

the atmosphere<sup>15</sup> in a mating disruption trial. With the assay, as little as 2 ng of (E)-11-tetradecenal deposited on 200 mg of Porapak Q (pre-cleaned by repetitive washing with hexane) was extracted and quantitated. A similar analysis by conventional packed column gas chromatography failed to detect the pheromone. High recoveries of pheromone (> 50%) from a stream of air were also measured by absorbing the pheromone onto Porapak Q and subsequently extracting. This technique is currently being used to measure pheromone released by female moths and from lures designed for pheromone traps. The rapidity and sensitivity of this bioluminescent assay provides a powerful new analytical tool for measuring aldehyde pheromones of insects.

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2 C.J. Sanders and J. Weatherston, Can. Ent. 108, 1285 (1976).

3 E.A. Meighen and R.E. MacKenzie, Biochemistry 12, 1482 (1973).  
4 J.W. Hastings, A. Rev. Biochem. 37, 597 (1968).  
5 G.W. Mitchell and J.W. Hastings, Analyt. Biochem. 39, 243 (1971).  
6 S. Ulitzur and J.W. Hastings, Proc. natl Acad. Sci. USA 75, 266 (1978).  
7 O. Shimomura, F.H. Johnston and H. Morise, Proc. natl Acad. Sci. USA 71, 4666 (1974).  
8 D. Riendeau and E. Meighen, J. biol. Chem. 254, 7488 (1979).  
9 A.S. Hill, R.T. Carde, H. Kido and W.L. Roelofs, J. Chem. Ecol. 1, 215 (1975).  
10 J.A. Klun, J.R. Plimmer, B.A. Bierl-Leonhardt, A.N. Sparks and O.L. Chapman, Science 204, 1328 (1979).  
11 B.F. Nesbitt, P.S. Beevor, D.R. Hall and R. Lester, J. Insect. Physiol. 25, 535 (1979).  
12 J.H. Gross, R.C. Byler, R.M. Silverstein, R.E. Greenblatt, J.E. Gorman and W.E. Burkholder, J. Chem. Ecol. 3, 115 (1977).  
13 J.H. Gross, R.C. Byler, R.F. Cassidy Jr., R.M. Silverstein, R.E. Greenblatt, W.E. Burkholder, A.R. Levinson and H.Z. Levinson, J. Chem. Ecol. 2, 457 (1976).  
14 P.J. Silk, S.H. Tan, C.J. Wiesner and R.J. Ross, Envir. Ent., in press (1981).  
15 C.J. Wiesner, P.J. Silk, S.H. Tan and S. Fullerton, Can. Ent. 112, 333 (1980).

The chromosomal location of phosphatase isozymes of the wheat endosperm

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Summary. The 7 phosphatase isozymes found in the endosperm of hexaploid wheat (*Triticum aestivum* L.) are related to chromosomes of homoeology group 4. At least 4 loci are related to phosphatase isozymes.

Hexaploid wheat phosphatase isozymes have been previously studied using the nulli-tetrasomic and ditelosomic series of the 'Chinese Spring' wheat cultivar<sup>2,3</sup>. The 1st work<sup>2</sup> reported that 35-day old euploid seedlings of 'Chinese Spring' showed 6 alkaline phosphatase isozymes (I-6 from faster to slower migration) after electrophoresis in starch gels; isozymes 1 and 2 were related to chromosome 4B, and isozymes 5 and 6 to 4D; no influence of chromosome 4A was reported. In further work<sup>3</sup> using a similar technique, again 6 bands of acid phosphatases were found in 7-day-old euploid seedlings, but this time the use of nulli-tetrasomic and ditelosomic strains revealed that these 6 bands were the results of the overlapping of a least 9 isozymes. Isozymes 4 and 8 were related to chromosome arm 4A $\beta$ , isozymes 2 and 3 to 4BS, and isozymes 5 and 6 to 4DL.

The purpose of the present work was to study alkaline and acid phosphatase isozymes of hexaploid wheat endosperm, the location of the chromosome arms related to these isozymes, and the comparison of these results with previous data reported for wheat seedlings<sup>2,3</sup>.

Materials and methods. The materials used in this study were the nullitetrasomic and ditelosomic strains of *Triticum aestivum* L. cv. 'Chinese Spring' supplied by Professor E.R. Sears. The chromosomal constitution of these nulli-tetrasomic and ditelosomic strains is indicated in table 1. The analysis was carried out with individual crude extracts of embryoless endosperms from dry seeds. Phosphatase isozymes were revealed by means of horizontal 10% polyacrylamide gel slab electrophoresis using buffers and staining solution previously described<sup>2</sup>. 2 different pHs, 8.5

(alkaline phosphatase) and 5.0 (acid phosphatase), were employed for staining.

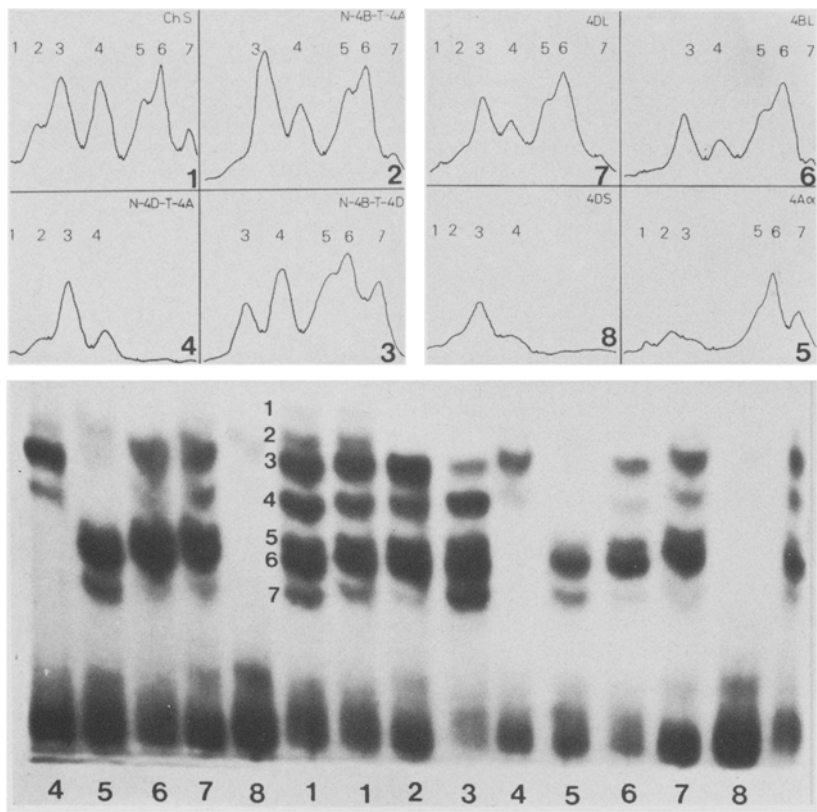
Results and discussion. The results obtained with euploid 'Chinese Spring' (genomes A, B and D) showed an identical 7-banded pattern at both pHs (8.5 and 5.0) for endosperm phosphatases, but better results were obtained at alkaline

Table 1. The chromosomal constitution of nulli-tetrasomic and ditelosomic strains of 'Chinese Spring'

Strains	Chromosomal dosage of nulli-tetrasomics			
	4A	4B	4D	
Euploid Ch.S.	2	2	2	
Nulli-4B-tetra-4A	4	0	2	
Nulli-4B-tetra-4D	2	0	4	
Nulli-4D-tetra-4A	4	2	0	
Chromosome arms present in ditellosomics				
Euploid Ch.S.	4A $\alpha$	4BS	4DS	4D
	4A	4B		
	4A $\beta$	4BL	4DL	
Ditello 4A $\alpha$	4A $\alpha$	4BS	4DS	4D
	4A	4B		
	-	4BL	4DL	
Ditello 4BL	4A $\alpha$	-	4DS	4D
	4A	4B		
	4A $\beta$	4BL	4DL	
Ditello 4DS	4A $\alpha$	4BS	4DS	4D
	4A	4B		
	4A $\beta$	4BL	-	

pH. Therefore, the location experiments were carried out at alkaline pH (fig.). The isozymes were numbered 1-7 from faster to slower migration. The following results were observed when nulli-tetrasomic and ditellosomic seeds were analysed (fig., table 2): a) lack of isozymes 1 and 2 and a decrease in the staining level of isozymes 3 and 4 when chromosome 4B, or its short arm, was absent; b) lack of isozymes 5, 6 and 7, very low staining intensity of isozyme 4, and a slightly decreased staining level of isozyme 3, when chromosome 4D, or its long arm, was absent; likewise tetraploid wheat endosperm (genomes A and B) also lacks isozymes 5, 6 and 7<sup>4</sup>; c) lack of isozyme 4 and a decrease in the staining level of isozymes 2, 3 and 5 when

the chromosome arm 4A $\beta$  was absent; and d) when the nulli-tetrasomic and ditellosomic strains for chromosomes other than the homoeology group 4 were analyzed, all 7 isozymes were found to be present. Thus, like previous results<sup>2,3</sup> for seedlings, the wheat endosperm phosphatases are related to the homoeology group 4. Because of this coincidence, and the coincidence in the relative staining level, the assumption that endosperm isozymes 1-6 are similar or identical to the 1-6 seedling isozymes<sup>2,3</sup> can be made. The exception is the isozyme 4 since neither a relation to the homoeology group 4<sup>2</sup> nor an influence of chromosomes 4B and 4D have been found<sup>3</sup>. Phosphatase isozymes have been previously described as



Electrophoretic and densitometric patterns of euploid, nullitetrasomic and ditellosomic 'Chinese Spring'. 1 Euploid 'Chinese Spring'; 2 Nulli-4B-tetra-4A; 3 Nulli-4B-tetra-4D; 4 Nulli-4D-tetra-4A; 5 Ditello 4A $\alpha$  (lacking arm  $\beta$ ); 6 Ditello 4BL (lacking short arm); 7 Ditello 4DL (lacking short arm); 8 Ditello 4DS (lacking long arm).

Table 2. The chromosomal location of wheat kernel phosphatase isozymes

	Isozymes						
	1	2	3	4	5	6	7
Euploid Ch.S.	+	+	+	+	+	+	+
Ditello 4A $\alpha$	+	+ - -	+ - -	-	+ - -	+	+
Nulli-4B-tetra-4A	-	-	++	+ -	+	+	+
Nulli-4B-tetra-4D	-	-	+ - -	+	+	+	++
Ditello 4BL	-	-	+ - -	+ - -	+	+	+
Nulli-4D-tetra-4A	+	+	+	+ - -	-	-	-
Ditello 4DS	+	+	+ - -	+ - -	-	-	-
Critical arm	4BS	4BS 4A $\beta$	4A $\beta$ 4BS 4DL	4A $\beta$ 4BS 4DL	4DL 4A $\beta$	4DL	4DL
Dimeric hypothesis	$\beta\beta$	$a\beta$	$aa$ $\beta\delta$	$a\delta$ $\beta\delta'$	$\delta\delta$ $a\delta'$	$\delta\delta'$	$\delta'\delta'$
Dimeric-monomeric hypothesis	$\beta\beta$	$a\beta$	$\beta\delta$ $a'$	$aa$ $\beta', \delta''$	$a\delta$	$\delta\delta$	$\delta'$

+, Isozyme present; ++, isozyme present with a higher intensity; + -, isozyme present with a low intensity; + - -, isozyme present with a very low intensity; -, isozyme absent.

dimeric enzymes in several species of higher plants: *Brassica oleracea*<sup>5,6</sup>, *Helianthus annuus*<sup>7</sup>, *Oriza sativa* and *O. perennis*<sup>8</sup>, *Zea mays*<sup>9</sup>, and even *Picea abies*<sup>10</sup>. However, in *Zea mays*<sup>11</sup>, *Hordeum spontaneum*<sup>12</sup> and *Secale cereale*<sup>4</sup> 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<sup>4,11,12</sup>, and some isozymes overlap. However, the behaviour of isozyme 4 again<sup>2,3</sup> fails to agree with the last 2 hypotheses as it is absent when the chromosome arm 4A $\beta$  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<sup>4</sup>, segregation genetic data are not available for the elucidation of the monomeric or dimeric nature of wheat endosperm phosphatases.

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- 2 G.J. Brewer, C.F. Sing and E.R. Sears, Proc. natl Acad. Sci. USA 64, 1224 (1969).
- 3 G.E. Hart and P.J. Langston, Heredity 39, 263 (1977).
- 4 J. Salinas, M. Pérez de la Vega and C. Benito, unpublished results.
- 5 A.B. Wills, S. K. Fyfe and E.M. Wiseman, Ann. appl. Biol. 91, 263 (1979).
- 6 S. Woods and D.A. Thurman, Euphytica 25, 707 (1976).
- 7 A.M. Torres and U. Deindenhofen, Can. J. Genet. Cytol. 18, 709 (1976).
- 8 C. Pai, T. Endo and H.I. Oka, Can. J. Genet. Cytol. 17, 637 (1975).
- 9 Y. Efron, Theor. appl. Genet. 43, 323 (1973).
- 10 K. Lundkvist, Hereditas 79, 221 (1975).
- 11 A. H.D. Brown and R.W. Allard, Crop Sci. 9, 72 (1969).
- 12 A.H.D. Brown, E. Nevo, D. Zohary and O. Gagan, Genetica 49, 97 (1978).

### 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.<sup>1</sup> 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<sup>2</sup> 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<sup>3</sup>. In *Saccharomyces cerevisiae* EB acts as a strong inducer of *petite* mutants<sup>4</sup>. 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<sup>5,6</sup>.

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<sup>7</sup>.

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<sup>8</sup>. 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