

Alcohol dehydrogenases in the pupae of *Drosophila melanogaster*¹

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Introduction

A study of protein differences in organisms of different developmental stages is a proper part of an analysis of differentiation. Such studies have been done on a number of species (9) including *Drosophila* (1). The analysis of pupal proteins of *Drosophila melanogaster* by polyacrylamide gel electrophoresis indicated that the major proteins do not vary greatly (1). Studies of enzymatic differences with respect to development have been done on many species (6); this report describes a study of alcohol dehydrogenases in the pupae of *Drosophila melanogaster*. Several enzymes or enzyme systems have been studied in *Drosophila*; these include acid and alkaline phosphatase, ATPase, esterase, xanthine dehydrogenase, α -amylase, aminopeptidase, glucose-6-phosphate dehydrogenase, alcohol dehydrogenase and octanol dehydrogenase (10). Alcohol dehydrogenases have been found to vary among and in strains of *D. melanogaster* (3, 4, 10).

Methods and Materials

Rearing and Aging of Pupae

The stock used in all experiments was Oregon R-C (obtained from E. B. Lewis). An egg producing population was maintained at $25 \pm 0.5^\circ\text{C}$ in a lucite cage (40" x 50" x 55") and fed daily on 10 cm diameter plastic petri plates of agar-oatmeal-molasses medium topped with a thick suspension of active yeast. In order to stimulate the laying of stored eggs, the feeder plates were changed two hours before egg collections were begun. Fresh plates were inserted then into the cage and allowed to remain for two hours. These plates were incubated at 25°C until some individuals reach the prepupal stage. The larvae and prepupae were washed onto a kitchen strainer and suspended in tap water. Those individuals, if any, which floated were discarded. Collections were then made by the flotation method which is based on the time of bubble formation; those individuals, which have formed a bubble in pupal case maturation, float. The flotation procedure was repeated at hourly intervals and the floating individuals were removed to paper towels. They were incubated at 25°C until the desired age had been reached and then were stored at -65°C .

Sample preparation

Individual pupae were put in a drop of homogenization medium in a spot plate depression and thoroughly homogenized with a glass stirring rod. The homogenization medium was 0.25 M sucrose-0.025 M KCl-0.005 M Tris, pH 7.8.

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Electrophoresis and staining

The method of electrophoresis was that of Ornstein (7) as modified by Davis (2). A 0.2 ml aliquot of the large pore sample gel was added to the homogenized pupa and transferred to a rubber grommet. The tube was inserted and the sample gel photopolymerized. The spacer gel and the running gel were successively photopolymerized and thermopolymerized, respectively. Electrophoresis was carried out at 4°C for about forty-five minutes with a constant current of five milliamperes per tube. The voltage across eight tubes rose from 220 to 400 volts during the course of the run. The gels were removed from the tubes by hydrostatic pressure from a rubber bulb after rimming the ends with a dissection needle. The gels were immersed in about 6 ml of substrate solution which contained the following per milliliter: 0.5 mg phenazine methosulfate (Sigma), 0.175 mg NAD (Sigma), 0.25 mg Nitro Blue Tetrazolium, 0.17 mg Trihydroxymethylaminomethane (Sigma) and 6.0 microliters of n-propanol. The pH of the substrate solution was 8.3. The gels were incubated at 37°C for 45-60 minutes and then stored in 7.5% acetic acid.

Results

The pupae gave eight types of staining patterns which are shown in Figure 1. Bands 5, 6, 7 and 8 are considerably fainter than bands 1, 2, 3 and 4; thus, there appear to be two "regions" of alcohol dehydrogenase

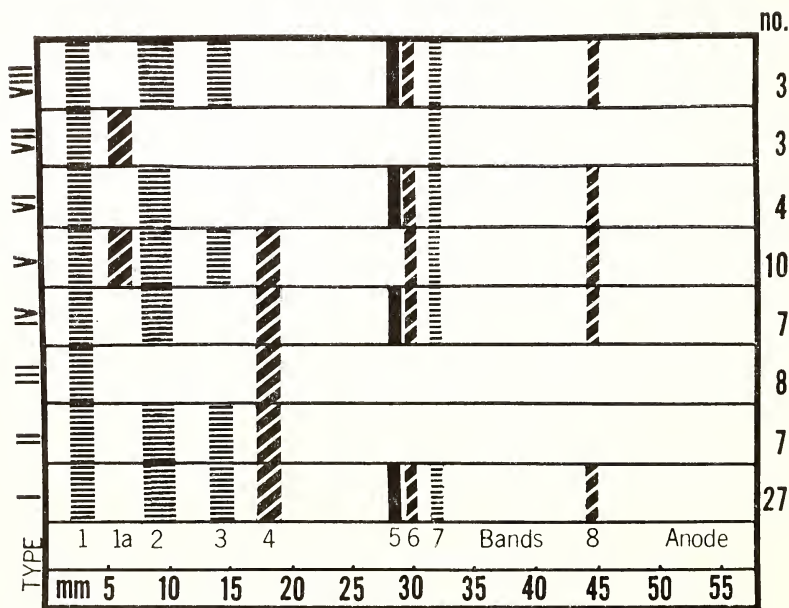


Figure 1.

activity in the gels. In addition to the eight types shown, seven other types were detected in low frequency (one or two cases). These types are modifications of types I and II:

- Type I with band 2 missing
- Type I with bands 2 and 4 missing
- Type I with bands 2 and 5 missing
- Type II with band 1 missing
- Type II with band 4 missing
- Type II with bands 2 and 3 missing

Discussion

These results show that the alcohol dehydrogenase system in the Oregon R-C strain is complex. This strain gives many more phenotypes than reported by others who have studied individual flies (3, 4, 10). This enzyme is clearly unsuited for study during development unless a homozygous strain is established. A differentiation of the bands might be attempted with a study of substrate specificities. The genetics of the alcohol dehydrogenase system could be studied by selective, pairwise matings. These results are similar to those of Scandalios (8) who studied the genetic variation of alcohol dehydrogenase in maize; the results with maize indicate that two regions or zones are present. It is not known if the two zones are due to two genetic systems. Johnson and coworkers (5) have analyzed polymorphisms among isozymic loci in *Drosophila* populations from American and western Samoa. The results reported here support those of Johnson and coworkers in indicating that *Drosophila* strains are more polymorphic at the level of genetic loci than formerly thought.

Finally, this work supports that of Ursprung and Leone (10) in that the alcohol dehydrogenases are polymorphic, but appear to be even more complex when analyzed by polyacrylamide gel electrophoresis.

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