

A COMPARISON OF THE FREQUENCIES OF VISIBLE MUTATIONS PRODUCED BY X-RAY TREATMENT IN DIFFERENT DEVELOPMENTAL STAGES OF DROSOPHILA

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INTRODUCTION

The problem of the production of mutations by artificial means has been partially solved. The scientific world was apprised of this fact when MULLER (1927) offered convincing evidence that irradiations are instrumental in producing heritable variations in *Drosophila*. Other investigators, experimenting with other forms of genetic material, have obtained similar results following treatment of the germ cells with irradiations. Thus STADLER, at the UNIVERSITY OF MISSOURI, induced gene mutations in barley and corn by means of X-rays and radium. Subsequently, P. W. and A. R. WHITING obtained positive results by the use of X-rays on *Habrobracon*. From the field of plant genetics BLAKESLEE and BUCHHOLZ offer a mass of interesting mutation results from X-rays and radium applied to the Jimson weed, *Datura*. The findings reported by the above, and many other workers who have resorted to the use of irradiations as a means of inducing changes in the gene, consist of an abundance of hereditary effects similar to every type of heritable variation obtained in untreated material. It can no longer be questioned that the effects which X-rays and radium produce upon genetic material are of the same nature as those produced by natural causes, whatever the natural causes may be.

In subsequent pages of this paper I hope to submit significant data upon the relative frequencies of the production of visible mutations in *Drosophila melanogaster* treated with X-rays at different stages of development, and to offer some evidence concerning the nature of the genic material of the chromosome.

MATERIALS AND METHODS

In the interest of simplicity it was deemed wise to use only those changes in the genes of the sex chromosomes of *Drosophila* as a criterion of the rate of mutation. Since most mutations are recessive to the normal, it is much easier to detect them if they occur in the sex chromosomes than if they are produced in the autosomes. The experiments were made critical by obtaining a recessive autosomal gene, in homozygous condition, in the treated flies. This permitted subsequent tests for contaminations.

To secure a stock suitable for the experiment, one male and one female, taken from a culture of brown-eyed flies were mated in a yeast culture. By this procedure a stock of flies was obtained having all the genes normal, except for the recessive mutant located in the second pair of chromosomes which is responsible for the brown eye-color. This latter gene, being homozygous in both males and females, was used as a marker against contamination of the cultures. Virgin females for testing the frequency of visible mutations produced by treating mature ova were obtained from this stock, treated with X-rays, and mated immediately to males, the X chromosome of which contained the mutant genes for scute, vermilion and forked. Any male offspring from this cross would receive its X chromosome from the treated mother and also the recessive gene for brown eyes. If a recessive, or a dominant mutation was produced in the sex chromosome, or a dominant in one of the autosomes, it would find expression in the male offspring receiving the mutant gene. When no mutations were produced, all the male offspring appeared normal.

In order to obtain information on the frequency with which mutations are induced by irradiating mature spermatozoa, young males were taken from the stock of brown (at the same time the virgins were obtained), treated with X-rays, and mated to yellow females having the X chromosomes attached together at the right hand ends and containing, in addition, a supernumerary Y chromosome. All male offspring from this cross resulted from the fertilization of eggs carrying the Y chromosome of the mothers by the X-bearing sperm cells of the treated fathers. These males also received one second chromosome from the treated male that contained the recessive gene for brown eye-color. Any abnormalities due to the mutation of genes in the treated X chromosome appeared only in the male offspring.

The method employed for securing immature germ cells for treatment was designed to enable the experimenter to procure masses of cells of a definite age,—theoretically in the same stages of development. Mass cultures of "brown" flies were made in vials. Yeast food was introduced into the vials on small paper spoons. Immediately after placing the flies in the vials a spoon of fresh food upon which a drop of dilute acetic acid had been spread to induce egg production, was inserted into each tube and allowed to remain for thirty to forty minutes. During this interval any fertilized eggs undergoing development in the oviducts of the females should have been deposited upon the food. At the end of this period those spoons were replaced by spoons of fresh food to which dilute acetic acid had been added. The females were permitted to deposit eggs upon this food for a period of one hour. The culture medium bearing the eggs was then removed from the spoons and placed in small culture dishes containing

yeast food where development of the larvae continued until the time for treatment. All developing individuals obtained by this method were within one hour of the same age. These developing cultures were irradiated at the various ages deemed desirable and the culture media transferred to pint milk bottles to await the development of imagoes. Males and females were separated before copulation occurred and the individuals were mated as in the case of treated adults.

Six experimental groups were examined for visible mutations: (1) females treated as freshly hatched adults, (2) females treated at the larval age of 71–72 hours, (3) females treated in the 35–36 hour larval stage, (4) males irradiated as adults, (5) males treated at 71–72 hours of age, and (6) males treated as 35–36 hour larvae. A series of controls consisting of untreated “brown” females mated to “scute-vermilion-forked” males, and “brown” males crossed to the yellow females, was reared as a check upon the mutation rate. Experiments upon each group were conducted simultaneously to avoid any unsuspected environmental influences and to distribute equally, among all the groups, any errors which might result from inexperience in observation.

All environmental conditions were controlled as accurately as possible. The age at which the flies were irradiated, or the stage of development of the germ cells at the time of treatment, was the only variable in the process. When variations appeared among male offspring of treated, or control individuals, the variant males were mated to a stock of females which gave all sons like the fathers with respect to the X chromosome.

EXPERIMENTAL RESULTS

Mutations

Frequency of Mutations in the X Chromosome

Experiments with which this paper treats were devised to insure constancy of all factors, except the stage of development of the germ cells at the time of irradiation. Since MULLER (1930) reported an absence of heritable variations when females were rayed while larvae three to four days old, it seems logical that the transmuting power of X-rays is largely if not entirely lost upon immature eggs. It was hoped that some cogent evidence upon the frequency of mutations in the sperm and egg might be obtained by observing large numbers of F_1 individuals from both males and females treated at the same time and with equal intensity. In other words, it offers the possibility of ascertaining the effect of sex upon mutability.

The sex-linked heritable variations and the calculated mutation rates expressed in percentage are tabulated in table 1. No more than a glance at the table is necessary to learn that no sex-linked heritable variations were found among the 12,633 F_1 male offspring of untreated control females.

Few more appeared among the 13,673 sons of the control males; only one sex-linked mutation was discovered. When the visible mutation rate is calculated for this group it appears as $.007 \pm .005$ percent, a very high natural mutation rate as compared with one of .002 percent based upon examinations of 20,000,000 flies (MULLER 1929).

TABLE 1

Mutation frequencies in the sex chromosome of treated and control flies and a comparison of mutation frequencies in sex.

SEX	AGE AT TIME OF TREATMENT							
	ADULTS		71-72 HOUR LARVAE		35-36 HOUR LARVAE		CONTROLS	
	MALE	FEMALE	MALE	FEMALE	MALE	FEMALE	MALE	FEMALE
Number of F ₁ flies	11,620	12,525	7,677	12,237	6,945	7,600	13,673	12,633
Number of sex-linked mutations	33	23	11	13	5	11	1	0
Percent of mutations	.282	.183	.143	.106	.070	.144	.007	.000
	$\pm .034$	$\pm .026$	$\pm .029$	$\pm .018$	$\pm .020$	$\pm .028$	$\pm .005$	$\pm .000$
Mutation frequency less control frequency	.276	.183	.136	.106	.063	.144	—	—
	$\pm .034$	$\pm .026$	$\pm .029$	$\pm .018$	$\pm .020$	$\pm .028$		
Difference divided by probable error	8.1	7.0	4.7	5.0	3.0	5.1	—	—
Difference in percent of mutations in sexes	$.093 \pm .042$		$.030 \pm .032$		$.081 \pm .034$			
Difference divided by probable error	2.21		0.94		2.39			

A perusal of the data from the treated groups is sufficiently convincing as to the effectiveness of X-rays in producing gene mutations even when administered in the light "dose" (1325 r units) employed. In the course of the experiment 11,620 F₁ males, offspring of treated adult males, were examined for mutations. Of the variant individuals detected, thirty-three contained heritable sex-linked mutations. Therefore, the rate of mutation of X chromosomal genes for this group appears as $.282 \pm .034$ percent, deviating from the percentage found in the control males by $.276 \pm .034$ percent. The mutations obtained by irradiating adult females were slightly, but not significantly, less numerous. The 12,525 male offspring of females treated as adults contained twenty-three individuals that bred true as sex-linked mutations. The twenty-three mutant individuals represent $.183 \pm .026$ percent of the total F₁ males.

A total of 7,677 F₁ males, offspring of males treated while larvae of from 71-72 hours of age, was examined for mutations. A large number of variant males were observed but only eleven, or $.143 \pm .029$ percent bred true as X chromosomal mutations. When the percentage of mutations detected in control males is deducted, the remainder is $.136 \pm .029$ percent, a differ-

ence 4.7 times the probable error. Females, treated as larvae of the same age, produced 12,237 F₁ males, thirteen of which were mutants. This number constitutes $.106 \pm .018$ percent in favor of the treated group. The difference, when comparison is made with the percentage found in controls, is 5 times its probable error.

Males treated at the larval age of 35–36 hours produced 6,945 F₁ male offspring. Of this number, five, or $.07 \pm .02$ percent developed from mutant sperm cells. This mutation percentage differs from that of the control males by $.062 \pm .0203$ percent. In this case the difference is only 3 times the probable error, a difference large enough to be generally accepted as significant. Females treated as 35–36 hour embryos gave 7,600 F₁ males, eleven, or $.144 \pm .028$ percent were mutants. Comparing this percentage to that found in control females the difference becomes 5.1 times the probable error and highly significant.

A Comparison of the Mutations in Sex

If the mutation rate obtained by treating a given group of males is compared with the rate observed when females of the same age are irradiated, no significant difference is observed. The comparisons of the sexes may be found in table 1. When the mutation frequency found for treated adult males is compared with the frequency obtained by testing females treated simultaneously, the difference is $.093 \pm .042$ percent; the difference, 2.21 times the probable error, is obviously without meaning. When the mutation frequency observed in males treated as 71–72 hour larvae is compared with the frequency occurring in females treated at the same age, the rate appears $.030 \pm .032$ percent higher in the males. The difference is less than the probable error. Males from larvae treated at 35–36 hours of age produced $.063 \pm .0203$ percent more mutant cells than were produced by the control males. Females, treated as larvae at the age of 35–36 hours, contained $.144 \pm .028$ percent more mutant germ cells than untreated controls. In this case irradiation failed to increase the rate of mutations more in one sex than in the other, the difference being $.081 \pm .034$ percent, or 2.39 times the probable error.

Comparison of the Mutation Frequencies of Different Groups of the Same Sex

In order to determine whether or not the age of the germ cell at the time of treatment plays any part in the mutability of the gene, the mutation frequencies of the different developmental groups of the same sex are compared. The results of these comparisons are briefly summarized in table 2. Males treated as adults gave $.140 \pm .044$ percent more mutations than males treated at the larval age of 71–72 hours. The difference, 3.20 times

the probable error seems to signify that mature sperm cells mutate more readily than immature cells of old larvae. The percentage of visible sex-linked mutations produced by irradiating adult males is $.213 \pm .037$ percent higher than the percentage found when males are treated as 35-36 hour larvae. This difference, 5.6 times the error, is evidence of the detectable

TABLE 2
A comparison of the mutation frequencies of the different treated groups.

AGE AT TIME OF TREATMENT	SEX	PERCENT MUTATIONS BY X-RAYS	ADULTS COMPARED WITH 71-72 AND 35-36 HOUR LARVAE	35-36 HOUR COMPARED WITH 71-72 HOUR LARVAE	DIFFERENCE DIVIDED BY PROBABLE ERROR
71-72 hour larvae	Male	$.136 \pm .029$			
Adults	Male	$.276 \pm .034$	$.140 \pm .044$	$.072 \pm .035$	3.20
			$.213 \pm .037$		5.75
35-36 hour larvae	Male	$.063 \pm .020$			
71-72 hour larvae	Female	$.106 \pm .018$			
Adults	Female	$.186 \pm .026$	$.077 \pm .030$	$.035 \pm .031$	2.56
			$.042 \pm .037$		1.13
35-36 hour larvae	Female	$.144 \pm .028$			1.14

increase of mutability of the gene residing in a mature sperm cell. If the mutability of the germ plasm of the two groups of males reared as larvae are compared, no difference in the mutation frequencies is found.

An examination of the mutation frequencies produced by treating females in different stages of development reveals no differences in the frequency of gene mutation, irrespective of the stage of development irradiated. Details of the various mutation frequencies may be obtained from the table.

"Partial" and "Radical" Mutations

The term "partial" mutation, as applied herein, refers to cases in which the mutation failed to show in all parts of the body of the organism that should have been affected by the mutant character; to individuals having mutant somatic cells but normal germinal tissues; or to flies which produced part mutant and part normal offspring. According to definition those flies apparently having the tissue mutant, and giving only mutant offspring if not sterile, are considered as "radical" mutations. The terms "partial" and "radical" are employed since they are descriptive of the amount of

genic material undergoing change at a given locus. In case a "radical" mutation occurs, all the genes at a given locus of the various chromatin threads of the chromosome undergo mutation, while a "partial" mutation involves only a part of the chromatin at any given locus.

"Partial" mutations were brought to attention by phenotypically mosaic individuals which bred as "radical," or complete mutations—giving offspring which showed the mutant character in all regions of the body in which the mutation was capable of phenotypic expression. For example, one male, obtained from mating a treated male to a female having attached X's and a Y chromosome, was selected for breeding because of the absence of the right postvertical bristle. All other bristles were present. All the male offspring produced by the mating of this male to a double yellow female showed scute-1. In this case the somatic cells covering the area of scute-1, except for the region of the right postvertical, were normal but all the germ cells contained the mutant gene. Bearing in mind that only the variations appearing phenotypically the same as those known to be dependent upon gene mutation are to be considered, it is possible to divide the anomalous individuals into groups of "radical" mutants, one-half mutant, one-fourth mutant, and "minute" mutations (individuals in which the character is visible in less than one-fourth the somatic tissues capable of expressing the mutant gene). The proportion of "partials" to "radicals" may then be obtained; the sterility of the types compared, and a comparison may be made of the numbers breeding true in each group. Table 3 is a composite of all the data obtained from the different groups.

TABLE 3
Classification of variants obtained from treated groups.

TIME OF TREATMENT	"RADICAL" MUTATIONS			"PARTIAL" MUTATIONS											
				1/2-MUTANT				1/4-MUTANT				"MINUTE" MUTATIONS			
	NO.	ST.	T.	NO.	ST.	T.	N.T.	NO.	ST.	T.	N.T.	NO.	ST.	T.	N.T.
Adult males	98	59	39	103	21	11	71	4	0	1	3	8	0	1	7
Adult females	77	55	22	92	20	12	60	3	1	0	2	6	3	0	3
71-72 hour males	27	15	12	29	9	0	20					2	1	0	1
71-72 hour females	34	26	8	42	7	2	33	2	0	2	0	5	0	0	5
35-36 hour males	18	11	7	22	8	2	12	2	1	0	1	1	0	0	1
35-36 hour females	27	17	10	25	4	1	20					4	1	1	2

No.—Number of cases; St.—Sterile; T.—Bred true for mutant character, and N.T.—Failed to breed true.

DISCUSSION

Mention has been made of the production of visible mutations in the X chromosome and the percent of mutations per treated group has been

noted in the foregoing section. By way of introducing a discussion of the mutation frequencies we may refer to table 1 for a review of the data. The mutation rate is increased in all the treated groups. The slightest effect is noticed when males are treated as 35–36 hour larvae, but the rate of mutations observed among offspring of this irradiated group is sufficiently high to be significant, the rate differing from that of the control males by $.063 \pm .0203$ percent,—a difference 3 times the probable error. A much greater increase in the mutation rate is observed when adult males are treated. The percentage of mutations found among offspring of males irradiated as adults is $.276 \pm .034$ percent above that of the control males. This figure is 8.1 times the probable error. As may be ascertained from table 1, the mutation rates found for all other groups are significantly higher than the rate observed in the control groups.

It is interesting to note that HANSON (1928) reported a visible mutation rate, obtained by treating mature males with a dose of X-rays equivalent to that employed by the writer, ten times that herein reported for males treated as adults. The apparently conflicting results can be brought to agreement by considering, in this experiment, the gene mutations that bred as such, irregularities resulting from chromosomal abnormalities, the anomalous sterile flies, and the aberrant flies that bred as normals under the heading of visible mutations. When such classes are grouped as visible mutations the rate becomes practically the same as that found by HANSON.

It has been observed repeatedly that the rate of induction of lethal mutations in mature sperm is much higher than in spermatogonia treated in the adult male but no comparative results, obtained by irradiating different developmental stages of cells in the female, have been reported. The visible mutations of the present experiment throw light upon the relative susceptibility of genic material, in different stages of development, to the transmuting power of irradiations. In male cells the rate of induction is apparently much higher when the mature cells are treated than if the immature cells are subjected to irradiations. Investigation of the sterility produced in flies treated as adults and in those irradiated while in the larval stages aids materially in explaining the increased rate of induction in adult males as compared with the transmutation frequency in immature cells of the male. The percentage of sterility among male flies treated as larvae is found to be 52 ± 1.7 percent when males are X-rayed as 71–72 hour larvae, and 55 ± 1.5 percent when males are irradiated at the larval age of 35–36 hours. Obviously, the sterility of the two groups treated as larvae is very high compared with 2 ± 1.7 percent of sterility observed among treated adults. Since there is good reason to believe that sterility is associated with physical variation (unpublished data) and is very likely linked with gene mutation or chromosomal aberration, it seems plausible to con-

clude that much of the gonadic tissue of sterile individuals is composed of mutant cells. It is also probable that the low rate observed when early sperm cells were treated is partially due to a differential reduplication of non-mutated as compared to mutated gonial cells. If the reduplication of non-mutant and mutant cells had proceeded at the same rates and if it had been possible for the sterile individuals to reproduce, many more mutant cells would have been detected among offspring of males treated as larvae, raising the mutation frequencies to the same (or perhaps a higher) level as that observed when adult males were irradiated. If the mutation rate observed for females, treated as adults, is compared with that induced by irradiating either larval stage, no importance can be attached to the slight difference in the rates. This lack of variation is satisfactorily explained by the fact that female germ cells contain two X chromosomes that protect them against differential reduplication. The differences existing between the mutation rate induced by raying adult males and the rate observed when females are treated as 71-72 hour, or 35-36 hour larvae are just within the bounds of significance. If sterility had been less frequent in these larval groups the induced mutation rates would probably have been approximately equal in value to that obtained by treating mature sperm cells. Hence, one can safely conclude that the genic material of a cell is equally susceptible to the transmuting effects of irradiations irrespective of the sex, or stage of development at the time of treatment, but associated factors render the detection of all mutations impossible when immature germ cells are irradiated. It is interesting to note that the results upon which this conclusion is based are in conflict with the findings of MULLER (1930) who reported that "the rate of induction in mature sperm is higher than that in adult females."

As regards the chromatin content, the question of the morphology of the chromosome in the mature germ cell is of extreme interest. KAUFMANN (1925) describes the chromosome of *Tradescantia pilosa* in somatic metaphase, insofar as the chromatic material is concerned, as a four-parted structure, a pair of parallel halves each containing duality of elements. NEBEL (1932), working with the same form, observed four chromonemata in each dyad of the meiotic metaphase. Our knowledge of the subsequent behavior, in the meiotic divisions, leaves doubt as to the number of chromonemata, or duplicate genes contained by the chromosomes of the mature sex cells. If there is no reduplication of chromonemata following the maturation divisions, the chromosomes of the mature sex cells of *Tradescantia* should contain two chromatin threads, or a duplicate set of genes. In case reduplication is the rule, four chromonemata, or the equivalent genic complex should be present in each chromosome of the mature sex cells. The cytological findings in *Tradescantia* stimulate interest as to

the conditions existing in *Drosophila*. KAUFMANN, in 1931, was unable to follow the chromonemata of *Drosophila* chromosomes through the various stages of cell division, but observed the presence of more than one chromatin thread. PATTERSON, in 1933, working on the production of mosaic flies by breaking the X chromosome of *Drosophila*, concludes that the X chromosome of the sperm cell is split in about one out of every seven sperms, and that the chromosome of the egg is a single thread at the time of treatment.

The results tabulated in table 3 offer a basis for the solution of the problem as to the genic complex in the X chromosome of *Drosophila*. The first line of table 3 records the data on mutations obtained from treated adult males. It has been pointed out that the various mutations, or variations, are divided into four distinct groups. The criteria for the classification have been explained. According to the data from treated adult males, ninety-eight "radical" mutations were detected. The sterile individuals (59) constitute approximately 60 percent of the total, while thirty-nine, or about 40 percent of the flies bred true. Among the "partial" mutations, 103 individuals were one-half mutant, of which twenty-one were sterile, eleven bred as mutant and seventy-one reproduced the normal type. Four quarter-mutants and eight "minute" mutations are listed. The number of "partial" mutations totals 115, thirteen of which bred true. This is interpreted to mean that two sets of genes are present in the X chromosome of the sperm at the time of treatment. Since nothing definite is known concerning the way irradiations affect the gene, one cannot predict the ratio of "radical" to "partial" mutants that should be obtained if the chromosome contains two chromonemata, or a duplicate set of genes. The ratio depends entirely upon the probability of single and simultaneous mutations at a given locus. However, it is impossible to account for the large proportion of individuals expressing the variations in one-half the tissues on the basis of a single thread of genes. Even the small number of flies that appear phenotypically "partial" and breed true, either as mosaics or as "radical" mutations, cannot be accounted for by such a simple assumption. Further, if more than duplicate genes exist in the chromosome of the sperm cells, many mosaics would occur with one-fourth, or less, of the tissues mutant. Actually twelve such cases were obtained from males treated as adults. Therefore, no alternative is at hand. An explanation of the results rests upon the assumption of a chromosome, the chromatic element of which consists of two chromonemata, or the genic equivalent. Such an assumption offers a complete explanation of the phenotypically half and half mosaics. It also lends understanding to the "radical" aberrant cases if we admit the possibility of transmutations occurring simultaneously in allelic genes,—the simultaneous mutation of two genes occu-

pying allelic loci in the chromonemata. It is obvious that complete, or "radical" mutations occur, and since it is improbable that the chromatin material of the sperm cell assumes a definite configuration in one cell and an entirely different condition in another, there is no choice but to concede the production of simultaneous mutations of allelic elements. Only the twelve mosaic individuals having one-fourth, or less, of the tissues mutant remain to be explained. These offer no difficulty when we recall that HUETTNER (1926) reports irregular cleavage as occurring frequently in *Drosophila*, and that STURTEVANT (1929) found cleavage to be indeterminate in gynandromorphs of *Drosophila simulans*. The individuals under consideration probably started development as half and half mosaics, in so far as genetic content is concerned, and may be accounted for on the basis of indeterminate cleavage which permits unequal distribution of mutant cells as compared to the non-mutant cells. Therefore, all things considered, the premise that the mature sperm cells bear chromosomes composed of duplicate genic threads supplies the only condition that fulfills the genetic requirement.

If adult females are treated the chromosomes of the egg cells lie in the equatorial plate of the first meiotic metaphase at the time of irradiation. Obviously, if we postulate one set of genes per chromosome, the appearance of mosaic individuals becomes, *a priori*, an impossibility. Likewise, if we assume each chromosome of this stage to consist of two genic strands, no mosaic individuals can be produced by irradiation. If four chromonemata, or the genetic equivalent, are present in each dyad of the first meiotic metaphase plate, the genetic result, with respect to the production of "radical" mutations and mosaic flies should parallel the result obtained by treating mature sperm. As the data of table 3 show, 77 "radical" mutations were obtained from females treated as adults, 55 of which were sterile and 22 bred true. Approximately an equal number of one-half mutant flies are recorded. A total of 92 such individuals contain 20 sterile flies, 12 breeding as mutants and 60 that produced normal offspring. As in the case of treated males, comparatively few mosaic flies are recorded that show one-fourth, or less, of the body mutant. Nine such cases are present, one of which bred as a mutation. This result is almost identical with that obtained by treating the mature males with irradiation, and therefore, fulfills perfectly the requirements of the assumption that the dyad of the first meiotic metaphase is composed of four chromonemata.

It seems that no conflict exists between the concept of duplication of genes, or the postulated number of chromonemata, and the inferences of PATTERSON'S findings on chromosome breakage. According to the view of the writer, the two results are the expressions of entirely different phenomena. Chromosome breakage is a phenomenon of the matrix. It involves

the physical property of separating the material bonds of the matrix substance as well as severing the inter-connections of the genes. The question of chromosome structure as revealed by breakage is apparently a question of the condition of the matrix material. Chromosomes in which the matrix is split may be broken in only one arm of the divided chromosome, behaving subsequently as if two chromonemata are present. A break of an undivided matrix results in the severing of all the chromonemata, the behavior in subsequent inheritance suggesting a single chromatin thread. Two, four, or more genic threads may be present in the chromosome and yet appear as one or two depending upon the condition of the matrix at the time the break occurred. One might expect the matrix to be unsplit in some cells and split in others, or vesiculated, which, if broken through one hemisphere of the vesicle, would react as if the matrix were divided, but it seems improbable that the chromatic elements of the cells would present varied pictures. Hence, it seems probable that breakage is a phenomenon of the matrix, and mutation a function of the genes which lie in the chromonemata. This being the case the data obtained by breakage and that derived from mutations compel no conflicting conclusions.

A word as to the behavior of mosaic flies in breeding tests is necessary to explain the preponderance of phenotypic "partials" that produce only normal offspring. The behavior of the mosaic individuals obtained by treating adult males is typical and serves as an example for discussion. In view of the predictions of HUETTNER (1926) concerning sex mosaics and of the findings of DOBZHANSKY (1930) in gynandromorphs, one would expect the gonads of 50 percent of the so-called bilateral mosaic individuals to be composed of one type of tissue,—either mutant or non-mutant. Therefore, since variation is frequently accompanied by sterility, the sterile half-mutants plus the half-mosaics breeding as "radical" mutations, should constitute about one-fourth the total population of the group tabulated as one-half mutant. Actually, 21 of the 103 individuals of this type are sterile and 6 prove to contain only mutant tissue in the gonads. The total of 21 individuals is slightly more than one-fourth the total number of males classified as one-half mutant. Five individuals of the class prove to contain both mutant and non-mutant cells in the testes, whereas, approximately 50 individuals should produce both normal and mutant offspring, and about 25 should give normal flies. Part of the 45 males that should have produced both normal and mutant offspring due to a mixture of tissues in the reproductive glands, but which bred as normal, were probably carriers of chromosomal abnormalities which were lethal in hyperploid and hypoploid offspring. Offspring develop from the normal germ cells but the abnormal cells are incapable of forming viable zygotes. The behavior of a part of the irregular individuals may be attributed to dominant

lethals carried by a comparatively small number of cells of the mosaic fly. Normal tissue renders the mosaic viable but the mutants are lethal to the offspring receiving them. Recessive lethal mutations associated with visibles of the X chromosome will account for a portion of the irregular individuals. Considering all the possibilities, the results of breeding tests compare favorably with the expectations.

I wish to express appreciation to Professor J. T. PATTERSON for the suggesting of the problem and for his helpful advice concerning the experimental work. I also express thanks to Professor H. J. MULLER for suggestions of method.

CONCLUSIONS

1. Irradiations produce mutations in *Drosophila melanogaster* irrespective of the developmental stage treated, or of the sex of the individuals to which the X-rays are administered. There is no difference in the mutation frequency induced by irradiating females and that induced by the treatment of males in the same stage of development. The sex of the cell exerts no influence upon the mutability of the gene.

2. The detectable mutation frequency obtained by treating mature males, or mature females with X-rays is significantly higher than that observed when certain groups of males, or certain groups of females are treated as larvae. All factors considered, the gene is probably as mutable in one stage of development as in any other stage, but all mutations cannot be detected when produced in certain stages of the germ cells.

3. The chromosomes of mature sperm cells contain two complete sets of genes as chromonemata, or potential chromonemata. It appears that each monad of the reductional metaphase of the egg is composed of two complete genic complexes in the form of chromonema threads.

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