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Evidence for the Adaptive Significance of Enzyme Activity Levels: Interspecific Variation in α-GPDH and ADH in *Drosophila*

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The activity levels of alcohol dehydrogenase and α-glycerophosphate dehydrogenase were compared among nine species of Drosophila *representing three phylogenetic groups. For any given life stage, interspecific variability in activity level was much greater for ADH than for α-GPDH. Patterns of ontogenetic expression of enzyme activity were also much more variable among species for ADH than for α-GPDH. These results are consistent with the interpretation that α-GPDH is involved with a relatively uniform adaptive function among species, whereas ADH levels may reflect variable adaptive capabilities. There is a significant correlation between ADH activities and survivorship on alcohol-treated media for these nine species.*

KEY WORDS: *Drosophila*; enzyme activity variation; α-glycerophosphate dehydrogenase; alcohol dehydrogenase.

INTRODUCTION

Levels and ontogenetic patterns of enzyme activities are known to vary within and between species (Avise and McDonald, 1975; Ward, 1974; Hewitt *et al.*, 1974; Birley and Barnes, 1973; Ward and Herbert, 1972; Pipkin and Hewitt, 1972). Since enzyme activities are subject to genetic control (Hewitt *et al.*, 1974; Ward and Herbert, 1972), they are susceptible to the action of natural selection. Although experiments have shown that activity levels can be important in determining rates of substrate breakdown

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and product formation that are crucial to major biological functions (e.g., O'Brien and MacIntyre, 1972*a*,*b*), the question of whether the variable patterns and levels that occur in nature are of evolutionarily adaptive significance remains largely unanswered.

One approach to this problem is to study the variation in enzyme activities between species of diverse ecology and phylogenetic affinity. Such a comparative approach may allow an assessment of the relative influences of phylogeny and adaptive function in the determination of enzyme activity levels and ontogenetic patterns in different species. We have assayed the activity levels of two enzyme systems, α-glycerophosphate dehydrogenase (α-GPDH; E.C. 1.1.1.8) and alcohol dehydrogenase (ADH; E.C. 1.1.1.1), in nine species representing three phylogenetic groups within the genus *Drosophila*. In addition, adult survivorship was measured for each species on media treated with a variety of alcohol concentrations.

MATERIALS AND METHODS

Species Studied

The nine *Drosophila* species examined represent three distinct phylogenetic groups within the subgenus *sophophora*. The *willistoni* group is represented by *D. willistoni*, *D. equinoxialis*, and *D. nebulosa*; the *obscura* group by *D. pseudoobscura*, *D. persimilis*, and *D. azteca*; and the *melanogaster* group by *D. melanogaster*, *D. simulans*, and *D. ananassae*. The geographical distribution and ecological preferences of the species studied are quite distinct. Species from the *willistoni* group are found in natural tropical environments. The *obscura* group species also occur preferentially in natural habitats but are confined to the temperate climates of western North America. The members of the *melanogaster* group occur in man-made habitats; *D. melanogaster* and *D. simulans* are found in temperate and tropical climates throughout the world. *D. ananassae* is pantropical but absent from colder climates. (Patterson and Stone, 1952).

Source of Strains

Adults of *D. pseudoobscura*, *D. persimilis*, *D. melanogaster*, and *D. simulans* were collected from McDonald Ranch, Napa County, California, in October 1974; *D. willistoni* and *D. equinoxialis* from Chetumal, Quintana Roo, Mexico, in September 1974; *D. ananassae* and *D. nebulosa*, respectively, from Merida and Peto, Yucatan, Mexico, in September 1974. Wild females of each species were used to initiate independent strains. F_1 second and third instar larvae, early pupae (prior to visible tissue differentiation), late pupae (eyes and

wing pads visible), early adults (2–3 days posteclosion), and late adults (>4 days posteclosion) collected from at least ten independent strains of each species were used in the activity studies. The viability studies were carried out with late adults.

Enzyme Assays

Six assays were made for each life stage of the nine species. For a single assay, 10 ± 0.5 mg of flies were carefully weighed and then homogenized with a glass tissue grinder in 0.6 ml of deionized water. The homogenate was centrifuged in a Sorval SR2 centrifuge at 5 C for 20 min at 48,000 *g*. The supernatant was removed and stored at 5 C until assayed within 24 hr after preparation. The reaction mixtures and spectrophotometric techniques were as previously described (Avise and McDonald, 1975) except that 0.05 ml of tissue homogenate and an additional 0.05 ml of 0.05 M tris-HCl buffer, *p*H 8.5, were employed. The assays were made on a Beckman Acta II spectrophotometer by measuring rate of conversion of NAD^+ to NADH at 340 nm for 2 min. One unit is defined as 1 μm NAD^+ reduced per 1 ml reaction mixture per minute per milligram live weight.

Adult Survivorship

Late adults were tested for ability to survive at room temperature (23 C) on standard cornmeal–molasses medium supplemented with propan-2-ol at concentrations of 1%, 2.5%, 5%, and 10% by volume. Ten replicate vials, each containing five females and five males, were started for each species at each alcohol concentration. Adult survivorship was recorded at 8-hr intervals over a period of 100 hr.

The alcohol was thoroughly mixed with the medium after it was poured. Flies were introduced as soon as the mixture cooled, usually within 1 hr after addition of the alcohol. This precaution was necessary since flies introduced at later times showed lower mortality, presumably because some alcohol had evaporated from the vials.

RESULTS

Enzyme Activities

The activity levels of α-GPDH and ADH during development are presented for each species group in Figs. 1 and 2, respectively.

α-Glycerophosphate Dehydrogenase

The developmental patterns of α-GPDH activity are similar among all nine

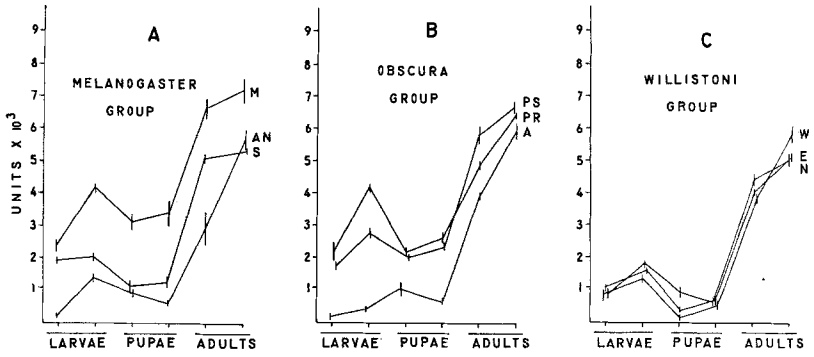

Fig. 1. Changes in activity of α-GPDH during ontogeny of nine species representing three phylogenetic groups of *Drosophila*. Mean activity ± SE is given for each of six developmental stages. Species abbreviations are as follows: M, *melanogaster*; PS, *pseudoobscura*; PR, *persimilis*; N, *nebulosa*; AN, *ananassae*; A, *azteca;* S, *simulans*; W, *willistoni*; E, *equinoxialis*.

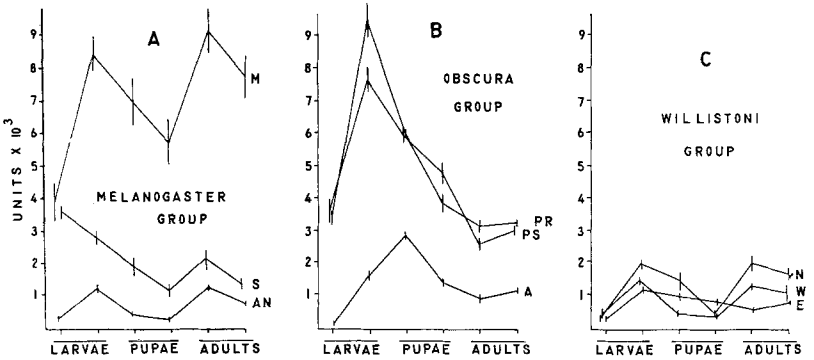

Fig. 2. Changes in activity of ADH during ontogeny of nine species representing three phylogenetic groups of *Drosophila*. Mean activity ± SE is given for each of six developmental stages. Species abbreviations are as in Fig. 1.

species. After a low peak in the third instar larvae, the activity levels drop in early pupae and then rise again to the highest level in adults. The general shape of the α-GPDH curves agrees with those previously reported for this enzyme (Rechsteiner, 1970; Karlson and Sekeris, 1964). Although significant variation in activity levels of α-GPDH exists even among species of the same phylogenetic group (e.g., during late larval stages *D. melanogaster* surpasses *D. simulans* and *D. ananassae* by better than 2 units), the overall spread in α-GPDH activity is never greater than 4 units at any life stage, for any of the species.

Alcohol Dehydrogenase

There is significant variation both in pattern and in level of ADH activity among the species examined. Members of the *willistoni* group display mildly U-shaped patterns[2] at low activity levels. *D. melanogaster* surpasses *D. simulans* in ADH activity during late larval and adult stages by about 8 units. *D. ananassae*, the third member of the *melanogaster* group, displays a slight U-shaped pattern and low activity. Among the *obscura* group flies, third instar larvae display the highest ADH activity. In later stages the activities gradually decline and reach their lowest level in adults. The average level of ADH activity in *D. pseudoobscura* and *D. persimilis* is intermediate between that of *D. melanogaster* and members of the *willistoni* group, while that of *D. azteca* is relatively low.

U-shaped curves have previously been reported for ADH in *D. melanogaster* (Rechsteiner, 1970; Ursprung *et al.*, 1970) and *D. hydei* (Imberski and Strommen, 1972). The downward pattern in the *obscura* group flies is similar to previous findings in *D. pseudoobscura* (Avise and McDonald, 1975).

Adult Survivorship

Survivorship curves for 1%, 2.5%, 5%, and 10% alcohol environments are presented in Fig. 3. Relatively little mortality occurred in vials with 1% alcohol (highest in *D. equinoxialis* with 15% mortality after 100 hr). At a 2.5% alcohol concentration, significant viability differences between species are apparent. *D. willistoni* and *D. ananassae* had the highest mortality of about 90% after 100 hr. *D. nebulosa*, *D. equinoxialis*, *D. azteca*, and *D. similans* showed intermediate mortalities of about 55% after 100 hr. *D. persimilis*, *D. pseudoobscura*, and *D. melanogaster* survived well at this concentration with a mean mortality of only about 25% after the same period of time. The alcohol severely affected all species at the 5% concentration. Mortalities after 100 hr were 60%, 70%, 80%, and 85% for *D. melanogaster*, *D. pseudoobscura*, *D. persimilis*, and *D. nebulosa*, respectively. The remaining five species left no survivors at 100 hr. *D. willistoni* was the first to succumb (30 hr), followed by *D. azteca* (40 hr), *D. equinoxialis* (60 hr), *D. similans* (98 hr), and *D. ananassae* (less than 1% survival after 100 hr). At an alcohol concentration of 10%, mortality was high for all species. Survivorship of *D. melanogaster* was highest with individuals alive to 50 hr. The remaining species succumbed at 30 hr or earlier.

[2] The term "U-shaped pattern" describes the ontogenetic pattern in which enzyme activity levels per unit body weight increase during larval development, fall during pupation, and recover to higher levels again in adults.

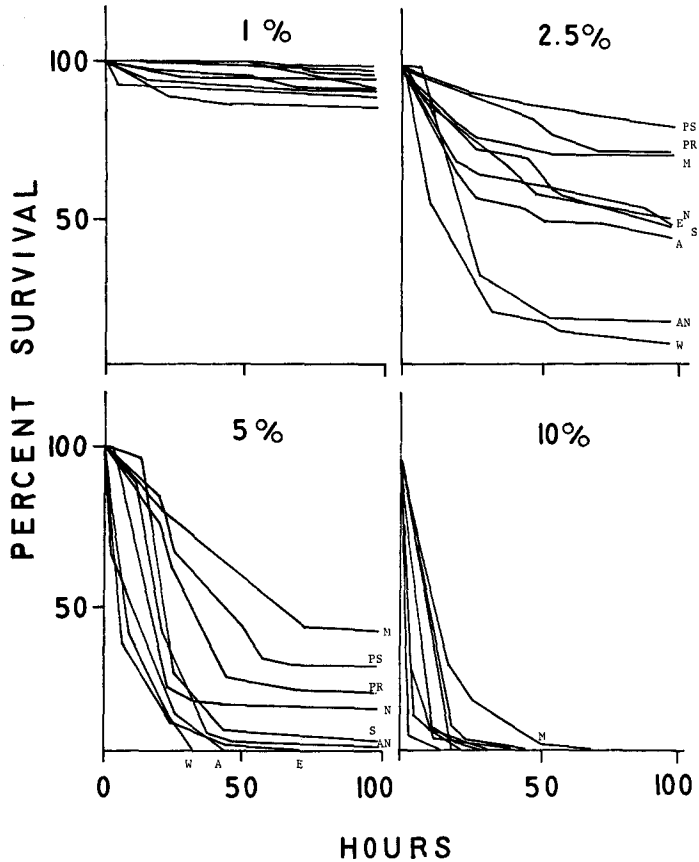

Fig. 3. Survivorship of adults of nine *Drosophila* species on media treated with four different concentrations of isopropanol. Species abbreviations are as in Fig. 1.

DISCUSSION

α-GPDH plays a crucial role in the cytosol–mitochondrial shuttle system (Sacktor 1965; Sacktor and Cockran, 1957; Chefurka, 1965). In addition to this soluble cytoplasmic enzyme, there is a mitochondrial, particle-bound oxidase, α-glycerophosphate oxidase (α-GPO; E.C. 1.1.99.5) (O'Brien and MacIntyre, 1972*a*). α-GPDH catalyzes the reduction of dihydroxyacetone phosphate while α-GPO carries out the reverse reaction in mitochondria. These two enzymes are coordinated to (1) regulate oxidized NAD in the cytoplasm for the continuation of glycolysis and (2) produce energy for flight by donating electrons to the respiratory chain in insect muscle mitochondria.

The role of the cytosol–mitochondrial cycle in larvae is believed to be involved with the production of ATP, most likely for utilization in lipid synthesis (O'Brien and MacIntyre, 1972*a*).

There is no obvious reason why the species of *Drosophila* examined should have grossly different requirements for phospholipid synthesis, ATP production, or energy production for flight. Thus if *in vitro* measures of enzyme activity levels and patterns accurately reflect physiological function we might expect similar α-GPDH levels and developmental patterns in the species surveyed. This indeed appears to be the case. The biological relevance of *in vitro* measures of α-GPDH activity is supported by the fact that there is a marked increase in observed activity as adults develop functional flight musculature. In addition, it has recently been found that mutant adult *Drosophila* deficient in measurable α-GPDH activity cannot initiate or sustain flight (O'Brien and MacIntyre, 1972*b*).

The function of ADH in *Drosophila* is presently unknown. Johnson (1974) has proposed a predominantly regulatory function for ADH in lipid metabolism. Other authors have stressed roles for ADH involving adaptive responses to environmental parameters. Pipkin *et al.* (1973) and Vigue and Johnson (1973) suggest a role in temperature adaptation. An important function of ADH may also include the detoxification and/or utilization of exogenous alcohols (McKenzie and Parsons, 1972, 1974; Gibson, 1970; Kojima *et al.*, 1970).

These proposed functions are not mutually exclusive. However, if at least one major function of ADH is related to environmental factors, ADH activities might be expected to differ between species depending on particular ecological requirements. Our results are consistent with this possibility. Very different levels of activity are apparent even among species showing close phylogenetic affinity. *D. simulans* and *D. melanogaster* are morphologically nearly indistinguishable and by all criteria are closely related phylogenetically, but the activity level of ADH is much higher in *D. melanogaster*.

The *in vivo* significance of spectrophotometrically measured differences in ADH activity is supported by our adult survivorship studies on alcohol-treated media. There is a significant correlation between level of adult ADH activity and survivorship at higher alcohol concentrations (Table I).

It is conceivable that some property other than ADH activity *per se* may allow these species to survive well on alcohol media. However, earlier studies also provide evidence for the biological significance of our *in vitro* measures of ADH activity. McKenzie and Parsons (1972, 1974) and David *et al.* (1974) report results of viability studies in *D. melanogaster* and *D. simulans*. They show significant differences between the species in ability to survive on alcohol media. This result corresponds to the differences in

Table I. Correlation Coefficients Between Adult Survivorship and Enzyme Activities[a]

Hours	Alcohol concentrations		
	1%	2.5%	5%
30	0.35	0.60	0.88[b]
100	0.42	0.65	0.91[b]

[a] The correlation coefficient among the nine *Drosophila* species is given for 30 and 100 hr of exposure to three different alcohol concentrations.
[b] $p < 0.01$.

ADH activity we find between these species. McKenzie and Parsons (1974) also report that in field collections in and around the maturation cellars of a wine vineyard *D. melanogaster* was found almost exclusively within the cellar while both *D. melanogaster* and *D. simulans* were found without. However, even if measured levels of ADH activity accurately reflect *in vivo* capacities of this enzyme in *Drosophila*, the question of whether the breakdown of exogenous alcohols is a major adaptive function of ADH in nature remains unanswered. The major evolutionary stimulus for the selective establishment and maintenance of ADH properties during development may or may not be related to ability to deal with environmental alcohols. The fact that we find significant variation in both activity levels and ontogenetic patterns for the enzyme among the nine species examined does suggest that at least one of its major functions is perhaps of an environmentally adaptive nature.

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