dimeric enzymes in several species of higher plants: *Brassica oleracea*[5,6], *Helianthus annuus*[7], *Oriza sativa* and *O. perennis*[8], *Zea mays*[9], and even *Picea abies*[10]. However, in *Zea mays*[11], *Hordeum spontaneum*[12] and *Secale cereale*[4] the presence of monomeric and dimeric phosphatases have been reported.

In relation to the behaviour of the hexaploid endosperm phosphatases the hypothesis of a dimeric structure and only three homoeologous loci can be rejected for 2 reasons, namely: a) There are 7 isozymes instead of the 6 expected, and b) all the chromosome arms are related to more than three isozymes (table 2) since more than 3 isozymes are absent or less stained when 1 chromosome arm is absent. Therefore, 3 alternative hypotheses can be put forward: 1) All the endosperm phosphatases are monomers; this hypothesis could be rejected on the basis of the general dimeric character of higher plant phosphatases and the high number of loci needed (at least 13) since several isozymes are related to 2 or 3 chromosome arms. 2) Endosperm phosphatases are dimers; in that case at least 4 loci are needed for 7 isozymes, and this number would be the result of the overlapping of some of the expected dimers; the postulated 4th locus would be in chromosome arm 4DL because this arm is related to the highest number of isozymes (5). 3) Some loci control dimeric enzymes and other loci control monomeric enzymes as in other graminaceous plants[4,11,12], and some isozymes overlap. However, the behaviour of isozyme 4 again[2,3] gails to agree with the last 2 hypotheses as it is absent when the chromosome arm 4Aβ is absent and it is present when the chromosome arm 4DL is absent (fig.; table 2). Therefore, none of the hypotheses suggested is really satisfactory, but evidently, to understand the electrophoretic behaviour of hexaploid wheat endosperm phosphatase isozymes, more than 3 homoeologous loci are needed, and at least an additional 4th locus should be located in chromosome arm 4DL. Since, to date, we have not found variability in the phosphatase isozyme pattern among 40 wheat cultivars scored[4], segregation genetic data are not available for the eludication of the monomeric or dimeric nature of wheat endosperm phosphatases.

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Effect of ethidium bromide on *Drosophila melanogaster* and *Drosophila simulans*

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Summary. Ethidium bromide was fed to *D. melanogaster* and *D. simulans* males in order to test its toxic capacity and potency for the induction of dominant lethals. Our results show that ethidium bromide has a high toxicity and likewise produces dominant lethals to a significant extent in both species, but more effectively in *D. melanogaster*.

Ethidium bromide (EB) is an intercalating agent usually used in molecular genetics and in structural studies of DNA and chromatin. Nishiwaki et al.[1] point out that in mice EB acts as an inhibitor of RNA-dependent DNA polymerase activity, and for this reason it can be considered as an antitumor agent. Furthermore, Heinen[2] shows that EB inhibits cell growth in tissue culture, even at very low concentrations; but in spite of this, EB is not used as an antitumor agent.

With reference to the mutagenic capacity of EB, the results in bacteria show that it is an effective frameshift mutagen if it is metabolically activated by liver microsomes[3]. In *Saccharomyces cerevisiae* EB acts as a strong inducer of *petite* mutants[4]. Its action is based on the inhibition of mitochondrial nucleic acid and protein synthesis and is probably due to specific intercalations between the base pairs of mitochondrial DNA[5,6].

In mice, EB apparently has little or no access to nuclear DNA, at least in vivo, while it intercalates perfectly well with isolated nuclear DNA in vitro[7].

No studies have been done on the mutagenic activity of EB in *Drosophila*. From the experiments quoted above there is not enough evidence that EB cannot act as a mutagenic agent (like many intercalating agents). In this paper we show the preliminary results on the effects of EB in *Drosophila*, comparing the effect on 2 sibling species: *D. melanogaster* and *D. simulans*.

Material and methods. The populations of *D. melanogaster* and *D. simulans* used come from a large number caught in June 1979 in Mirasol (Barcelona).

1. Toxicity test. In this test, males were fed with different concentrations of the test compound in a 30-ml glass filter. The filter is placed in a 100-ml beaker which contains 15–20 ml of test solution. The test solution should be nearly in contact with the lower part of the filter plate[8]. The EB (Merck) was prepared at different concentrations in an aqueous solution which contained 5% sucrose (Merck). Every 12 h the number of dead flies are scored. The treatment lasts for 72 h.

2. Dominant lethal test. In this test we used 2 feeding techniques: a) Treatment during 6 h placing the males in an empty vial with Whatman N.4 paper soaked in the different solutions; b) treatment during 2 days with the feeding technique used in the toxicity test.

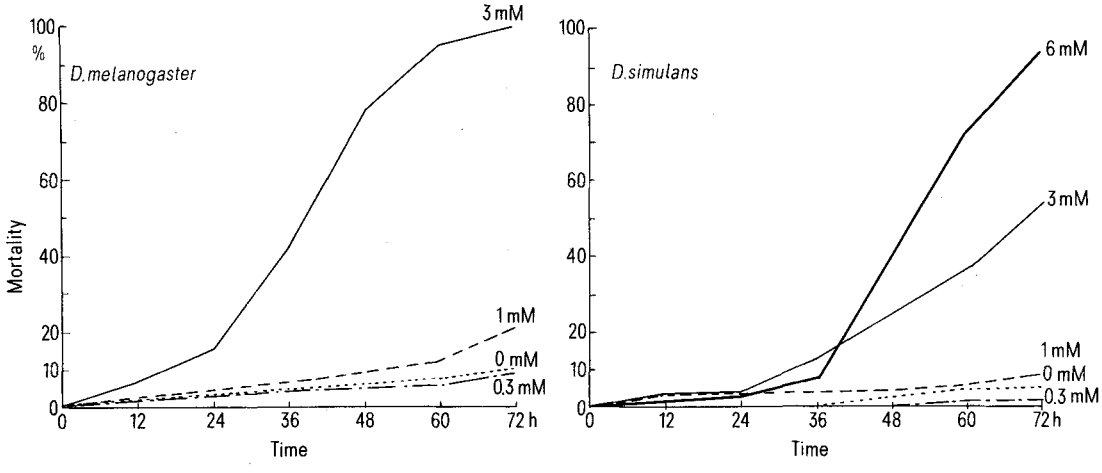
The treated males were coupled (1:1) with females in vials open at both ends. In the upper one, we placed a cotton plug and, in the lower one, a piece of agar-agar darkened with powdered charcoal. Every day, the agar was removed and replaced with fresh piece. The eggs laid were counted, kept at 25 °C and counted again 48 h later in order to check the unhatched eggs.

Crossing the males with different females at different periods, it was possible to score mature spermatozoa, late spermatids and early spermatids. For each brood 15–20

The induction of dominant lethals in male germ cells of *D. melanogaster* and *D. simulans* exposed to ethidium bromide

Experiment	Concentration (mM)	Brood	No. eggs laid		Egg hatchability (%)		Relative hatchability		Dominant lethals (%)	
			D.m.	*D.s.*	*D.m.*	*D.s.*	*D.m.*	*D.s.*	*D.m.*	*D.s.*
1[a]	0	1	7175	7045	96.00	97.87	100	100	0	0
	0.03	1	2412	1053	86.52	92.02	90.01	94.02	9.99	5.98
	0.30	1	1534	1193	84.81	93.46	88.34	95.49	11.66	4.51
1[b]	0	1	4207	2265	96.55	95.94	100	100	0	0
	1.00	1	3501	2954	94.17	93.97	97.53	97.95	2.47	2.05
2[a]	0	2	8217	4872	97.24	93.28	100	100	0	0
	0.03	2	2701	1752	86.78	80.93	89.24	86.76	10.76	13.24
	0.30	2	1853	1317	58.80	86.48	60.46	92.71	39.54	7.29
2[b]	0	2	1264	512	98.66	97.66	100	100	0	0
	1.00	2	450	162	96.22	98.77	97.53	-	2.47	-
3[a]	0	3	7776	5841	95.57	94.14	100	100	0	0
	0.03	3	2570	1756	90.42	93.84	94.61	99.68	5.39	0.32
	0.30	3	1628	1653	80.95	91.47	84.70	97.16	15.30	2.84
3[b]	0	3	2112	456	98.47	98.68	100	100	0	0
	1.00	3	1197	301	98.40	99.44	99.93	-	0.07	-

D.m., Drosophila melanogaster; D.s., D. simulans. [a] and [b] refer to the 2 treatments employed.



Exposure-mortality relationship to EB of *D. melanogaster* and *D. simulans* males after adult feeding.

females were allowed to oviposit during 10 days and all the eggs laid were counted.

Results and discussion. Concentration-mortality relationships are useful to express the biological reactivity of substances tested. From the figure, we can deduce that *D. simulans* is more concentration-resistant than *D. melanogaster*. The LC_{50} values at 48 h i.e., the concentration at which 50% of treated males died within 48 h, were 2.16 and 8.50 for *D. melanogaster* and *D. simulans* respectively. LC_{50} values were calculated assuming linearity of concentration and mortality. These toxicity values are high in comparison with those found for many monofunctional alkylating agents[9].

The table shows the dominant lethal values for the 3 early broods, which correspond to mature spermatozoa, late spermatids and early spermatids. The difference between treatments is striking; thus, the (b) treatment (theoretically the 'most effective' and with the highest concentration) produces fewer dominant lethals than the (a) treatment.

In spite of the differences found between treatments, our results are significant ($p \leqslant 0.05$) in both species, except in brood 3^b in *D. melanogaster* and in broods 2^b and 3^b in *D. simulans*. Generally, it is clear that the induction of dominant lethals is higher in *D. melanogaster* than in *D. simulans*, this would be in agreement with the concentration-resistance pattern. The strong reduction of offspring in the 3rd brood in *D. melanogaster* and in the 2nd and 3rd broods in *D. simulans* indicates that ethidium bromide can act as a mitotic and meiotic poison and it blocks the process of spermatogenesis. However, the real capacity for induction of genetic damage by EB must be measured by the sex-linked recessive lethal test, which is being done.

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