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.

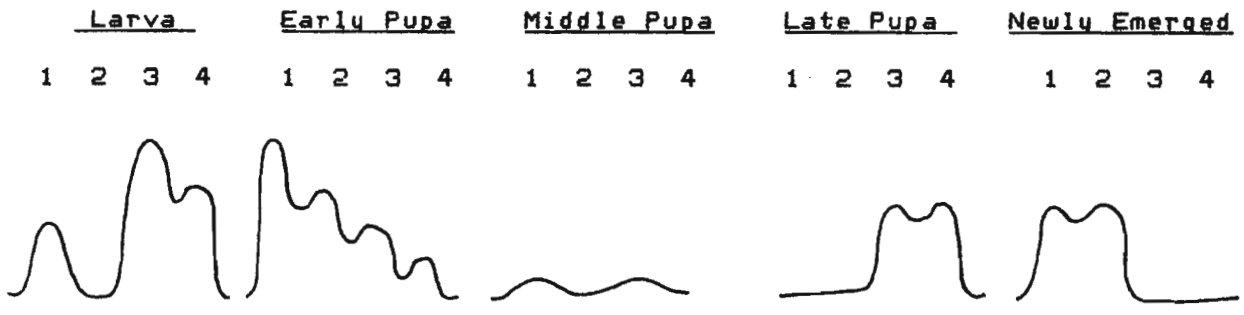

Fig. 1. Densitometric scan of prominent patterns for each stage. Peak height is correlated to amount of isozyme activity.

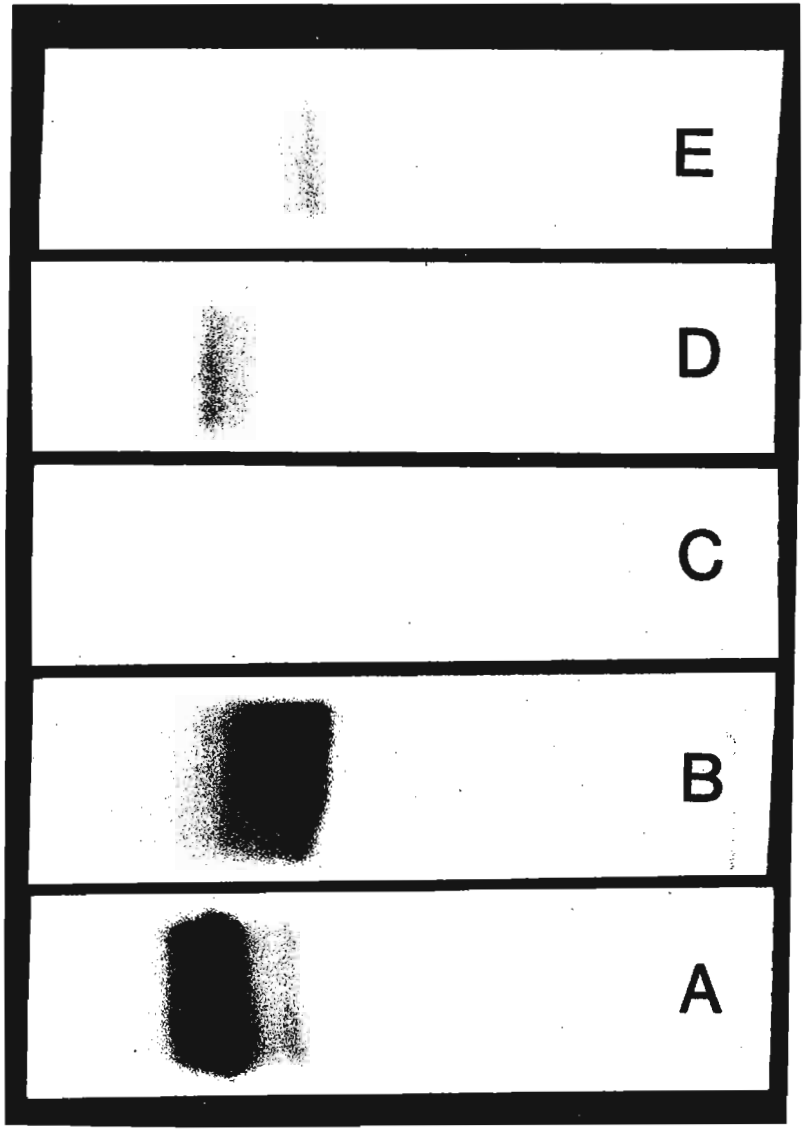

Fig. 2. Zymograms of prominent patterns of each developmental stage in D.melanogaster. A-Larva, B-Early Pupa, C-Middle Pupa, D-Late Pupa, E-Newly Emerged Fly.

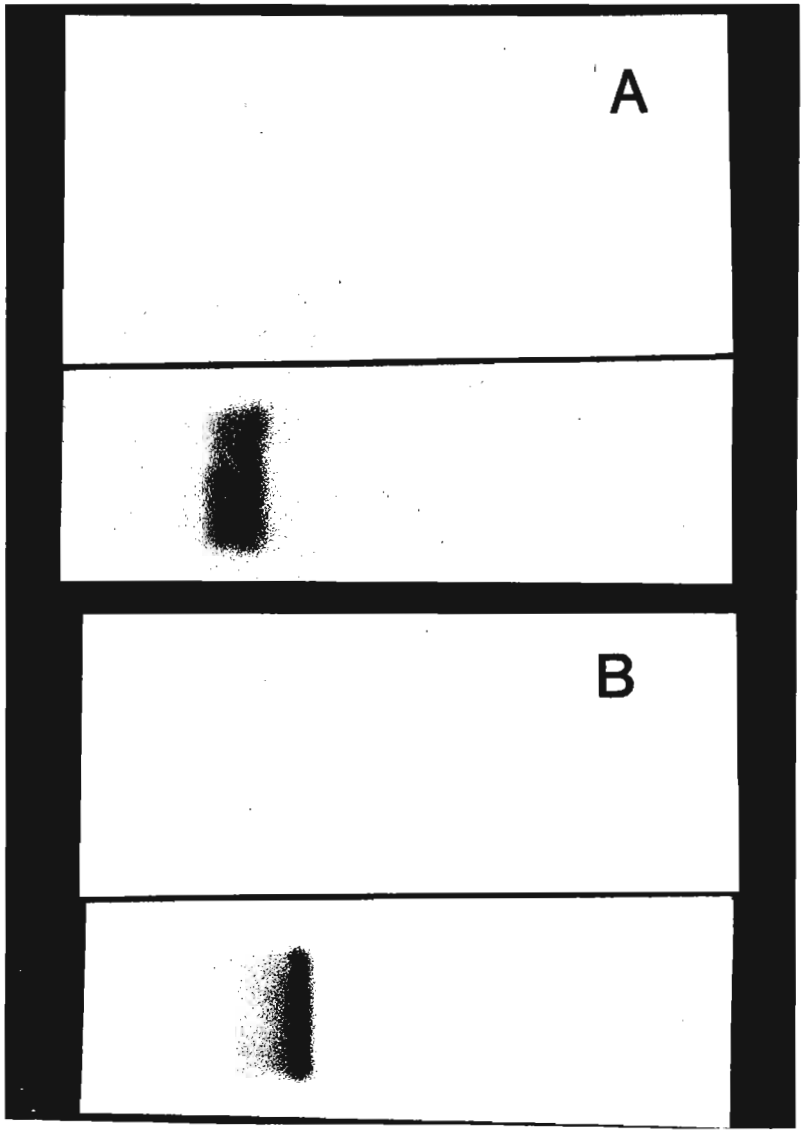

Fig. 3. Comparison between Zymogram and X-IEP patterns. A-Larva, B-Early Pupa.

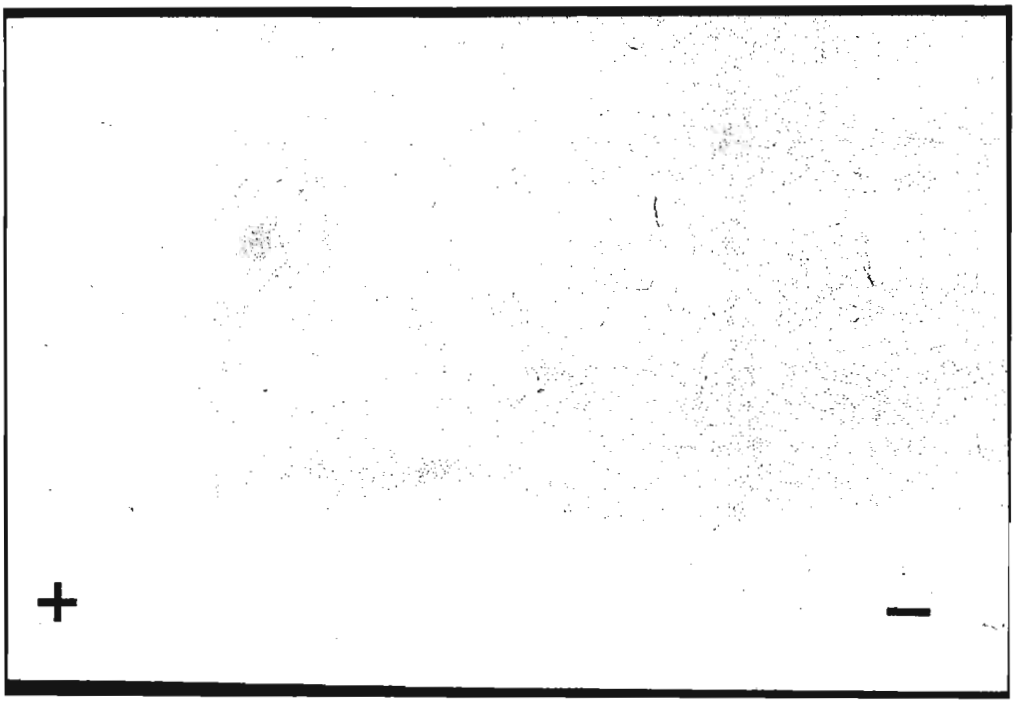

Fig. 4. X-IEP protein pattern of Early Pupa

Yet, though these authors found an activity peak in adult flies at 3 weeks of age, electrophoresis studies yielded insignificant activity in the adult stages. Again, females stained with more frequency than males. The color formation was diffuse and lacked distinct banding patterns. Furthermore, the staining regions tended to shift either anodically or cathodically. This shift did not demonstrate predictability with age, sex, or individual polymorphism. Thus far, it is not certain whether the random adult patterns are due to enzyme processing, gel interaction, substrate preference, protein carriers, membrane-binding, or activity below detectable limits.

Crossed immunoelectrophoresis (X-IEP) confirmed the presence of peroxidase isozymes. In the first dimension, the isozymes were separated electrophoretically, as in the zymograms. The agarose slides were then turned 90 degrees, and the separated proteins were further electrophoresed into a section of gel containing rabbit antibodies to Drosophila proteins from all developmental stages to produce loops of precipitate (Emmett & Crowle 1982). As shown in Figure 3a, both zymogram and X-IEP analyses of a single larva indicated the presence of isozymes 3 and 4, while an early pupa (Figure 3b) demonstrated 3 peaks for isozymes 1, 2 and 3. Since all peaks in the same patterns were the same height and dimensions, the isozyme proteins were approximately of the same concentration. Therefore, the difference in intensities in the zymograms, probably represented differences in enzyme activity. Fusion of the isozyme precipitates indicated that these isozymes were different forms of the same protein, as determined by their immunological identity. An X-IEP for the early pupa in Figure 3b did not detect peroxidase peaks upon staining for protein (Figure 4), but did reveal numerous other proteins against which to compare the peroxidase.

Studies of peroxidase alterations and aging in adult Drosophila must await further developments (i.e., enzyme purification). Nevertheless, zymogram and X-IEP analyses of the 4 detected isozymes offers a unique opportunity to examine the early developmental stages of this organism.

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