Table 1. Specific activities of ADH, ALDH and ALDOX in D.melanogaster and D.simulans.

	D.melanogaster	D.simulans
ADH mU/mg prot.	74.0±4*	23.0±0.8
ALDH mU/mg prot.	25.7±1.5	11.3±0.6
ALDOX U/mg prot.	0.74±0.02	1.41±0.13

* each value represents the mean ±SD of 4 independent experiments.

Table 2. Ethanol and acetaldehyde toxicity in D. melanogaster and D.simulans.

	D.melanogaster	D.simulans
Ethanol LC_{50}	11.3	4.2
Acetaldehyde LC_{50}	2.6	1.5

at -80°C until assayed. ADH and ALDH activities were determined spectrophotometrically by monitoring the formation of NADH at 340 nm (see Garcin 1979; Garcin et al. 1983). ALDOX activity were determined according to Dickinson (1971). Protein concentrations were measured according to the method of Bradford (1976) using serum albumin as the standard. Enzymatic specific activities are expressed in Units (or milliUnits) per mg. protein.

Table 1 shows the results obtained in the two sibling species for the activities of ADH, ALDH and ALDOX. These data were obtained from four independent experiments. ADH and ALDH in D.melanogaster are respectively threefold and two fold higher than those in D.simulans. In contrast ALDOX activity is twofold higher in D.simulans.

For comparison purposes we present in Table 2 in vivo data obtained in previous experiments on ethanol and acetaldehyde toxicity in the two sibling species. It can be seen from the concentrations inducing 50% lethality in the population, (LC 50s) that D.melanogaster is much more tolerant to both agents than D.simulans.

Thus it appears from our biochemical data that both dehydrogenases(ADH and ALDH) play a significant biological role for the expression of alcohol and acetaldehyde tolerance in the two species. The data on ALDOX activity confirm our previous hypothesis that this enzyme is possibly not involved in the ethanol metabolic pathway. Though the biological role of ALDOX is not yet precisely known, our data suggest that the higher ALDOX activity in D.simulans could confer to this species an adaptive advantage over D.melanogaster in environments where other aldehyde substrates are present in large concentrations.

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Latorre, A., R.deFrutos & L.Pascual. Universidad de Valencia, Espana. Loci activity in three A chromosomal arrangements of Drosophila subobscura.

The authors who have studied the patterns of puffing activity in several Drosophila strains carrying different chromosomal aberrations found that, in general, chromosomal arrangements do not affect puffing (see revision of Ashburner & Berendes 1978). A great similarity in the puffing patterns of species carrying different inversions was also obtained by Moriwaky & Ito (1969). However, deFrutos & Latorre (1982) found some differences in the puffing patterns of two different U chromosomal arrangements.

In the present work, patterns of puffing activity of the sex chromosome of three strains are statistically compared. The strains studied were: H271, from a locality near of Helsinki (Finland), which is homozygotic for the A_{st} arrangement; Ra121, from Las Raices, Canary Islands (Spain), which is homozygotic for the A_2 arrangement and R225, from Ribarroja, Valencia (Spain), which was fixed in homozygous for the A_1 chromosomal arrangement. The study was carried out at the 0h. prepupa stage, which coincides morphologically with the eversion of the anterior spiracles. A total of 50 preparations were prepared per strain. Of these, only the females were analyzed. Thus, a total of 33 preparations for A_{st} chromosome,

Table 1. Mean values and standard error (based on two repetitions) of the number of active loci in the A chromosome at 0h. prepupa stage in H271, Ra121 and R225 strains.

Puffs	H271	Ra121	R225
1C	0.020±0.020	0.000±0.000	0.000±0.000
2C	0.005±0.005	0.155±0.005	0.095±0.005
4A	0.025±0.025	0.230±0.100	0.000±0.000
5D	0.535±0.055	0.340±0.050	0.530±0.000
6E-7A	0.005±0.005	0.060±0.010	0.025±0.015
8E/9A	0.025±0.025	0.000±0.000	0.005±0.005
9B	0.160±0.020	0.060±0.050	0.025±0.025
9D	0.010±0.010	0.150±0.060	0.020±0.020
10AB	0.310±0.030	0.300±0.077	0.310±0.051
11D	0.045±0.045	0.085±0.045	0.120±0.040
12	0.440±0.150	0.395±0.045	0.175±0.085
13A	0.005±0.005	0.115±0.085	0.060±0.020
13BC	0.285±0.045	0.340±0.150	0.735±0.025
13E	0.030±0.010	0.135±0.095	0.035±0.005
14B/C	0.095±0.085	0.000±0.000	0.025±0.015
14CD	0.005±0.005	0.120±0.090	0.015±0.015
15B/C	0.010±0.010	0.025±0.025	0.000±0.000
15DE	0.195±0.005	0.140±0.070	0.050±0.050
16B	0.275±0.015	0.370±0.140	0.105±0.045

Table 2. Analysis of Variance.

Source of Variation	SS	d.f.	MS	F
Strain	0.0277	2	0.0139	0.79 ns
Puff	2.3840	18	0.1324	7.57 *
Strain x Puff	0.6300	36	0.0175	3.24 *
Error	0.3052	57	0.0054	

ns: not significant; * $P < 0.001$

29 for A_2 chromosome and 32 for A_1 chromosome were analyzed. In each preparation five nuclei were observed. The obtention of the cultures as also the dissection of the salivary glands and the method of chromosome squash preparation used were identical to those described by deFrutos & Latorre (1982). In each locus only the presence (+) or absence (-) of activity were considered, and the results are given in frequencies out of the total of chromosomes analyzed.

On considering as a puff all the loci found active even once among the total chromosomes studied, a total of 19 puffs were found in A_{st} chromosome, 17 in A_2 chromosome and 16 in A_1 chromosome. The list of puffs is given in Table 1. In A_2 chromosome only 16 puffs were taken into account, because puff 12D was not analyzed. This locus is found in an active state as a consequence of the A_2 inversion (Stumm-Zollinger 1953) that divides the 12 region into 12AC and 12D. In the A_{st} and A_1 chromosomal arrangements the whole region becomes active (puff 12) whereas in A_2 chromosomal arrangement activity was found at the two loci (12AC and 12D) and their moments of activity did not always coincide in the same chromosome. That is to say, it is possible that through the effect of the inversion a differential activity in 12 region was originated. A more detailed study of this region, in order to compare the results in a greater number of strains, is being carried out.

In this species the low activity of the A chromosome in 0h. prepupa is remarkable, and differs from that obtained in the autosomes. A chromosome is the only one that presents a peak of activity in third instar with a decrease of activity in 0h. prepupa (Pascual et al. Genetica, in press).

To compare the results among the strains, two-way analyses of variance with repetition were carried out. Repetitions were obtained taking two sets of fairly equal number of preparations at random. The frequency of appearance of each puff is calculated in each repetition. Mean values and errors are given in Table 1. In Table 2 the results of the variance analysis are shown.

Differences were not found between the chromosomes of the three strains. On the other hand, as was hoped, there do exist differences among puffs. Nevertheless, the existence of a significant interaction strain x puff must be interpreted. That is to say, while a similarity in the total activity of the three strains at 0h. prepupa exists, and the greater or lesser total activity of the puffs is independent of the strain, there are puffs which are more active in some strains than in others and puffs which are less active in some strains than in others.

Further analysis is required before it can be understood whether the existence of the interactions are due to a specific effect of the inversions more than to a strain effect.

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Lichtenstein, P.S.[1,2], M.Emmett[1], L.Dixon[2], and A.J.Crowle[1]. [1]Webb-Waring Lung Institute, University of Colorado Health Sciences Center and [2]University of Colorado at Denver, Colorado USNA. Developmental changes in activity of peroxidase isozymes of D.melanogaster.

Recent studies have demonstrated developmental and age-related changes in total peroxidase activity of D.melanogaster (Armstrong et al. 1978; Poole 1983). Peroxidase commonly exists in multiple isozyme forms throughout the plant and animal kingdoms. Therefore, the purpose of this investigation was to demonstrate and characterize the presence of peroxidase isozymes in D.melanogaster as well as to correlate the total age-related activity changes to the individual isozymes.

The peroxidase substrates DAB (diaminobenzidine) and Hydrogen peroxide produce an insoluble brown product which can easily be trapped in an agarose gel yielding high resolution of peroxidase activity (Graham & Karnovsky 1966). The peroxidase specificity of the stain was demonstrated in two ways: 1. horseradish peroxidase (HRP) and Drosophila extract yielded the brown insoluble product in the gel, whereas neither catalase nor polyphenol oxidase produced any color formation, and 2. both the catalase inhibitor, 3-amino-triazole (Samis et al. 1972; Bewley & Lubinsky 1979), and the polyphenol oxidase inhibitor, phenylthiourea (Dickinson & Sullivan 1975; Smith & Shrift 1979), failed to prevent the color formation by HRP or Drosophila extract.

Electrophoresis of singly fly extracts (1 fly/20 ul) in 1.4% agarose/1.0% dextran gels using a Tris Glycine discontinuous buffer and stained with DAB/H_2O_2 at pH 4.4 (Herzog 1973) yielded 2 to 4 peroxidase isozymes (depending on the developmental stage). Though these zymograms indicated that the transition between isozymes was a continuous process throughout the developmental stages, certain predominant patterns could be correlated to each stage as seen in Figures 1 and 2.

The transition from larva (3rd instar) to early pupa (untanned, newly pupated) was associated with a shift from isozymes 3 and 4 to isozymes 1 and 2. As the pupa tanned (middle pupa), there was a large loss of activity in all isozymes, and only small residual activity from isozyme 1 and/or 3 was detected. In the late pupal stage (darkly pigmented with wing and eye development), there was an increase in total activity, though not as intense as in the larval and early pupal stages. The activity was mostly due to isozymes 3 and 4. Newly emerged flies (0-6 hr) also produced observable activity, though staining more intensely in female flies and again shifting toward isozymes 1 and 2. The above data agreed well with the total peroxidase activity changes found by Armstrong and Poole.

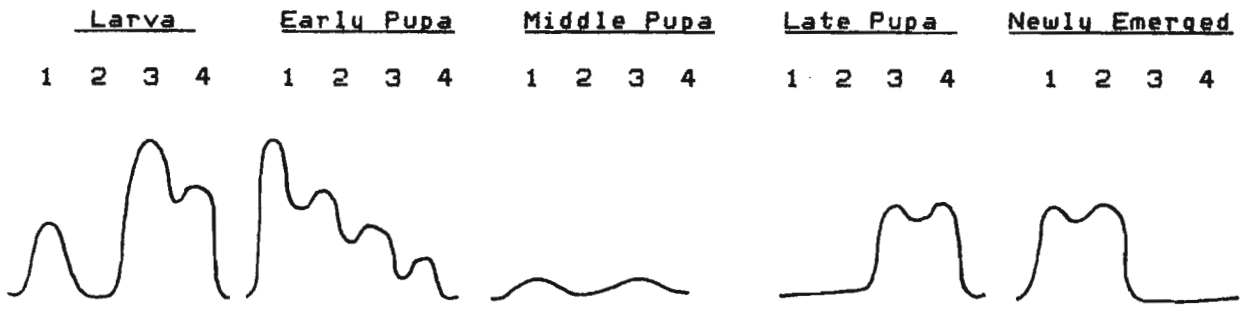

Fig. 1. Densitometric scan of prominent patterns for each stage. Peak height is correlated to amount of isozyme activity.