THE RELATIVELY HIGH FREQUENCY OF WHOLE-BODY MUTATIONS COMPARED WITH FRACTIONALS INDUCED BY X-RAYS IN DROSOPHILA SPERM¹

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MUTATIONS arising in the mature sperm cells of *Drosophila* might often be expected to be fractionals, on the assumption that they would, as a rule, involve only one strand of the double strand DNA molecule. The object of the present studies was to determine the proportion of whole-body *versus* fractional mutations induced by X-rays in the sperm of mature *Drosophila*, as well as the proportion arising under treatment by chemical mutagens and spontaneously for comparison.

METHODS

Three series of experiments were run. In the first, males were treated with 3000r X-rays and the F₁ females examined for mutant traits, as revealed by MULLER's "Maxy" technique (to be considered more fully below). In the second series, the adult males were injected abdominally with a chemical mutagen (one set with phenylalanine mustard, another with dimethyl myleran, and a third with 2,5 bis-ethyleneiminohydroquinone). In the spontaneous series the males received no treatment. Only sperm ejaculated during the first week after the male hatched was used in these studies. Hence only sperm cells treated in post-meiotic stages were sampled.

As above indicated, mutations were detected by means of the Maxy technique (MULLER 1954, 1955). This technique is a means of detecting visible mutations at 14 selected loci in the X chromosome of the male. Maxy females (shown below) have an X which carries the recessive alleles at these 14 loci. In addition, this X carries inversions (*sc*⁸¹ and *sc*⁸) and also *v*, which will be considered later, but which is not at one of the loci under examination for mutations. If we omit *sc*⁸¹, *sc*⁸, and *v*, the remaining alleles are the recessives at the loci under consideration. These recessives, with abnormal order due to inversions, are yellow (*y*), carnation (*car*), outstretched wings, small eyes (*odsy*), forked (*f*), garnet (*g*²) dusky (*dy*), raspberry (*ras*²), singed (*sn*³), cut (*ct*⁶), carmine (*cm*), ruby (*rb*), echinus (*ec*), white (*w*), and prune (*pn*). These 14 comprise virtually all the better known visibles in the X. Seven are for eye color and the rest for body color, wings, and bristles. The females receive the X with these recessives from their maternal parent. The other X is of paternal origin and ordinarily contains the normal

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alleles. Females homozygous for the paternal X (the one with the normal alleles) are inviable because of a lethal (l^{v1}). However, males with this chromosome survive because they have a special Y chromosome which contains the normal allele of l^{v1} . The recessive chromosome (of the females) gives a very weak phenotype because of all its recessives and in addition contains a lethal. Therefore, both males with this chromosome and females homozygous for it are inviable. Inversions prevent crossing over (sc^{s1} and sc^s in the recessive chromosome, $In(1)dl-49$ and B^{M1} in the one with the normal alleles). As a result of all this, the Maxy stock produces only two classes of flies (apart from the v locus, to be considered later): (1) females heterozygous for the X with the 14 recessives and an X with the corresponding normal alleles and (2) males having an X with the normal alleles; thus:

$$\frac{\gamma \ sc^{s1} \ car \ odsy \ f \ g^2 \ dy \ v \ ras^2 \ sn^2 \ ct^6 \ cm \ rb \ ec \ w \ pn \ sc^s}{l^{v1} \ sc^{s1+} \ In(1)dl-49 \ v^+ \ B^{M1}} \quad \text{and} \quad \frac{l^{v1} \ sc^{s1+} \ In(1)dl-49 \ v \ B^{M1}}{l^{v1+}}$$

Maxy female Maxy male

The X chromosome with the normal alleles at the loci under examination may contain either v or v^+ . These two kinds of X's (with v and v^+) alternate between males and females in successive generations. Thus, if the male had the X with v and the female the corresponding X chromosome with v^+ in one generation (as shown), then the two sexes would have the opposite X's in the next generation (female v and male v^+). The X with the 14 recessives (in the female) contains v . In one generation the females would therefore be homozygous for v and in the next heterozygous (and accordingly vermilion and red, respectively, in phenotype). This alternation of v and v^+ in successive generations is a means of detecting and guarding against nondisjunction.

Any mutations which show in an F_1 female at the 14 loci under examination would have arisen in an X of paternal origin (the chromosome with l^{v1}). If, for example, a mutation to white eyes had occurred in the paternal X, then the F_1 female would now be homozygous for white and would be white-eyed. Of course, if the mutation had been a fractional, then only part of the body would be mutant, as one eye white and the other normal.

The Maxy technique is unusually well suited to the detection of mosaics, since Maxy females are heterozygous at virtually all the better known visible loci in the X chromosome. This technique also makes possible the detection not only of ordinary visibles but also of visibles which have recessive lethal effects (and which might ordinarily escape detection) as well as isoalleles (which by themselves are of normal expression but which have a mutant expression in females heterozygous for them and the regular recessive allele). In the present studies mutant F_1 females (both whole-body and fractional) were genetically tested for the types of alleles here mentioned (ordinary visibles, visibles with recessive lethal effects, and isoalleles).

In the event that a mutation was a visible with a recessive lethal effect, it

would be lost before it could be identified (as to locus) if the mutant F_1 female were bred to a Maxy male, for then all F_2 offspring which received the visible lethal (from their F_1 mother) would be inviable: the males because of the lethal in question, and the females because of the l^{v1} in both their X's (as usual for homozygous l^{v1} females in the Maxy stock). The mutant F_1 females, therefore, have to be crossed to a male which does not contain the l^{v1} chromosome. In addition, the male should contain the normal allele of l^{v1} in his Y chromosome in order to allow F_2 males (with the l^{v1} chromosome) to survive in the event the visible is not a lethal. Therefore, in order to preserve newly arisen mutations for further testing, the F_1 mutant females were crossed to males which had the normal allele of l^{v1} in both their X and Y. In the present studies, the F_1 mutants were crossed to $sc^s.Y/X^{c2} \gamma f$ males (the $sc^s.Y$ of which it contains the normal allele of l^{v1}). From this cross, a balanced stock with the mutant chromosome of the F_1 female could be derived, and further tests could then be made to identify the locus of the mutant gene. For this latter purpose, "tester" stocks were used, each containing a mutant gene at one of the loci under examination. Thus, if an F_1 female had a mutant eye color, the balanced stock with the mutation was crossed to each of seven tester stocks, each having one of the mutant eye colors. If, now, one of these crosses produced, say, prune offspring when the tester stock was prune, then the new mutation would have been identified as prune.

A certain number of F_1 females of mutant appearance (both whole-body and mosaic) were sterile or died before they could be bred. It could, therefore, not be genetically proved that they contained mutant tissue.

A mutant with one of the darker eye colors (*car*, g^s , *ras*^s, *cm*, *rb*, or *pn*) is somewhat more difficult to identify than is a lighter (as white) in an otherwise red culture. However, vermilion sensitizes these darker mutants in that it makes them a distinct bright apricot which can readily be detected. If, in the Maxy technique, the male parent is vermilion, then the F_1 females are homozygous vermilion, and the darker eye color mutants (which now would be bright apricot) can more readily be identified than if the male had been v^+ (and the F_1 females heterozygous for v and v^+). In the X-ray and chemical series, only v males were treated; but in the spontaneous series, v^+ males were used as P_1 in about half the experiments when it was not yet realized that v sensitizes the darker eye color mutations.

In all three series of experiments, the P_1 were bred in mass cultures. In the X-ray series, from 100 to 200 Maxy males (less than 30-hours old) were irradiated at one time (3000r). They were immediately mated in mass to from 300 to 500 Maxy virgins and the cross was cultured in a vat (having the food capacity of about a dozen half-pint milk bottles). After three days the males were mated to fresh virgin females, and on the sixth day they were discarded thus insuring that only sperm of the first week were being sampled. A yield of from 2,500 to 8,500 F_1 daughters per experiment was obtained by this means, with the number of mutants appearing among them varying from one to five. Similar culture methods were employed in the other two series of experiments, except that bottles instead of vats were used. In these, the number of males tested in a given experiment was

usually smaller—not sufficient to utilize the large amount of food in a vat. The counts of F_1 females were based on careful weighing of all the dehydrated flies coming from a single mass culture, a time-saving method that has been found to be accurate to within three percent.

Whenever two or more F_1 mutant females of the same type were derived from the same male parents (in a single experiment) and were found to represent identical mutations, they were considered as being a cluster and were therefore regarded as representing a single mutation of premeiotic origin (in the male parent).

In the developing *Drosophila* egg, the first cleavage plane might be either longitudinal or transverse. Accordingly, it might divide the egg either into left and right halves, or into front and hind. In the case of a fractional mutation, mutant and normal tissue might be correspondingly distributed. Accordingly, in recording the mutants, a fractional was classified as “left-right” if, for example, one eye was white and the other red; as “front-hind” if both eyes were mutant but the gonads normal.

RESULTS

In the X-ray treated series, among a total of 157,000 F_1 females, there were 43 nonlethal, and 38 lethal, whole-body visibles which in both cases were “proved” in the sense that they bred and transmitted the mutation to their offspring (Table 1). The total proved, then, is 81. Of these, 68 (38 plus 30 in the table) were identified as to locus (the rest being lost before they could be further tested). In addition there were 32 F_1 females which died before they could be tested or were sterile but which to outward appearance were whole-body mutants. It is possible that some of these were fractionals of the “front-hind” type. However, as will be apparent later, there were probably very few, if any, of these front-hind mosaics in the X-ray series. If, then, we ignore them and regard all 32 of the mutants in the “died or sterile” column under discussion as whole-body, then the total whole-body mutant F_1 females in the X-ray series is 113. The total fractionals was nine, or about seven percent of the total mutants (Table 1). By contrast, in both the chemically treated and spontaneous series, the percent of identified fractionals was much higher (between 30 and 67 percent, Table 1).

In view of the small percent of fractionals in the X-ray series (seven percent), and further, since only part of these are of the front-hind type, it is not probable that there were more than one or two front-hind mosaics among the 32 mutants listed as whole-body in the “died or sterile” column in Table 1. In the chemical and spontaneous series, overlooked front-hind fractionals would have made the percent of fractionals even higher than listed in Table 1, and so would have made the difference between the X-ray and the other two series even greater than shown under “percent fractionals” in the table. However, there were comparatively few mutants in the “died and sterile” category in these two series, and therefore overlooked front-hind fractionals here would not significantly have changed the results as listed in Table 1.

In all three series, most of the mutants (at the visible loci under study) were

TABLE 1

Whole-body mutations versus fractionals in the X chromosome of Drosophila originating in post meiotic stages in the male

Series	No. F ₁ females	Whole-body				Fractionals							Percent fractional (of total mut.)
		Non- lethal	Lethal	Died or sterile	Total whole- body	Left-right†			Front- hind‡		Total frac- tional	Total mutants (wh-b & fr.)	
						Non- lethal	Lethal	Not trans- mitted	Died or sterile	Not trans- mitted			
X-rays (3000r)	157,000	43* (38)	38 (30)	32	113	1 (1)	0	2	2	4	9	122	7
Chemicals§													
(A)	24,000	3 (3)	5 (3)	3	11	3 (0)	1 (0)	5	1	2	12	23	52
(B)	17,000	2 (2)	7 (1)	4	13	3 (1)	5 (1)	6	2	10	26	39	67
(C)	31,000	6 (3)	11 (6)	4	21	1 (1)	1 (0)	3	0	4	9	30	30
Spon- taneous	77,000	2	1	1	4	1	0	3	1	2	7	11	64
	(<i>v</i> ⁺ / <i>v</i>)	(2)	(1)			(1)							
	74,000	5 (3)	5 (1)	3	13	1 (1)	3 (1)	7	1	1	13	26	50

* Total above; genetically identified below in parentheses.

† As, for example, one eye white, one red.

‡ As, for example, both eyes white, gonads normal.

§ (A) Phenylalanine mustard; (B) dimethyl myleran; (C) 2,5 bis-ethyleneiminohydroquinone.

|| All other F₁ females (those above and below) were ν/ν (which makes the darker eye colors bright apricot).

ordinary visibles, but there were also many visibles with lethal effects (between one third and one half of our total tested visibles), and the two kinds (nonlethal and lethal) were found in about the same proportion to each other among the whole-body and the fractional mutants. There were a few isoalleles.

The majority of the fractionals were left-right mosaics, but about a third of the total in the three series (23 in a total of 76) were front-hind. The finding of this segmental distribution of mutant tissue is in line with earlier work of others on gynanders, indicating that the first cleavage plane of the fertilized egg is usually longitudinal (into left and right halves of the body) but that it is sometimes transverse.

Clusters were found in all three series. In the X-ray series there were three (consisting of two, three, and 27 mutations of common origin). In the chemical series there were three (of six, two, and four). In the spontaneous series there was one cluster which was genetically identified as usual, and in addition there were two suspected clusters (of three and four) which were lost before they could be identified. The clusters in every instance were represented by F₁ females of whole-body mutant appearance, as was to be expected, since even if they had originated as fractionals, they would upon replication in the germ track of the male have become whole-body, in the sense that the mutant nucleotide base in the original DNA molecule would have attracted its complementary base in the

process of replication and would then no longer have been fractional. In any event, the clusters would represent spontaneous mutations of premeiotic origin and are therefore not included in the present studies.

Among the mutants that died before reproduction or were sterile, it was impossible to identify possible clusters. But since clusters are rather rare, these "died or sterile" are regarded as mutations of separate, postmeiotic origin (not clusters) and are included in Table 1.

Many variants for non-Maxy traits were found among the F_1 females of a kind not uncommonly seen in *Drosophila*. Some, probably partial gynanders, had one wing noticeably shorter than the other, or a smaller eye or thin, short bristles on one side (or combinations of these traits on one side). Other familiar variants were notch wings, crumpled wings, missing bristles, females with one half thorax missing, blisters on wings, deformed abdomens and bulging eyes. A few complete sex gynanders were found. None of these are included in the table.

DISCUSSION

The relative proportions of whole-body mutations to fractionals in X-ray and chemically treated material confirms the work of AUERBACH (1946) in which a more limited array of markers (mostly bristle mutations) was used.

The preponderance of whole-body mutations induced by X-rays poses a problem in connection with the WATSON-CRICK model of the DNA molecule, since, as previously indicated, it might offhand seem that as a rule only one strand of the double helix would be changed at a given level by X-rays; or if two, not both in a complementary manner. In either event, fractionals rather than whole-body mutations would be expected.

Conceivably, the mutations here being referred to as whole-body might be masked mosaics, in the sense that their superficial tissues and gonads might be mutant, but some underlying tissue normal. The mutant, therefore, would appear whole-body and test genetically as such, but actually would be a fractional. The proportion of fractionals which were masked mosaics would depend on the distribution of the nuclei in the segmenting egg; in particular, on whether or not a nucleus, formed at the first cleavage, went below the surface. The treatment of the male parent would probably have no influence on this proportion. Therefore, if, for example, one third of the fractionals in the chemical series were masked mosaics, then in the X-ray series one third would also be. In view of this fact, the percent of masked mosaics in the X-ray series would not have been very great, as the following consideration will show. In the chemical series, the classifiable mosaics were as high as 67 percent of the total mutants so that even if all remaining of the total were masked mosaics (leaving no whole-body at all), the masked mosaics could not have been over 33 percent of the total or about half as frequent as the classifiable mosaics. Thus, even if we increased the fractionals (classifiable) in the X-ray series by as much as one half (i.e., one half of seven percent to include the possible masked, making the total fractionals about 10.5 percent), this would still leave the whole-body mutants greatly in excess of the fractionals in

the X-ray series. We are then left with the problem of how to account for the high percent of the whole-body mutants in the X-ray series.

In order to account for the preponderance of whole-body mutations produced by X-rays, MULLER, CARLSON and SCHALET (1961) suggest that a single ion track (or even single ionization) might break the bonds holding the two bases of a nucleotide pair to the double strand DNA backbone, thereby allowing their "rotational substitution", i.e., the rotation and substitution of the base pair in a direction opposite from the original, so that now each base is in the strand formerly occupied by the complementary base. The result would be a whole-body mutation. A coincidence of the two breaks would not be so very unusual since single ion tracks are known to cause two breaks separated by even greater distances than those separating the bonds at opposite ends of a nucleotide pair.

The relatively high percent of fractionals in the spontaneous series confirms the high rate previously reported by SCHALET (1958). Studies on untreated sperm by SCHALET and MULLER have shown that the fractionals are carried for the most part by sperm ejaculated during the first 3-4 days after the male hatches, and that they arise mostly during the spermatid stage in the germ track, a stage which is especially mutagenic and which is prolonged for several days in the pupal stages, in the case of those spermatids which are going to give rise to the first formed sperm (those used during the first 3-4 days after the male hatches).

In all three series, most of the fractionals seemed to be one half mutant, rather than one fourth or less. If we are right in considering them one half mosaics, then, as pointed out by MULLER, they could hardly represent delayed effects leading to missteps in the replication of the DNA molecule at the first division of the fertilized egg, for then they would have been only one fourth mutant. In order to be one half mosaics they must have arisen as permanent changes in one of the strands of an already existing double strand DNA molecule of a postmeiotic chromosome of the male parent. This would apply to all three series, including the chemical. The mutagenic effects of chemicals are undoubtedly sometimes delayed, as demonstrated by AUERBACH. However, delayed effects would usually lead to mosaics which were mutant in less than one half of the body.

Our material was not internally examined for the possibility of mosaics that were less than one half mutant, and we have by no means excluded the possibility that some of our mutants were one fourth mosaics. In a fly with one white eye and one red, for example, it is possible that only one fourth of the body might be mutant, particularly if the gonads were normal as evidenced by the fact that the mutation was not transmitted. Among the left-right fractionals, in fact, out of a total of 46 left-right, 26 were not transmitted, and the great majority of these were for eye color. Even when the gonads contained mutant tissue, a left-right eye-color fractional might occasionally have been only a one fourth mosaic, for in *Drosophila* cell lineages are sometimes mixed up. The present evidence from mosaics therefore does not exclude the possibility that mutations might sometimes be due to errors in replication. However, it seems reasonable to assume that many of the apparent one half mosaics were actually such.

In summary, mutations in all three series (X-ray, chemical, and spontaneous)

agree in representing, in large part at least, permanent changes in an already existing DNA molecule in a spermatid or sperm cell (rather than transient changes which cause errors in replication at the division of the fertilized egg). However, the X-ray-induced mutations differ from the chemically-induced and the spontaneous in that the first (X-ray-induced) much more often are whole-body than are the chemically-induced and spontaneous.

We might account for the relatively small proportion of fractionals produced by X-rays on the assumption that when breakages of chemical bonds separate only one member of a base pair from a DNA molecule, the resulting loss would often be restituted since the complementary base would still be present to attract back the lost base (or another base of the same kind). The only remaining breakages would then be those which resulted in the separation of a base pair from a DNA molecule. These would give rise only to whole-body mutations, by rotational substitution.

However, if losses of single bases are often restituted, then we are left with the problem of how fractional mutations are produced, and in particular, how chemicals produce them. It is highly improbable that a chemical could cause a direct change of one base into another; that is, while the original base remained attached to the DNA molecule. More probably, it would first have to remove the base. If then, the base is removed, it would be restituted through the agency of the complementary base, with no resultant genetic change, as assumed in the case of X-ray-induced breakages. Substitutions of base analogues in the DNA molecule might conceivably produce mosaics, but in the present studies no such analogues were employed.

SUMMARY

Among the visible mutations of postmeiotic origin in the sperm cells of *Drosophila* treated with X-rays (3000r), 93 percent appeared to be whole-body, the remaining seven percent being fractionals. Among the mutations detected in a chemically treated series and those of postmeiotic spontaneous origin, the percent of fractionals was relatively high (30–67 percent). The high proportion of whole-body mutations in the X-ray treated series could be accounted for on the theory of MULLER, CARLSON and SCHALET that radiation may break the bonds of both bases of a nucleotide pair, thereby allowing their “rotational substitution” with resultant whole-body mutation.

In all three series the mutant tissue of the fractionals as a rule seemed to involve half of the body (not a quarter or less), so far as could be determined upon external examination of the fractionals. This half-body amount of mutant tissue indicates that these fractionals arise not as delayed effects followed by errors in replication at the first or later divisions of the fertilized egg (events which would lead to one quarter or less mutant tissue), but rather that the fractionals in question arise as permanent changes in an already existing DNA molecule, these observations thus supporting the recent conclusions of MULLER and his co-workers as to the usual nature of the mutational event. However, some of the mutants may

possibly have been one fourth mosaics. If they were, they would indicate that mutations might sometimes be due to an error in the replication of the gene.

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