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The production of visible mutations in *Drosophila* by chloroethyl methanesulphonate (CB 1506)

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The relative frequencies with which various mutagens produce visible and lethal mutations in *Drosophila* have recently been discussed in a number of publications (Fahmy & Fahmy, 1960; Carlson & Oster, 1961; Browning & Altenburg, 1962). It therefore seems pertinent to publish and discuss in full data that so far have been reported only in a preliminary fashion (Auerbach, 1957). This experiment had been designed as an independent test of the observation by Fahmy & Fahmy (1956) that chloroethyl methanesulphonate (CB 1506) produced a higher ratio of visible to lethal mutations than any other mutagen tested by them or others.

MATERIAL AND METHODS

CB 1506 was kindly provided by the Chester Beatty Institute for Cancer Research in London. It was made up into a 10^{-2} M solution in 0·4% NaCl. Two-day-old wild-type (Oregon-K, *Ork*) males were given 0·2 microlitre as abdominal injections. They were mated in mass to females of the genotype $sc^{sl}InS\ w^a sc^8$ (*Muller-5*, *M-5*). Every 3 or 4 days the males were remated to fresh females; but since CB 1506 is known to produce most of its mutations in late broods (Fahmy & Fahmy, 1956), no progeny were kept from matings done before the 12th day after treatment. If this selection of broods for testing should introduce a bias into the result, it would be in favour of visible mutations; for in the experiments by Fahmy and Fahmy the visible-to-lethal ratio increased in late broods, as a consequence of germinal selection against lethals (Purdom, 1957). On Day 12, at the beginning of brood *d*, the treated males were divided into two groups and mated individually, those in group I to 3 *M-5* females, those in group II to 3 attached-X females. On Day 16, at the beginning of brood *e*, the males were remated, those in group I now receiving 3 attached-X females and those in group II 3 *M-5* females. On Day 19, the males were discarded.

In broods *d* and *e*, both sex-linked lethals and visibles were scored, the latter in two different ways. (1) the F_2 cultures of the *M-5* series were examined through the binocular and without etherization by an experienced assistant, who had been told to pay special attention to visible mutations. Only cultures in which all males with the treated chrosome showed the same abnormality were scored as visibles; no attempt was made to detect visibly abnormal males among the late hatchers of cultures that had produced sufficient normal males for unambiguous clasisfication as lethal or non-lethal by our usual criterion (10 or more *M-5* males and no wild-type

ones). (2) In the F_1 of the attached-X test, all males were examined carefully under the binocular by myself and, as far as possible, every male showing even a slight abnormality was tested by a backcross to attached-X females. Mutants that arose in clusters were scored as so many separate mutations, since obviously in these broods the non-mutant spermatozoa, too, must have formed clusters of similar sizes. The results are shown in Tables 1–4. Slight deviations from those reported in a preliminary way (Auerbach, 1957) are due to incompleteness of the records at that time and to the fact that in the present table the brood periods are counted in days after treatment rather than in days after the first mating.

In each brood, the frequency of sex-linked lethals has been compared with that of sex-linked visibles as calculated in three different ways: (1) from the F_2 of the *Muller-5* tests, (2) from the F_2 males in the attached-X test, counting only fully penetrant and confirmed mutations, (3) from the same males, including also those unconfirmed aberrants that from their phenotypes were judged to be probable mutants. Since this group may include not only phenocopies but also autosomal dominants, it provides a maximal estimate of the frequency of sex-linked visibles. Mutants of incomplete penetrance could not be used for the calculation since lethals of incomplete penetrance will go undetected. In brood *d*, the first two estimates

Table 1. *Frequencies of sex-linked visible mutations in Muller-5 test*

Brood	Days	No. of P_1 ♂♂	No. of tested X-chromosomes	% lethals	No. of visibles	% visibles
d	12–16	19	386	5·5	2	0·5
e	16–19	18	379	13·1	2	0·5

Table 2. *Frequencies and types of sex-linked visible mutations in attached-X test*

				No. of and % visibles*		
Brood	Days	No. of P_1 ♂♂	No. of F_1 ♂♂	Type I	Type II	Type III
d	12–16	18	864	4 = 0·5%	5 = 0·6%	2
e	16–19	19	878	8 = 0·9%	3 = 0·3%	18

* Type I: fully penetrant. Type II: no progeny, possibly genetic. Type III: incompletely penetrant.

Table 3. *Ratio of frequencies of visibles as estimated in various ways to lethal frequencies in Muller-5 test*

		Visibles in attached-X test	
Brood	Visibles in Muller-5 test	Type I*	Types I and II*
d	0·5:5·5 = 1/11	0·5: 5·5 = 1/11	1·1: 5·5 = 1/5
e	0·5:13·1 = 1/23	0·9:13·1 = 1/14	1·2:13·1 = 1/11

* See explanation to Table 2.

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Table 4. *Description of visible mutants; in brackets: bunch sizes*

	Brood *d*	Brood *e*
Muller-5 test	*r* *N*	*tw* (2/23)
Attached-X test		
Type I*	*fu* (2/49) *w* ^{satsuma} small, wings folded	*w* ^{satsuma} (7/62)
Type II*	rough eyes, scalloped wings small yellow abdomen white eyes folded wings	scalloped wings wings held back small
Type III*	short bristles spread wings	small (6/92) scalloped wings (4/39) wings held back (6/67 rough eyes roofed wings

Note that the males producing brood *d* of the Muller-5 test produced brood *e* of the attached-X test, and *vice versa*.

Gene symbols: *r* = rudimentary; *N* = Notched; *tw* = twisted abdomen; *fu* = fused; *w* ^{satsuma} = white-satsuma.

* See explanation to Table 2.

agree in yielding a visible-to-lethal ratio of 1:11, which is increased to 1:5 by the inclusion of unconfirmed presumed mutants. In brood *e*, all three calculations give visible-to-lethal ratios that are lower than the corresponding ones in brood *d*; but the statistical error introduced by the presence of large bunches makes the results of brood *e* less reliable than those of brood *d*.

Of special interest was the repeated independent occurrence of the same mutation. In the attached-X test, mutation to *w*^{satsuma} (kindly identified by Dr M. M. Green) occurred twice in two different males. In the *Muller-5* test, a mutation to rotated abdomen, located close to *sc* and therefore probably an allele of twisted abdomen (*tw*), occurred as a bunch of 2 in brood *e*. The same or an allelic mutation was found in three other tests with CB 1506, once after treatment of *OrK* males, once after treatment of *Muller-5* males (Reddi, personal communication), and once after treatment of *vg* females.

DISCUSSION

These results do not support the claim that CB 1506 is exceptionally effective in the production of sex-linked visible mutations. In the experiments by Fahmy & Fahmy (1956) the ratio of sex-linked visible to sex-linked lethal mutations was about 4:10 in early broods and approached 1:1 in late ones. In the present experiment, the visible-to-lethal ratio was less than 1:10 in both broods when only confirmed mutations were counted; this is similar to ratios obtained in older X-ray

work (Schultz, 1936; Timoféeff-Ressovsky & Delbrück, 1936). When unconfirmed but presumably genetic aberrants were included, the ratio remained less than 1:10 in brood *e*, but rose to 1:5 in brood *d*; even this maximal estimate is still within the range found in X-ray experiments (Spencer & Stern, 1948).

An analysis of the procedure by which visible mutations are scored suggests the causes of this discrepancy. Fahmy and Fahmy usually score visible mutations in the F_2 of *Muller-5* tests. It seems that they classify three types of culture as mutations. (1) Cultures in which all males with the treated X-chromosome show the same sex-linked abnormality. This is the only type that by conventional techniques is scored as a visible. (2) Cultures which at first appear to have a lethal but in which towards the end one or a few visibly abnormal males appear. By most workers this type of culture will be scored as a semi-lethal with visible effect on the survivors. Alternatively, it may be classified as a full lethal; for the usual practice in *Muller-5* tests is to score an F_2 culture without wild-type males as a lethal as soon as a standard number of *Muller-5* males has been obtained. Re-testing in the next generation will be done in the same way and has the same chance of missing late-hatching males. Thus, while the inclusion of this type of culture among visible mutations is legitimate, it will result in discrepancies with the results of other workers, and these discrepancies will be in the direction of a higher ratio of visibles to lethals. Indeed, when Fahmy & Fahmy (1957) redetermined this ratio for X-radiation, they obtained a value (2:10) that was slightly above the highest recorded one (Spencer & Stern, 1948). (3) Cultures in which males with the treated X-chromosome segregate into normal and mutated ones. This may have various causes: incomplete penetrance of a sex-linked mutation; maternal effect on manifestation; mosaicism of the F_1 female; interaction between a sex-linked and an autosomal gene. Whatever the cause for segregation of mutated and non-mutated males in an F_2 culture, since a culture that segregates for lethal and non-lethal males will not be scored as a lethal, cultures that segregate for a visible mutation cannot legitimately be used for calculation of the visible-to-lethal ratio. Disregard of this precaution will bias the data in favour of visible mutations, and Carlson & Oster (1961) have suggested that this is what happened in the calculation of visible-to-lethal ratios by Fahmy and Fahmy. That this interpretation is correct is shown by the fact that Fahmy and Fahmy repeatedly list *m-l*^{bronzy} among the visibles obtained in their *Muller-5* tests, although this mutant—because of the strong maternal effect on its manifestation (Glassman, 1959)—must always have occurred as a minority of mutant males among non-mutant ones.

In experiments with chemical mutagens, mosaicism will be the main source of segregating F_2 cultures. The higher the ratio of mosaic to complete mutations, the greater will be the error in the visible-to-lethal ratio when mosaic visibles are included in the calculation. Browning and Altenburg (1962) have, in fact, shown a positive correlation between frequency of mosaics and proportion of visibles among mutants induced by a number of different mutagens. In their experiments, mosaics were scored by the specific-loci technique, by which small mosaic areas often will be missed. The technique adopted by Fahmy and Fahmy of scoring individual

mutant males in whole F_2 cultures is much more efficient in detecting even very small mutated areas of the gonads. The fact that in their experiments the highest apparent ratio of visibles to lethals occurred after treatment with CB 1506 agrees with evidence suggesting that this substance produces an unusually high proportion of mosaics, many with small mutated areas (Auerbach, unpub.). Since in later broods mosaics would be expected to be less frequent, lethals would be expected to form a higher proportion of all mutants in these broods. It is, therefore, surprising that the opposite was true in the experiments by Fahmy and Fahmy. However, Purdom (1957) has shown that the increase in the visible-to-lethal ratio in the late broods of these experiments was due to germinal selection against sex-linked lethals. Whether correction for germinal selection would have resulted in the decrease of this ratio, expected on the present interpretation, cannot be decided from the published data, in which all clusters of visibles have been recorded as single mutations while the frequencies of lethals have been corrected for clustering in a way that has not been described.

The repeated occurrence of the same two mutations among small numbers of tested chromosomes points to the much discussed but still unproven possibility of mutagen specificity in *Drosophila* (Auerbach & Woolf, 1960). In particular, the occurrence of two independent mutations to $w^{satsuma}$ in 1700 chromosomes is reminiscent of the type of allele specificity which by now is well known from mutation work on micro-organisms. Specific-loci tests for these mutations might give clearer results than have been obtained so far on *Drosophila*.

SUMMARY

The claim that CB 1506 produces an unusually high proportion of visible to lethal mutations could not be confirmed. The ratio of visible to lethal sex-linked mutations was less than 1:10 in both *Muller-5* and attached-X tests. In one brood it rose to 1:5 when unconfirmed visible mutants were included. The much higher ratio obtained by Fahmy and Fahmy is interpreted as a methodological artifact, due to the inclusion in their calculations of mosaic visibles but not of mosaic lethals.

Two visible mutations—twisted abdomen and $w^{satsuma}$—occurred more than once. This suggests the possibility of a locus or allele specific effect of CB 1506.

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