muller has only two cases of return mutations—both involving the same factor locus, *scute*. This raises the problem of why it is more difficult to find mutations in one direction than in another.

Mutations by X-rays are also fortuitous or chance occurrences at the present time. The operator may be likened to a hunter shooting birdshot into a flock of ducks. As the hunter "accepts with natural piety" what comes down, so the investigator shooting X-rays into a flock of genes accepts what is given. For it is impossible to aim at any particular gene at the present time.

However, in spite of the infrequency of return mutations to the normal condition and the impossibility of controlling results, it appeared to the writer that an experiment carried out on sufficiently large scale might give the mutation rate of mutant genes to normal and the relative frequency with which certain specific genes are hit.

The problem then was: Return mutations at specific loci due to the action of X-rays.

The males used in the experiment carried five mutant genes in their X or sex chromosome; those for yellow body, white eye, forked bristles, bar eye and Beadex wings. The first three of these are recessive to normal, the other two are dominants. Part of these males were exposed to X-rays, using a dosage of 50 K. V.; 5 M. A. M.; 15 cm from the target and forty-eight minutes' exposure (known in Muller's Lab. as the T-4 treatment). The remainder were treated for twice this length of time (T-8). During treatment the flies were placed in gelatin capsules punctured by a fine needle to admit air.

Immediately following treatment these males were mated to virgin double-X yellow females. These females are peculiar in that the two sex chromosomes are attached at the right hand end and go together into the same gamete, which is equivalent to 100 per cent. non-disjunction. The double-X yellow females also carry a male Y-chromosome. In such a cross the sons get their X-chromosome from their fathers and their Y-chromosome from their mothers, a reversal of the usual procedure in this species. There are several advantages in using a stock made up in this way. Practically all mutations occurring in the sex chromosome of the treated fathers show up in the first generation of sons, whether recessive or dominant, as they are not covered by normal allelomorphs in the Y-chromosome.

One thousand such virgins were mated to irradiated males, two pairs to a bottle. This gave five hundred bottles and after seven days the parents were transferred to new bottles and remained there for seven days more. This gave one thousand bottles of offspring among which to look for changes in the five specific loci described above. Such heavy dosages of X-rays as were used in this experiment decrease productivity to a marked extent. The writer has shown elsewhere that following dosages of the magnitude used here only 12 per cent. of the eggs laid complete their life history.

The following table gives the count of the young hatching in the one thousand mating bottles. The average young per bottle was only 10.7 per cent. Many bottles contained no offspring at all.

	Males	Females	
T–4	3,796	4,811	
T–8	866	1,243	
	4,662	6,054	10,716

Gene mutations apparently are produced by a dosage which is just under that rendering complete sterility.

While an experiment involving one thousand mating bottles is not exactly small in scale the poor viability of rayed males reduces the offspring to a point where extensive results could hardly be expected. This defect is being remedied by a repetition of the work on an even larger scale. However, the results secured at the bar gene locus throw considerable light on a mooted question and seem worthy of record at the present time.

[1] This work was done in the Zoological Laboratory of the University of Texas during a recent sabbatical leave of absence from Washington University. My appreciation for the many courtesies received is hereby expressed to Professor J. T. Patterson and Professor H. J. Muller.

There were no return mutations at the yellow, white or forked loci. Considering the small number of offspring examined and the rarity of return mutations this is not a matter for surprise. There were eight cases of normal wings instead of the expected Beadex wings. These were either extreme overlaps of the beaded characters or somatic mutations. They did not breed true when mated to double-X yellow virgins, but gave again the beaded wings. There were four cases of reversion to full eye, and these bred true to full. Two were in the T-4 group and two in the T-8 group. This is a ratio of one reversion in 433 males in the T-8; one in 1,898 in the T-4. These are the results of the observations upon the five definite loci studied in the X-chromosome of the male offspring.

In addition, approximately one hundred other mutations, mosaics and abnormalities of various sorts were observed. These occurred in both males and females and affected all parts of the body. The strong temptation to preserve and breed everything that turned up was resisted and the original plan of rigidly concentrating on the five loci described above will be adhered to in further work now under way.

The four reversions to full eye at the bar locus are of considerable importance. Zeleny[2] has studied the bar-eye gene very extensively. He found that bar eye reverts to round eye about once in 1,600 times; and also discovered an allelomorph of bar, called ultra-bar, whose reversion rate is approximately the same. Zeleny considered the bar-eye character to be due to a gene mutation not different from other gene mutations in Drosophila. Upon evidence based on the sex ratio Zeleny concluded that mutations to full eye may occur in the male germ tract as well as in the female.

Sturtevant[3] assumes that Zeleny's data indicate that reversion occurs only in the female and reports extensive experiments which seem to show that reversions at the bar locus are due, not to gene changes at all, but rather to unequal crossing-over between the two X-chromosomes of the female. Hence no reversion in the male is possible, since no crossing-over occurs.

According to Sturtevant crossing-over in the bar region occurs in such a way that the respective points of interchange lie to the left of the bar locus in one chromosome, but to the right of it in the other one. Sturtevant made many crosses and all his data, compiled in nineteen tables, indicated that both reversion to round and to ultra-bar eye (called by him double-bar) was due to unequal crossing-over. He also made a count of 10,179 males derived from a cross with double-X yellow females, in which all males got their X-chromosome from the father, and in these males no rounds or double-bars were observed.

Furthermore, Muller and Dippel[4] counted 35,000 sons that had derived their X-chromosome from a bar-eyed father, and in this large number not a single case of reversion to full eye occurred. These results of Sturtevant and Muller and Dippel seemed to warrant the conclusion that mutations to round eye occurred exclusively in the female. And Sturtevant's experiments indicated that reversion to round eye was due to unequal crossing-over in the female and not to a gene mutation such as is responsible for character changes at other loci.

However, that reversions to round eye *are* possible without crossing-over seems proved by the work with X-rays. Muller (unpublished data) got two reversions to round eye in females in cases where there was no crossing-over near the bar locus (between forked and Beadex), one in control material and the other from lightly X-rayed flies. My results (described above) give one reversion to round in 433 males from heavily treated fathers and one in 1,898 males from fathers less heavily treated. These reversions were obtained under conditions which clearly rule out Sturtevant's theory of unequal crossing-over.

It is of interest that a mutation rate of one in 433 males is probably the highest rate of gene change yet reported in *Drosophila melanogaster*.

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[2] Zeleny, Charles, 1921. "The Direction and Frequency of Mutation in the Bar-eye Series of Multiple Allelomorphs of *Drosophila*," *Jour. Exp. Zool.*, 34: 203–233.

[3] Sturtevant, A. H., 1925. "The Effects of Unequal Crossing-over at the Bar Locus in *Drosophila*," *Genetics*, 10: 117–147.

[4] Muller, H. J., and Dippel, A. L., 1926. "Chromosome Breakage by X-rays and the Production of Eggs from Genetically Male Tissue in *Drosophila*," *British Jour. Exp. Biol.*, 3: 85–122.

A SPONTANEOUS MODIFICATION OF THE VISCOSITY OF FRESH BLOOD SERUM

IN order to study such unstable solutions as blood serum, it is important to dispose of methods which enable us to follow continuously the evolution of a phenomenon, as a function of time, or temperature, for example, without introducing uncontrollable or disturbing factors. The viscometer described in 1923 (based on the principle of coaxial cylinders) was devised in order to fulfil these requirements, and was used recently to determine the viscosity of fresh normal horse serum, as a function of time. It was found that such a serum, centrifuged immediately after separation from the clot and placed in the viscometer, behaved in the following way: At first, its viscosity, which is