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Gian Antonio Danieli, Guido Barbujani, Carla Cravera, Italo Barrai

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SEZIONE III

(Botanica, zoologia, fisiologia e patologia)

Genetica. — *Sex ratio in* Drosophila *as a mutagenicity test*(*). Nota di GIAN ANTONIO DANIELI(**), GUIDO BARBUJANI(***), CARLA CRAVERA(**) e ITALO BARRAI(***), presentata(****) dal Corrisp. B. BATTAGLIA.

RIASSUNTO. — Vengono presentati i risultati preliminari ottenuti saggiando l'efficacia del test sex-ratio in *Drosophila melanogaster* nell'individuare l'azione mutagena di composti chimici. La mutagenicità dell'etilmetansulfonato, saggiata in una serie di esperimenti che utilizzano tale test viene rivelata anche a dosi molto basse del mutageno e l'effetto del trattamento appare proporzionale alla dose.

In the first tests used for the detection of mutagenicity, the X chromosome of *Drosophila* was chosen as a preferred acceptor for the induction of revealable mutations [1-2].

An extension of this experimental design is the sex ratio test, which was also used to assess induced mutation in man. It has been known for a long time that the induction of dominant sex linked lethals in *Drosophila* males will result in an increase in the sex ratio of the progeny of such males, and that the induction of recessive sex linked lethals in *Drosophila* females results in a decrease of the ratio in their progeny. Usually the variations of the sex ratio are tested against a control value. We propose here a minor modification of the sex ratio test which might be more efficient than the system based on the comparison of the progeny of treated and control individuals for the detection of mutagenicity [3].

The modification is simple, and based on the following consideration.

A chemical may or may not be a mutagen. If it is a true mutagen it might induce either dominant or recessive mutations, or both. This last case might be the general case, although it would require careful assessment to determine the relative rates of induction of the two types of mutation.

We believe that it would be easier to detect significant variations when the sex ratio in the progenies of treated males, crossed with normal females, is compared to the sex ratio in the progeny of treated females. Therefore, we established a

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(**) Institute of Animal Biology, University of Padua.

(***) Institute of Zoology, University of Ferrara.

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system in which larvae from a single cross are chronically exposed to a substance, the males and the females are separated at hatching, crossed with untreated individuals, and the sex ratio in the progeny of such crosses is compared in 2×2 tables.

The possible increase in the sex ratio of the progeny of treated males, and the possible decrease in the progeny of treated females, due to the simultaneous induction of dominant and recessive mutations, will increase the chance of detecting mutagenicity; even if only one type of mutation is induced, the system will automatically provide a control group.

2. Materials and methods

For the experiments, a wild type strain (Alexandria) of *Drosophila melanogaster* was used. Cultures were routinely grown on standard cornmeal medium at 24 °C.

Larvae were collected at hatching time and set (200 larvae per dish) in synchronously developing cultures on Bakker's medium [4]. In this medium they were treated by feeding.

The chemical was dissolved, at the desired concentration, in a suspension of heat-killed yeast (1.0 gr/ml). The treatment was effected by administering the chemical twice during larval life: 24 and 40 hrs after hatching time.

Since the killed yeast suspension containing the chemical to be tested is the only source of food for the larvae, we may consider the treatment as chronic.

Four experiments were set up with different concentrations of EMS: 1×10^{-6}, 1×10^{-5}, 4×10^{-3}, 1×10^{-2} M.

Imagins of the treated individuals were collected at hatching and singly crossed with individuals of the same strain raised simultaneously and in similar conditions, except for the EMS treatment. Progenies from crosses were grown on standard medium in glass vials, at 24 °C. Fifteen days after the appearance of the first pupae in the vials, the progeny of each cross was scored for males and females. The results were arranged in 2×2 tables which were analyzed by a chi square test.

3. Results

The results of the experiments are given in Table I. It seems that in all the experiments at increasing doses of EMS the system is capable of detecting significant changes in the sex ratio of the progeny of treated individuals of different sexes. We observed that at doses as low as 10^{-6} M, a significant mutagenic effect is already detectable.

TABLE I

Sex ratio in the progeny of males and females treated with Ethyl-methan-sulphonate.

EMS	Parents		Progeny		TOTAL	Sex ratio	2.78 d
	Treated	Untreated	Males	Females			
1×10^{-6} M	Male	Female	1989	1876	3865	1.0602	0.3014
	Female	Male	3117	3275	6392	0.9518	
	$\chi_1^2 = 7.014$ $P < 0.01$						
1×10^{-5} M	Male	Female	714	655	1369	1.0900	0.4278
	Female	Male	220	235	455	0.9361	
	$\chi^2 = 1.96$						
4×10^{-3} M	Male	Female	687	642	1329	1.0701	0.5805
	Female	Male	801	930	1731	0.8613	
	$\chi_1^2 = 8.83$ $P < 0.01$						
1×10^{-2} M	Male	Female	1942	1803	3745	1.0771	0.6021
	Female	Male	1919	2230	4149	0.8605	
	$\chi_1^2 = 24.74$ $P < 0.001$						

In order to test for a dose effect, we used the indicator $\sqrt{\chi^2/n}$, where n is the total number of progeny individuals raised in an experiment. This indicator is the square root of the contingency coefficient of Pearson, and is equivalent to a correlation coefficient in a 2×2 table.

The correlation coefficient between sex ratio and treatment was therefore plotted on a semilog scale in Fig. 1, and the points obtained in the experiments seem to lie on a straight line; it would be therefore possible to cautiously

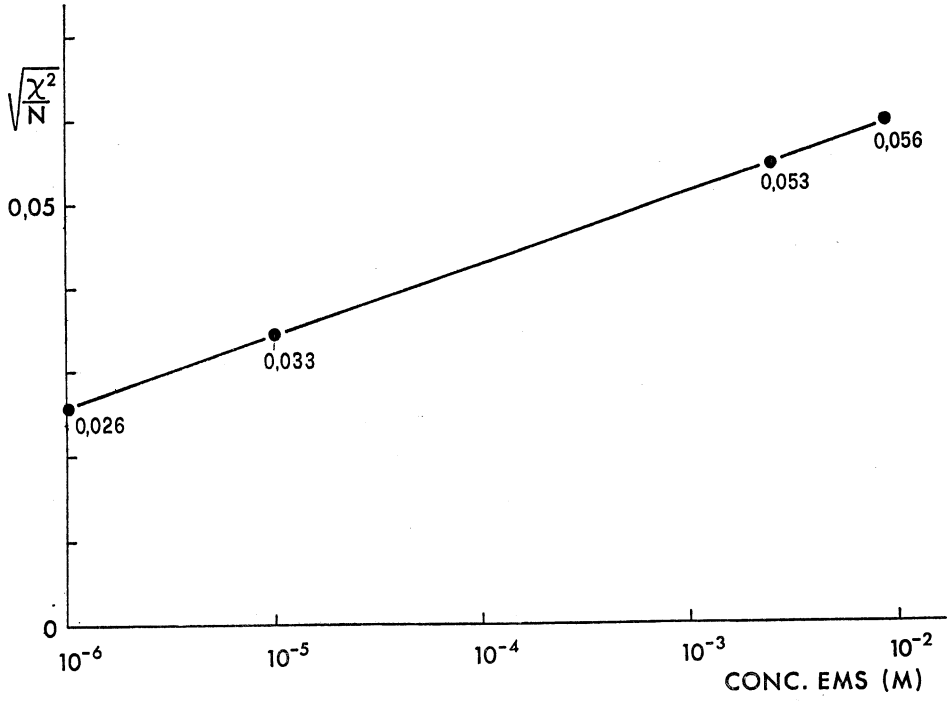

Fig. 1. – Relationship between $\sqrt{\chi^2/N}$ and dose in EMS treatment experiments.

suggest that the strength of the association increases linearly with the log. dose for EMS, namely that the mutagenic effect increases linearly with log. dose.

Estimate of induced mutation rates.

It is possible to suggest an attempt at estimating induced mutation rate per genome from the values of the sex ratio after treatment. Since in *Drosophila melanogaster* the X chromosome represents about 12 percent of the genome, if mutations are induced at random on all chromosomes, the sex ratio in the

progeny of treated males in expected to be:

$$r_M = \frac{p}{q\,(1 - 0.12\,\mu_D)}$$

where p is the proportion of males, q the proportion of females, and μ_D the total rate of induced dominant mutations. On the other hand, the sex ratio in the progeny of treated females is

$$r_F = \frac{p\,(1 - 0{,}24\,\mu_R)}{q}$$

where μ_R is the total rate of recessive mutations induced per genome. Therefore, the difference d between sex ratios in the progeny of males and females which were treated is expected to be

$$d = \frac{p - p\,(1 - 0.12\,\mu_D)\,(1 - 0.24\,\mu_R)}{q\,(1 - 0.12\,\mu_D)}$$

from which, disregarding products between mutation rates and between mutation rates and d, and assuming that p and q do not deviate greatly from 0.5, one may obtain

$$d = .24\,\mu_E + .12\,\mu_D\,.$$

Now, considering the average rate of mutation per genome, either dominant or recessive, one might write

$$2.78\,a \simeq \bar{\mu}\,.$$

From this it would be possible to express the average mutation rate induced per locus under the hypothesis that there are K loci in the *Drosophila* genome.

The mutation rate is expected to vary linearly with the difference between the sex ratio in the progeny of treated individuals.

4. CONCLUSION

Most tests used for the revelation of induced mutation in *Drosophila* are based on the X chromosome.

Now, it seems from results using the mutagen EMS as a standard that significant variations in the sex ratio may be correlated with the rate of induced mutation.

The administration of the mutagen to *Drosophila* as described, and the scoring of the sexes in the progeny of crosses between treated and untreated individuals, seems a simple enough operation to be performed by persons having only a moderate degree of specialization.

The test proved to be sensitive to very low doses of EMS, so is likely to respond also to weak mutagens. If these results were confirmed on different standard mutagens, the sex ratio test might represent a tool in the first level assessment of mutagenicity of chemical compounds in eucaryotes.

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