

MUTAGEN SENSITIVITY OF AN INSECTICIDE-RESISTANT STRAIN OF *Drosophila melanogaster* TO ETHYL METHANESULFONATE. COMPARISON WITH A STANDARD STRAIN

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ABSTRACT

Two strains of *Drosophila melanogaster*, one resistant (*MRA*) and the other sensitive (*BK*) to several insecticides, were examined for mutability after treatment with the well known alkylating agent ethyl methanesulfonate (EMS).

The results of sex-linked recessive lethal induction indicated that, although the standard strain is more sensitive to the mutagenic effects of EMS, with a mutation induction level nearly twofold the value obtained for the *MRA* strain, both strains are sufficiently sensitive, showing a dose-dependent increase in mutability in post-meiotic male germ cells.

INTRODUCTION

Insecticides are chemicals that pose special problems in their mutagenic evaluation in a battery of assays, principally due to their high toxicity. This is particularly true when an insect such as *Drosophila melanogaster*, is used as test system. In spite of the intrinsic advantages that the fruit fly shows in genetic toxicology studies (Sobels, 1980), the current use of wild-type *D. melanogaster* strains in mutagenicity testing (*Berlin-K*, *Canton-S*, *Oregon-R*) has not permitted testing sufficiently high concentrations for an adequate exposure time, in order to obtain conclusive results (Valencia, 1981).

Several authors (Fahrig, 1974; Wild, 1975; Vogel, 1980) have pointed out that these difficulties could be overcome by the use of special insecticide-resistant

Drosophila strains. Using this strategy, several insecticides have been shown to be mutagenic in *Drosophila*, especially in the sex-linked recessive lethal (SLRL) test (Vogel, 1974; Velázquez *et al.*, 1984, 1986; Batiste-Alentorn *et al.*, 1986). Nevertheless, the use of an insecticide-resistant strain is not necessarily an advantage, since if the higher amounts of insecticide administered are enzymatically degraded to non-mutagenic compounds, or if the resistance is due to a mechanism preventing the entry of excess insecticide, then approximately the same amount of chemical will reach the target as in sensitive strains (Hanna and Dyer, 1975).

These considerations have led us to question whether the resistance mechanism could influence the sensitivity of such strains in detecting mutagens. Thus, we undertook the present study to compare the response of two *Drosophila* strains (respectively insecticide-resistant and insecticide-sensitive) to the well-known mutagenic compound ethyl methanesulfonate (EMS).

MATERIALS AND METHODS

Strains

MRA: Wild-type strain selected for resistance to malathion. *Berlin-K (BK)*: wild-type strain. *Basc*: In(1) sc^{S1L} sc^{8R} + S, sc^{S1} sc⁸ w^a B. A complete explanation of the genetic symbols is given by Lindsley and Grell (1968).

Culture conditions

Standard food medium enriched with living yeast was used. The standard growth temperature was $25 \pm 1^{\circ}\text{C}$.

Test compound

Ethyl methanesulfonate (CAS No. 62-50-0, Merck) was dissolved in a 5% sucrose solution in distilled water.

Route of administration

For adult feeding, males were treated for 24 or 48 hours in glass filter special feeding units after 4 hours of starvation.

Mating scheme

The standard *Basc* scheme described by Würigler *et al.*, (1984), was used for the lethal mutation test.

Brooding scheme

Treated (and control) males were mated with three new virgin females for each of three broods (3, 2 and 2 days).

RESULTS AND DISCUSSION

The insecticide-resistant *MRA* strain used in this study was obtained in an experiment of selection for increased resistance to the organophosphorus insecticide malathion (Velázquez, 1983). Subsequent studies have indicated that this strain also shows cross-resistance to other organophosphorus and pyrethroid insecticides (Velázquez, 1987; Batiste-Alentorn *et al.*, 1987).

A preliminary step in testing for differential mutagen sensitivity between strains is the comparison of spontaneous mutation rates. Thus, our first experiment established the spontaneous frequency of sex-linked recessive lethal mutations for our insecticide-resistant *MRA* strain and the *BK* strain (Table I). These data indicate that the spontaneous mutation frequencies do not differ between the 2 strains for the 3 post-meiotic broods analyzed.

Table I - Spontaneous frequencies of sex-linked recessive lethals in *Drosophila* male germ cells of the insecticide-resistant *MRA* and standard *BK* strains.

Strain	Brood	No. lethals	Total tests	% SLRL
<i>MRA</i>	1	3	3181	0.09
	2	8	3409	0.23
	3	9	3405	0.26
	1 + 2 + 3	20	9995	0.20
<i>BK</i>	1	4	2608	0.15
	2	8	2189	0.37
	3	3	1812	0.17
	1 + 2 + 3	15	6609	0.23

The data from the two parallel experiments carried out to measure mutation induction by 30 and 100 ppm EMS after treatment via adult feeding are presented in Table II. Low concentrations were tested in order to detect possible differences between the two strains for the lowest effective concentration. On the basis of our data, both strains showed an apparent increase in the frequency of induced mutations directly related to exposure time and concentration. These findings are in good

agreement with results previously obtained by other authors with EMS (Aaron and Lee, 1978; Vogel, 1979).

Table II - Frequencies of sex-linked recessive lethals from insecticide-resistant MRA and BK males treated with EMS.

Strain	Conc. (ppm)	Time (h)	Brood	Lethals	Chromosomes tested	% SLRL	P value ^a
MRA	30	24	1	0	1150	—	—
			2	1	1119	0.09	—
			3	2	966	0.21	—
			1+2+3	3	3235	0.09	—
	30	48	1	11	1140	0.96	—
			2	15	1212	1.24	—
			3	10	1065	0.94	—
			1+2+3	36	3417	1.05	—
	100	48	1	16	1150	1.39	—
			2	30	1079	2.78	—
			3	24	951	2.52	—
			1+2+3	70	3180	2.20	—
BK	30	24	1	4	1099	0.36	>0.05
			2	2	1137	0.18	>0.05
			3	6	1113	0.54	>0.05
			1+2+3	12	3349	0.36	0.02
	30	48	1	22	1069	2.06	0.03
			2	22	1059	2.08	>0.05
			3	17	1121	1.52	>0.05
			1+2+3	61	3249	1.88	<0.01
	100	48	1	42	1063	3.95	<0.001
			2	45	1155	3.90	>0.05
			3	50	1164	4.29	0.02
			1+2+3	137	3382	4.05	<0.001

^a P value determined by Fisher's exact test.

In the present study, treatment with 30 ppm EMS for 48 hours corresponded to the lowest effective dose assayed, the mutation rates for each of the three broods being significantly higher than the respective control values. Taking into account that the same concentration of 30 ppm EMS was ineffective in increasing the spontaneous mutation frequency when applied for a period of 24 hours only, our data reinforce the importance of the duration of treatment for adequate testing of chemical mutagens. In this context, we emphasize the ambiguous results generally obtained in the mutagenicity testing of highly toxic chemicals due to the low concentrations and/or short exposure times used. Thus, the use of resistant strains may be important to test adequate doses that will permit obtaining conclusive results.

The results of mutation induction in post-meiotic germ cells of *MRA* and *BK* males, summarized in Table II, clearly show that the frequency of SLRL mutations induced in the *BK* strain was higher than in the *MRA* strain, *i.e.*, treatments with 30 and 100 ppm EMS lasting for 48 hours induced approximately twofold mutation rates in the *BK* strain when compared with the rates obtained for the *MRA* strain.

For comparison, data were corrected by Abbott's formula (Abbott, 1925) to reflect the real induced mutation rate for each of the post-meiotic germinal stages analyzed. The corrected values are graphically represented in Figure 1, where it is clearly shown that the induction of SLRL mutations by EMS was significantly higher in the *BK* strain than in the *MRA* strain.

To obtain a more comprehensive picture of the mutagen sensitivity of the two strains to EMS, brood-by-brood statistical analysis of the data from Table II was performed using Fisher's exact test. For the lowest dose tested (treatment with 30 ppm EMS for 24 hours) there were no significant differences; for the intermediate dose (30 ppm for 48 hours), *BK* males showed a significantly higher mutation rate in brood 1, mainly corresponding to metabolically inert mature sperm; for the highest dose assayed (100 ppm for 48 hours) *BK* males exhibited a significant increase over *MRA* males for mutations induced in broods 1 and 3, corresponding to mature sperm and spermatocytes (and spermatids), respectively.

Interestingly, when we used the same statistical analysis to compare the mutation rates of the two strains for the pooled data (total of the three broods), we found that the frequency of mutation in *BK* males was significantly higher than in *MRA* males for the three doses tested. Even if this significance was due to the increase in the sample size, it reinforces the experimental evidence supporting a somewhat higher mutation induction in the *BK* strain.

When we compared the mutation rates induced with 30 ppm EMS (48 hours) with those induced with 30 ppm EMS (24 hours), we found a significant difference in both strains. Thus, even though the *BK* strain appears to be more sensitive to the mutagenic effects of EMS, both strains are sufficiently sensitive, showing a dose-dependent increase in mutability in post-meiotic male germ cells.

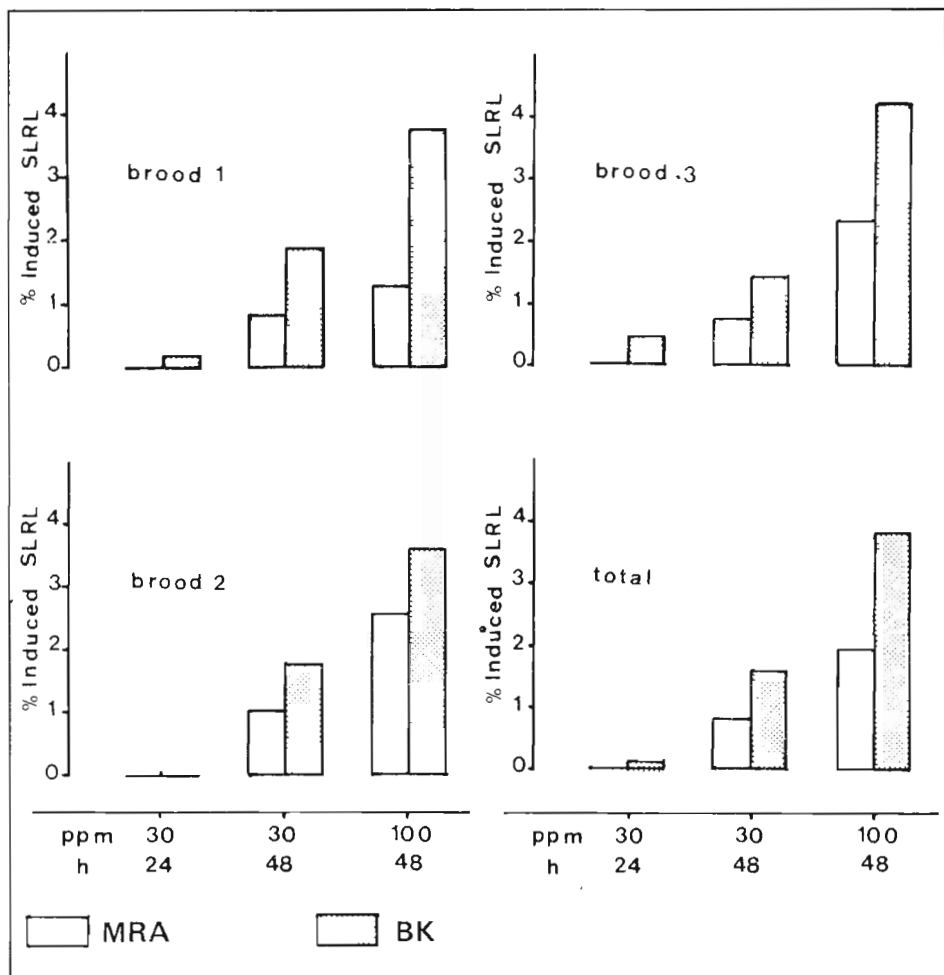


Figure 1 - Induced percentages of sex-linked recessive lethals from insecticide-resistant *MRA* and *BK* standard males treated with different doses of EMS.

Considering that the insecticide-resistant *MRA* strain is sufficiently sensitive to detect the mutagenic effects of EMS and that using this strain we were able to show the mutagenicity of several highly toxic compounds (Batiste-Alentorn *et al.*, 1986; Velázquez *et al.*, 1986; Velázquez, 1987), it may well be that with some chemicals, the use of the *MRA* strain may offer certain advantages. However, more general conclusions in terms of a testing strategy cannot be drawn until a more elaborate study of the influence of metabolism on the mutagenic effectiveness of chemicals is done, comparing more strains and/or more mutagens.

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RESUMO

Duas cepas de *Drosophila melanogaster*, uma resistente a vários inseticidas (*MRA*) e a outra sensível (*BK*), foram analisadas quanto à mutabilidade face ao conhecido agente alquilante metanosulfonato de etila (EMS).

Os resultados relativos à indução de letais recessivos ligados ao sexo indicam que, embora a cepa padrão seja mais sensível aos efeitos mutagênicos do EMS, com uma taxa de mutação induzida que é aproximadamente o dobro do valor obtido para a cepa *MRA*, ambas as cepas são suficientemente sensíveis, apresentando um incremento da mutabilidade em células germinais masculinas pós-meióticas dependente da dose.

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