

An Analysis of Pupal Proteins of *Drosophila melanogaster* by Polyacrylamide Gel Electrophoresis

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Introduction

The classical object of genetic study, *Drosophila melanogaster*, provides a desirable system for studying biochemical events related to differentiation: these Dipterans may be grown in synchronous culture, a wealth of genetic information exists about them and the life cycle is relatively short. The dearth of *Drosophila* biochemical genetics has recently been discussed (2). The demonstration of protein differences with respect to age is a proper part of a study of developmental processes. This report includes a description of the methods employed, the results of the analysis of various pupal ages and a short discussion of the results.

Methods and Materials

Rearing of Pupae

The stock used in all experiments was Oregon R-C (obtained from Dr. 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 plastic petri plates of standard banana-agar medium topped with a thick suspension of active yeast. In order to stimulate the laying of stored eggs, these feeder plates were changed two hours before egg collections were begun. Half-pint milk bottles containing about 35 ml of banana-agar medium topped with yeast were inserted into the population cage and then incubated at $25 \pm 0.05^\circ\text{C}$ until the pupae were ready for collection. Pupae were collected by filling the bottles with tap water, loosening the pupae from the walls with a camel's hair brush and pouring the floating pupae onto a kitchen strainer. The pupae were then resuspended in water and washed several times until the wash water was clean. The pupae were allowed to dry on paper toweling, weighed and incubated until the age needed for experimentation. At the proper time the pupae were stored at -65°C .

Homogenization and Centrifugation

The pupae were homogenized in first distilled water in a Kontes glass cone grinder driven by a stirring motor. The homogenization volume was 2.5 x the weight of pupae (ml/gm); the homogenizer was rinsed with an aliquot 1.5 times weight of pupae (ml/gm). The homogenate is brown and viscous but can be drawn up in a machine-manufactured capillary pipette. The homogenate was centrifuged at 45,000 rpm (average force = 122,249 x g) for 30 minutes at 4°C in a model L Spinco centrifuge (type 50 rotor). Centrifugation yields a granular dark brown sediment covered by a whitish middle layer and a yellow-brown supernatant with a pellicle of lipid on its surface. The lipid layer is removed and the supernatant is used directly for electro-

phoretic analysis. The supernatant was stored at -65°C ; electrophoresis of stored supernatants (6 weeks) gives results identical to fresh preparations.

Electrophoresis

Polyacrylamide gel electrophoresis was done on an apparatus constructed from two plastic wash basins supported on a plyboard rack. The method of electrophoresis was that of Ornstein (3) as modified by

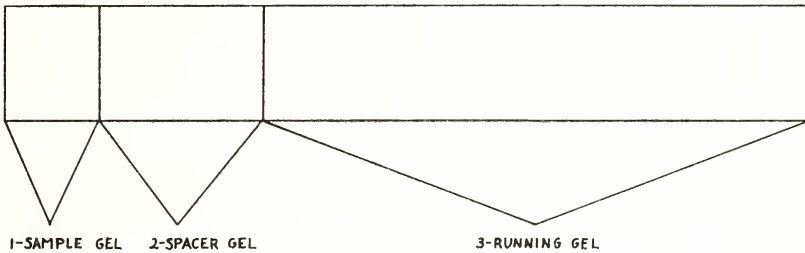


Figure 1

Davis (1). Figure 1 shows the basic relationships of the gel which is polymerized inside glass running tubes (5.3 cm, 5 mm i.d. tubing). A constant current of 5 milliamperes was provided by a Beckman Duostat power supply for about forty minutes at 4°C . The run was stopped when the tracking dye band had proceeded to about 0.5-0.75 cm from the bottom of the tube. The gels were loosened by rimming the ends with a dissection needle and were then forced out by hydrostatic pressure from a medicine dropper bulb. The gels were cut off at the tracking dye band and immersed in Amido-Schwarz stain for one hour or more. The samples were destained electrophoretically in 15% HOAc at 15 ma per tube. The destained gels were stored in 7½ percent HOAc.

Results

About 25 bands were obtained. A composite diagram of the bands is shown in Figure 2. The majority of the bands did not change during the pupal stage; some changes were observed, however. The following results were obtained:

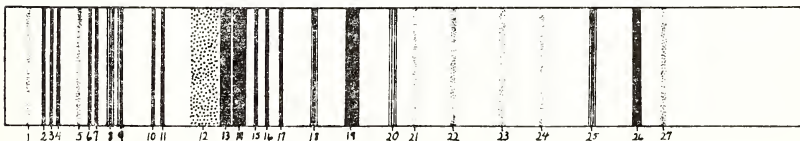


Figure 2

- | | |
|-------------|---|
| Bands 1-7 | Constant |
| Band 8 | Reddish staining band; appears at 132 hours, strongest at 150, fades and disappears by 174. |
| Band 9 | Constant. |
| Bands 10-11 | Did not always separate. |
| Bands 12-14 | Constant. |

Bands 15-18	Bands 15 and 16 may be the same. Bands 17 and 18 grow stronger from 168-192 hours.
Bands 19-24	Constant.
Band 25	Weak until 144; then becomes much stronger and persists through to the end of the pupal stage at 192 hours.

Discussion

These results show that the major protein components of the pupal stage do not vary greatly. Thus, few proteins which are involved in metamorphosis probably can be detected by this method. Some bands (8, 17, 18, 25) may provide useful systems for study. Other experiments that are pertinent to this work could concern the analysis of various *Drosophila* mutants. These data may serve as a reference for future studies on *Drosophila* pupae proteins particularly protein synthesis.

Literature Cited

1. DAVIS, BARUCH J., *Annals of the New York Academy of Sciences*, **121**, 401 (1964).
 2. FUCHS, MORTON S., *Drosophila Information Service*, **40**, 105 (1965).
 3. ORNSTEIN, LEONARD, *Annals of the New York Academy of Sciences*, **121**, 321 (1964).
- SUPPORTED BY RESEARCH GRANT GM-11860, UNITED STATES PUBLIC HEALTH SERVICE