FURTHER EVIDENCE FOR DIFFERENTIAL EFFECTS OF MUTAGENS IN *DROSOPHILA MELANOGASTER*

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1. Introduction

The early work on mutagenesis, mainly through the agency of radiation, seemed to indicate that the mutation process was random and indeterminate. This view is widely held by many geneticists, and its history has been recounted recently by Muller (1952, 1955). The analysis of mutagenesis in micro-organisms, however, did not support the principle of random mutability. The work of Demerec (1955) on *Escherichia coli* has revealed that the rate of reversion of several nutritional deficiency loci varied markedly under similar treatments with different mutagens. There was conclusive evidence also, that this differential response was a function of the genes themselves, rather than the cell environment, since there were no particular strains which were conducive to high, or low, mutation rates for all loci. The results with *Neurospora* were closely parallel to those in bacteria. Here again the rate of back-mutations of different loci, or even different alleles of the same locus, depended on the mutagen. This has been demonstrated for different radiations, such as ultra violet and X-rays (Giles, 1951), as well as for different chemical mutagens (Kölmark & Westergaard, 1953). That the differential response in *Neurospora* was a function of the locus has been shown by Kölmark (1953) on a double-mutant strain (adenineless and inositolless). Diepoxybutane proved to be markedly more effective on the adenine locus.

The evidence for selective mutagenicity in higher plants and animals has been very scanty, practically non-existent. In fact there were only the reports of the Gustafsson group (Gustafsson & Mackey, 1948; Gustafsson & Nybom, 1949); that the yield of mutations in cereals, especially barley, is dependent on the mutagen and the method of treatment. Quite recently, however, the work on the comparative mutagenic effects of the alkylating compounds and X-radiation (Fahmy & Fahmy, 1956*a*) has yielded the first decisive and comprehensive evidence for the differential action of various mutagens in *Drosophila*. This phenomenon does not only manifest itself in the different relative frequencies of the various types of mutations induced, but is sometimes of a much finer nature, being a specificity for certain gene loci. It has been shown, for example, that the '*N*-mustard' derivative of phenylalanine (*p-N-di*(chloroethyl)aminophenylalanine) is most effective in the induction of 'visible' (morphologically detectable) mutations, and that most of these visibles are 'new', in the sense that they have not been recorded in the literature as induced by other mutagens.

The clearest differences in mutagenic effects, however, seemed to occur between chemical agents and radiation. In a recent paper (Fahmy & Fahmy, 1956*a*) we have outlined these differences on the basis of our results with the alkylating agents as com-

pared to the radiation mutagenesis results available in the literature. The most obvious differences manifested themselves in the following respects:

(1) The frequency, properties, and distribution of the chromosome breaks.

(2) The mechanism of induction, and frequency of small deficiencies (involving up to 10 bands of the salivary gland X-chromosome).

(3) The ratio of 'visible' (morphologically detectable) to lethal recessive mutations induced in the same sample of treated chromosomes.

(4) The distribution of the genetically placed recessive lethals along the X-chromosome.

(5) The phenotypic expression and genetic position of the visibles induced.

In a note published in this journal, Stern (1957) has inquired into the degree of reliability of the last two of our criteria for difference between mutagens, viz. those based on genetical localization of lethals, and the detection of visibles. It would be of value, therefore, to examine whether any of the pitfalls he pointed out, could have affected our conclusions.

2. Distribution of recessive lethals

Stern (1957) has elaborated the well-known and accepted fact that heterogeneities of recombination do occur in linkage tests. It is equally known, however, that such heterogeneities can be reduced by adequate selection of gene markers, by the scoring of a reasonably high number of individuals, and by the use of optimal cultural conditions during experimentation. That is why, when we wanted to compare the distribution of the chemically induced lethals with the radiation mutations, we chose the Spencer & Stern (1948) linkage pattern, since their experimental procedure and criteria of accuracy were roughly the same as in our experiments. In both cases a five-point marker chromosome was used and a minimum of 200 males were scored for each lethal placing. Furthermore, like ourselves, these authors corrected the placings of their mutants in relation to the markers by reference to the standard map distances.

Stern (1957) objected to the comparison of the distribution of our chemically induced lethals with the X-ray mutations, on the grounds that the chromosome utilized in the placing of the radiation mutations gave recombination values between the utilized markers which varied more markedly from the standard map distances than was the case in our linkage experiments. This is undoubtedly true, but it is highly doubtful whether this heterogeneity could have resulted in any serious artifacts of distribution, particularly in the coarser groupings of our analysis. The average deviation from standard distances, even in the heterogeneous data of Spencer & Stern (1948), is 0·124 per unit recombination, with a maximum of 0·2 per unit, in the region *cv* (13·7)–*v* (33·0). It is hard to visualize how an error of that magnitude could be, (*a*) large enough or (*b*) unidirectional enough for a large number of the placed loci, to alter *grossly* the overall relative distribution of the chemical and radiation lethals among large segments (3 units in Table 6 A and 12 units in Table 6 B—Fahmy & Fahmy, 1956*a*).

It has always been realized however, that, the crucial evidence for the difference in the distribution of the sex-linked recessive lethals, under the effect of the radiation and chemical mutagens, must come from our own laboratory with work undertaken on both classes of mutagens with our own stock and with the comparison carried out on homogeneous data. This is now being undertaken and has already been partially accomplished. So far seventy X-ray sex-linked recessive lethals have been placed, and their distribution along the

X-chromosome has been compared with that of the same mutations induced chemically in our own laboratory, as well as with Spencer & Stern's (1948) data (Table 1). Because of the smallness of our sample of X-ray lethals, the data had to be pooled very drastically, and it was thought that the most objective way would be a grouping between the common marker used in linkage experiments for each set of comparisons. The marker genes in our experiments with both the chemically and radiation-induced lethals were: scute (*sc*), cut (*ct*), vermilion (*v*), forked (*f*) and carnation (*car*). These are the same as those of Spencer & Stern (1948), except that the latter authors used cross-veinless (*cv*) instead of cut (*ct*). In comparisons between our data and those of Spencer & Stern (1948), we pooled the lethals between the common markers used in both sets of experiments, viz. *sc–v–f–car–centromere*. Needless to say that with such a grouping the relative frequency in the successive segments would be utterly objective and trustworthy, and completely independent of errors of placing within segments.

Table 1. *The distribution of the sex-linked recessive lethals induced by the alkylating compounds and X-radiation between the localization markers*

	Genetical map of the X-chromosome					
	sc	*ct*	*v*	*f*	*car*	centromere
Mutagens and (authors)	0–19·9	20·0–32·9	33·0–56·6	56·7–62·4	62·5 +	Total
Alkylating compounds (Fahmy & Fahmy, 1956*a*)	172	92	182	42	79	567
X-radiation (Fahmy & Fahmy)	24	16	21	6	3	70
X-radiation (Spencer & Stern, 1948)	116		80	21	12	229
Alkylating compounds (regrouped)	264		182	42	79	567
Pooled X-radiation data (Fahmy & Fahmy; Spencer & Stern, 1948)	156		101	27	15	299

Table 2. *The significance of the variation in distribution (expressed in* χ^2*) of the sex-linked recessive lethals induced by the alkylating compounds and X-radiation between the localization markers (based on data in Table* 1)

	Genetical map of the X-chromosome						
	sc	*ct*	*v*	*f*	*car*	centromere	
Comparison (and authors)	0–19·9	20·0–32·9	33·0–56·6	56·7–62·4	62·5 +	Total χ^2	*P*
Alkylating compounds and X-radiation (Fahmy & Fahmy)	4·563	1·945	0·123	0·121	5·170	11·925	0·018
X-radiation (Fahmy & Fahmy and Spencer & Stern)	0·904		0·584	0·023	0·102	1·613	0·660
Alkylating compounds (Fahmy & Fahmy) and X-radiation (Spencer & Stern)	1·096		0·594	0·695	12·16	14·545	0·004
Alkylating compounds and pooled X-radiation data	2·470		0·251	0·703	16·10	19·524	<0·001

It is convincingly clear from Table 2 that the distribution of the chemically and radiation-induced sex-linked recessive lethals is significantly different whichever data are used for the comparison. The greatest differences, on the basis of our own data, occur at the free and centromere ends of the chromosome. At the proximal end of the X-chromosome, near the centromere, the chemical agents induce more mutations than X-radiation, whereas at the distal free end the reverse is true, radiation being more effective.

It would be desirable to compare our own data for X-radiation and chemically induced lethals for various degrees of grouping along the X-chromosome. This is obviously not possible with the present small sample of X-ray lethals, but will undoubtedly be undertaken and published later. It was thought worth while, however, to pool our X-ray data with those of Spencer & Stern (1948), and compare the overall results with the data on the alkylating compounds for the same degree of grouping as was undertaken in our previous publication (Fahmy & Fahmy, 1956*a*), i.e. in 3-unit segments. The data were subjected to the Ψ test (Smith, 1952) as before, and both sets of analysis are presented in Table 3. It is of interest to note that the inclusion of our sample of lethals with that of Spencer & Stern (1948) has no pronounced effect on the degree of significance. This is further proof that the heterogeneity of the linkage data of the latter authors for the radiation mutations in comparison with our own data with the chemically induced lethals is perhaps not worth the emphasis given by Stern (1957).

Table 3. *The distribution of sex-linked recessive lethals along the genetic map of the X-chromosome under the effect of the alkylating compounds and X-radiation*

Mutagens (and authors)	Genetical map of the X-chromosome (in 3 unit segments)																									P	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	Total		
Alkylating compounds (Fahmy & Fahmy, 1956*a*)	100	10	16	17	17	9	9	23	33	20	10	21	18	17	27	22	24	29	32	27	27	26	26	7	567	0·008	0·003
X-radiation (Spencer & Stern, 1948)	31	12	3	1	10	4	13	15	10	11	6	9	16	7	10	6	11	11	19	9	4	7	4	0	229		
Pooled X-radiation data (Fahmy & Fahmy and Spencer & Stern)	42	18	4	4	12	5	15	20	13	13	10	15	17	7	11	11	12	13	25	11	6	8	5	2	299		

Another point raised in Stern's (1957) note on linkage data concerns the effects of what he calls 'regional differentials in recombination' and how they might operate in producing artifacts of distribution in the process of correcting genetical placings to standard map distances. Of course, his arguments do not hold at all when comparisons of the linkage results are based on data pooled between the common markers used in placing experiments by different workers in the manner presented in the present paper. Yet, it is often desirable to make comparisons between the distributions of mutations placed by different workers without reference to the markers used. Most authors agree that in such cases it is satisfactory to give the position of the locus corrected to the standard map distance on the basis of the recombination values it gives with the marker genes on either side of it. As Stern (1957) points out, this procedure assumes that the variation in the recombination values occurring in the localization experiment is evenly distributed over the region between the marker genes. While this assumption is probably acceptable if the region between the markers is small, it could, if incorrect, be serious if the distance between the markers is large, as is the case with some segments used in the lethal placings (*v–f* for example, covering a distance of 23·7 units). It must be pointed out, however, as Stern (1957) himself admits, that there is no experimental evidence whatever to help the assessment of the seriousness of regional differentials in recombination. We do know, however, that with the placings of the chemical lethals undertaken in our laboratory this phenomenon could not have caused serious disturbances in distribution. It was ascertained that the overall distribution of the corrected and uncorrected sex-linked lethals, even in the finest grouping tested (3 units),

did not differ significantly. Whether this situation holds true for the X-ray lethals as well, still remains to be seen. It would have been desirable to determine how different is the overall distribution of the corrected and uncorrected X-ray data of Spencer & Stern (1948) at different degrees of pooling. Unfortunately, however, the uncorrected data of these authors seem to be at present inaccessible.

3. 'Visible' mutations

The detection of 'visible' mutations has long been recognized to depend to a large extent on the acuity of the observer. This large 'personal factor' in visible mutation work necessitates that the scoring should be undertaken by highly skilled workers with special aptitude for the recognition of morphological changes and with many years' experience with *Drosophila* material. In comparative mutagenesis results based on 'visible' detection, it is essential to restrict the scoring of the mutations induced by different agents to a few workers, preferably one. That is why, when the discovery of the differential yield of visibles by different mutagens passed the stage of impression and approached the stage of reality, we restricted all scorings of 'visible' mutations to one of us only (M.J.F.). For technical convenience all the mutation experiments in our laboratory are coded, and uncoding is not undertaken until the end of the experiments with each particular mutagen. Often experiments with various mutagens are interwoven and undertaken simultaneously, so as to make it impossible for the observer to predict which treatment the scored flies had received. All these precautions make the 'visible' data published from our laboratory as objective as is humanly possible, and they obviously meet the requirements stipulated in Stern's (1957) note.

In view of the above remarks it is considered that the strongest evidence for selective mutagenicity is demonstrated by the differential yield of 'visible' mutations by different agents. The ratio of visibles to lethals induced in the same sample of treated X-chromosomes varied considerably not only between radiation and chemical mutagens, but also between some of the different classes of the alkylating compounds themselves.

Most of these compounds induce 1 visible to 10 lethals. One exception is the '*N*-mustard' derivative of phenylalanine, which yields 3 visibles to 10 lethals (Fahmy & Fahmy, 1956*a*). A new compound, viz. 2-chloroethyl methanesulphonate (Fahmy & Fahmy, 1956*b*), proved to be most effective in mutating morphogenesis loci, giving an overall ratio of visibles to lethals of 4:10. For the very young germ cells which are most susceptible to the action of this sulphonate the ratio of the two types of mutations reaches unity. A sample of X-ray mutations detected in the F_2 cultures of Muller-5 tests has also been analysed in our laboratory and by the same observer. In three experiments with various doses of X-rays the number of sex-linked visibles to lethals were 36:167, 10:84 and 15:95, giving an overall ratio of roughly 2 visibles to 10 lethals. This is the same ratio as that ascertained by Spencer & Stern (1948) in similar experiments with the same radiation.

Attention has already been drawn (Fahmy & Fahmy, 1956*a*) to the fact that the alkylating compounds seem to mutate 'new' morphogenesis loci which are different in phenotypic expression and genetic position from those induced by other agents. This selectivity for 'new' loci does not always go hand in hand with the overall effectiveness on the morphogenesis genes in general. Thus while 2-chloroethyl methanesulphonate proved to be the most effective agent in the induction of visibles, it is less selective on 'new' loci

than the '*N*-mustard' derivative of phenylalanine. The percentage ratio of 'new' to known visibles under the effect of three alkylating compounds is as follows: *p–N–di*(chloroethyl)-aminophenylalanine, $80{\cdot}3 \pm 2{\cdot}0 : 19{\cdot}7 \pm 2{\cdot}0$; 2-chloroethyl methanesulphonate, $68{\cdot}5 \pm 3{\cdot}9 : 31{\cdot}5 \pm 3{\cdot}9$; 2:4:6-tri(ethyleneimino)-1:3:5-triazine, $59{\cdot}9 \pm 4{\cdot}2 : 40{\cdot}1 \pm 4{\cdot}2$.

Practically all the visibles referred to above as 'known' have been induced by radiation, especially X-rays. It must be emphasized, however, that the sample of X-radiation visibles recorded in the literature, or maintained in the private files of the radiation geneticists, is by no means a full list of all the mutations that can be induced by this agent. Actually as our own sample of X-ray mutations accumulates, it has become apparent that some of the 'new' visibles which are induced chemically can also be induced by radiation and must have been missed by the earlier workers. We discovered also some 'new' X-radiation visibles which were neither described by the earlier workers nor detected in our own work on chemical mutagenesis. These cases, however, are not sufficiently frequent to account for the fact that by the use of the alkylating mutagens we have nearly tripled the number of loci giving clear (rank 1 and 2) sex-linked recessive visibles (from 115 to more than 300) and that nearly two thirds of these have not been mutated by other mutagens including radiation, as far as can be ascertained.

4. Mutability of specific 'visible' loci

To test how far the mutability of a locus is a function of the mutagen, we analysed the response of seventeen chemically induced visibles under the effect of radiation. The selected loci were spread along the whole length of the X-chromosome, were most easily recognizable, and each has occurred more than once in the chemical mutagenesis work. All mutants were fully viable and fertile in the homozygous condition. It was further possible to combine these mutants in sets of 2–4 loci per stock without impairing either the fertility or the viability. Care was taken to combine mutants which do not show any phenotypic overlap. For the sake of comparison we simultaneously tested the mutability of seven X-chromosome loci which are known to be affected (to various degrees) by radiation: viz. scute (*sc*), cut (*ct*), vermilion (*v*), wavy (*wy*), garnet (*g*), forked (*f*) and carnation (*car*).

Females, homozygous for the recessive visibles, or heterozygous for two sets of visibles one on each X, were mated to irradiated males carrying the normal allelomorphs, and the F_1 daughters were scored for the different genes tested. The number of chromosomes tested per locus is given by the total number of daughters when a homozygous mother is used, and only by half that number when the mothers are heterozygous. About 30,000–80,000 daughters receiving the marked and treated X-chromosomes were scored per locus. A daughter could show one of the characters tested for, if the paternal X has been affected so as to carry:

(*a*) a 'visible' allelomorphic to the marker, or (*b*) a 'visible' as above together with a lethal somewhere else on the chromosome, or (*c*) a deficiency or a deletion (in itself a lethal) covering the locus of the marker. Extensive genetic tests were undertaken to differentiate between the above three possibilities.

Table 4 summarizes the results. The left half of the table represents the mutability of the 'new' chemically induced visibles, whereas the right half represents the response of the known visibles for control purposes. Of the seventeen chemically mutated visibles

only one has been affected intragenically by radiation and also eliminated within deletions; eight others have been eliminated in deletions, six at a low frequency, and two at a much higher frequency, but were not mutated intragenically. The remaining eight were stable to radiation in samples of the size utilized. That this size of sample is reasonably adequate is shown by the fact that it was sufficient for the induction of intragenic mutations, as well as deletions for the tested known loci, except *car* which was only eliminated.

Table 4. *Mutability at specific 'visible' loci under the effect of X-radiation*

'New' chemically mutated loci	Genetic position	Mutation rate per locus per r × 10^{-8}: Deficiencies and deletions	Mutation rate per locus per r × 10^{-8}: Point mutations	Known loci (control)	Genetic position	Mutation rate per locus per r × 10^{-8}: Deficiencies and deletions	Mutation rate per locus per r × 10^{-8}: Point mutations
v. 1230	0·0	—	—				
				scute	0·0	1·74	3·49
v. 638	2·7	—	—				
v. 1276	4·1	1·24	—				
v. 1243	5·7	1·82	—				
v. 1998	6·3	1·27	—				
v. 1255	14·8	1·82	—				
				cut	20·0	5·23	0·87
v. 1287	23·4	1·52	—				
v. 1726	29·9	—	—				
				vermilion	33·0	0·7	1·44
v. 1892	40·5	1·17	—				
				wavy	41·9	6·10	3·49
v. 1523	43·1	—	—				
				garnet	44·4	5·23	4·36
v. 2161	51·7	—	—				
v. 2100	53·6	—	—				
				forked	56·7	0·87	2·62
v. 2212	59·1	—	—				
v. 1225	59·4	8·26	—				
v. 2073	61·6	1·05	8·42				
				carnation	62·5	3·06	—
v. 1663	64·5	—	—				
v. 1920	65·0	6·07	—				

Tested sample: 30,000–80,000 chromosomes per locus.
Dose range: 2500–4250 r.
v: symbol for 'visible' mutation.

The mutability of the same chemically induced and known visibles will be tested by the same technique under the effect of the phenylalanine mustard, and the full comparative results and their statistical analysis will be presented in due course. Nevertheless, it is perhaps worth mentioning that in a sample of 50,000 X-chromosomes treated with various alkylating compounds and scored in Muller-5 and attached-X experiments, all the 'new' visibles were detected at a minimum frequency of 2 and a maximum of 5. Most interesting is that *car*, which has not been mutated intragenically by X-rays in our sample (viz. 67,000 chromosomes for this locus), occurred 3 times in the chemical experiments (i.e. 3 in roughly 50,000 chromosomes). Also *sc* and *ct* which are mutated fairly frequently with radiation have never been encountered in the chemical experiments.

It can be safely concluded, even at this stage in our investigation, that the results of mutability at specific loci support the principle of differential gene response to mutagens. They also indicate that the detection of a large number of 'new' visibles under the effect of the alkylating compounds is a genuine reflexion of the higher response of some loci to these compounds and, therefore, perhaps not entirely due to a higher 'observational acuity'.

5. Summary

A sample of X-ray sex-linked recessive lethals has been localized by the same marker chromosome, and under identical experimental conditions, as prevailed in the placing of the same mutations induced by the alkylating compounds, so as to insure homogeneity of localization. The distribution of this sample of X-ray lethals along the X-chromosome varied significantly from that for the chemically induced mutations.

Differential production of visibles has been demonstrated by observations made by the same worker on coded experimental cultures. The ratio of visibles to lethals induced in the same sample of treated X-chromosomes, as well as the phenotype and genetic position of some of the visibles themselves, varied with the mutagen. Tests of mutability at specific chemically mutated visible loci under the effect of X-rays supported selective mutagenicity.

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