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Gurbachan Miglani
Franklin Ampy, *Howard University*

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***Drosophila* Alcohol Dehydrogenase: Developmental Studies on Cryptic Variant Lines**

Gurbachan S. Miglani¹ and Franklin R. Ampy²

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*Thirty-five cryptic variant lines were used to examine the mechanisms involved in genetic modulation of alcohol metabolism in *Drosophila*. Late third-instar larval, preemergence pupal, and adult stages cultured at 18 and 28 C were examined. Spectrophotometric analyses for native alcohol dehydrogenase (ADH) activity and residual ADH activity after treatment with guanidine hydrochloride and heat were performed. Differential response of cryptic variants to treatment with the denaturants during development suggested that this variation may have an adaptive significance.*

KEY WORDS: alcohol dehydrogenase; *Drosophila*; cryptic variants.

INTRODUCTION

A theory proposed by Johnson and Schaffer (1973) suggested that enzyme polymorphisms are often associated with regulatory reaction in metabolism. It was further suggested that the regulatory role of a particular enzyme reflected the degree of heterozygosity at that enzyme locus (Johnson, 1974). The examination of several natural populations of *Drosophila* revealed a remarkable degree of polymorphism at specific loci (Courtright, 1976). The number of alleles per locus, allele frequency, proportion of polymorphic loci, and average heterozygosity per population for the enzymes involved directly with primary reactions (e.g., glucose metabolism) are substantially lower than for gene-enzyme systems that are involved with secondary reactions (e.g., alcohol metabolism) (Kojima *et al.*, 1970).

¹ Department of Genetics, Punjab Agricultural University, Ludhiana, Pb. 141001, India.

² Department of Zoology, Howard University, Washington, D.C. 20059

The number of variant proteins that are revealed in a population depended on the methodologies used, the intensity of effort, and the geographical diversity of the strains examined (Koehn and Eanes, 1979). Spectrophotometric analyses revealed that enzyme activities varied among and within species (Avisé 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 might be responsive to the action of natural selection. Enzyme activity levels could be important in determining rates of substrate breakdown and product formation crucial to major biological (developmental) functions (O'Brien and McIntyre, 1972 a, b).

McDonald and Avisé (1976) suggested that at least one of the major functions in the variable levels of enzyme activity might, perhaps, be of an adaptive nature. The extent of biochemical, genetic, and developmental studies on alcohol dehydrogenase (ADH) makes this system probably one of the most broadly characterized gene-enzyme systems in *Drosophila* (Dickinson and Sullivan, 1975). Since Mexican strains of *D. melanogaster* showed a cline in the frequency of *Adh* alleles from coastal to central highland regions (Pipkin *et al.*, 1973, 1975, 1976), they appeared to be an ideal system for investigating the regulatory mechanisms which operate to control enzyme levels during development.

From the 16 Mexican *D. melanogaster* populations (Pipkin *et al.*, 1973, 1976) available, 52 isochromosomal lines (12 homozygous for *Adh*^I and 40 homozygous for *Adh*^{II}) were extracted (Miglani, 1980). The isochromosomal lines homozygous for the *Adh*^I or *Adh*^{II} allele were varified electrophoretically (Miglani, 1980). Significant variations in native ADH activity were detected among and within Mexican populations. The existence of an enormous amount of additional genetic variation in ADH activity in isochromosomal lines having approximately the same native ADH activity was revealed subsequent to treatment with guanidine hydrochloride (GuHCl), urea (UR), and heat (Miglani, 1980). Ursprung and Carlin (1968) found that with concentrations of 6 M GuHCl and 10 M urea the activity of ADH was completely lost, as indicated by the absence of isozyme bands after electrophoresis. Therefore, the concentrations used by those workers would be inappropriate for the present study, whose purpose was to investigate cryptic variation in ADH activity. The concentrations of GuHCl (0.7 M) and urea (1.0 M) and the temperature (45 C) used in the present study were, on the average, found to be statistically effective in reducing ADH activity. Isochromosomal lines (cultured at 25 C) which differ significantly in the percentage residual ADH activity after treatment with GuHCl, UR, and heat were classified as "resistant" or "susceptible" and were designated "cryptic variant" lines (Miglani, 1980).

Developmental studies were undertaken using the cryptic variant lines to examine the mechanisms involved in genetic modulation of metabolism compensating for variable reaction conditions.

MATERIALS AND METHODS

The isochromosomal lines were extracted from the following Mexican *D. melanogaster* populations: Puebla (Pu), San Luis Potosi (SLP), Tahuacan (Teh), Saltillo (Sa), Actalan (At), Oaxaca (Ox), Cuatla (Cu), Orizaba (Oz), Cordoba (Co), Yanga (Ya), Palenque (Pe), Acayucan (Ac), Coatzacoalcos (Cz), Merida (Me), and Rio Papalopan (Rio). The 35 cryptic variant lines used in the present investigation were described by Miglani (1980). Lines where the percentage residual ADH activity after treatment with denaturants was 80% or greater were designated "resistant," while those lines where the residual ADH activity was 20% or less were termed "susceptible." Only male adult flies raised at 25 C were utilized in determining the cryptic variant lines. The flies from these lines were allowed to lay eggs for 6 hr at 25 C and allowed to develop at either 18 or 28 C. Late third-instar larval, preemergence pupal, and adult (males only) stages were selected for the developmental studies. The three stages of *D. melanogaster* above were collected on day 4 (larval), day 8 (pupal), and day 16 (adult) after oviposition when the flies were raised at 28 C. The physiologically comparable ages for flies raised at 18 C were collected on day 10 (larval), day 20 (pupal), and day 32 (adult) after oviposition. Spectrophotometric analyses for native (prior to treatment) ADH activity and residual ADH activity after treatment with GuHCl and heat were conducted as previously described (Miglani, 1980). ADH activity was expressed as nanomoles of NADH produced per milliliter per minute per milligram of live weight.

RESULTS

The mean ADH activity levels were calculated during three developmental stages for all Adh^I/Adh^I and all Adh^{II}/Adh^{II} cryptic variant lines raised at 18 and 28 C. No significant differences in mean ADH activity were observed between the larval and the pupal stages when Adh^I/Adh^I lines were raised at 18 C (Table I). However, a significant difference was observed when these flies were raised at 28 C ($P < 0.001$) (Table II). A significant difference in the mean ADH activity was observed between the pupal stages when Adh^I/Adh^I cryptic variant lines were raised at 18 and 28 C. The differences in the mean ADH activity between the larval and the pupal and the adult stages for Adh^{II}/Adh^{II} lines were found to be significant ($P < 0.001$) regardless of whether the flies were raised at 18 or 28 C. The mean ADH activity levels during the larval stage of the Adh^I/Adh^I lines and during the

Table I. Native ADH Activity (nmol of NADH produced/ml/min/mg of live weight) During Different Developmental Stages Collected on Day 10 (Larval Stage), Day 20 (Preemergence Stage), and Day 32 (12-Day-Old Adult) After Oviposition from Cryptic Variant Lines Raised at 18 and 28 C

Isochromosomal line	Developmental stage		
	Larval	Pupal	Adult
<i>Adh^I/Adh^I</i> lines (10) ^a			
Average body weight at 18 C	75.0 ± 2.19	58.1 ± 2.03	38.3 ± 1.04
Average body weight at 28 C	62.6 ± 2.61	40.8 ± 1.25	27.0 ± 1.05
Average ADH activity at 18 C	2.7 ± 0.24	1.9 ± 0.13	5.6 ± 0.33
Average ADH activity at 28 C	2.3 ± 0.13	1.5 ± 0.06	7.0 ± 0.46
<i>Adh^{II}/Adh^{II}</i> lines (40) ^a			
Average body weight at 18 C	78.8 ± 1.37	55.6 ± 1.06	36.5 ± 0.58
Average body weight at 28 C	57.4 ± 2.61	41.2 ± 0.44	31.8 ± 0.50
Average ADH activity at 18 C	1.5 ± 0.08	1.1 ± 0.06	2.4 ± 0.13
Average ADH activity at 28 C	1.5 ± 0.05	1.0 ± 0.05	2.3 ± 0.54

^aActual body weights and ADH activity values by specific lines are available upon request.

larval, the pupal, and the adult stages of the *Adh^{II}/Adh^{II}* lines raised at 18 C showed no difference from the ADH activity levels of the corresponding stages raised at 28 C. However, the pupal and the adult stages collected from the *Adh^I/Adh^I* lines raised at 18 C were significantly different ($P < 0.05$) in mean ADH activity levels from the corresponding stages raised at 28 C.

Table II. Average ADH Activity (nmol of NADH produced/ml/min/mg of live weight) and Average Percentage ADH Activity Subsequent to Treatment with 0.7 M GuHCl for 40 sec and Heat for 15 min at 45 C During Different Developmental Stages Collected on Day 10 (Larval Stage), Day 20 (Preemergence Stage), and Day 32 (12-Day-Old Adult) After Oviposition from Cryptic Variant Lines Raised at 18 and 28 C

Isochromosomal line	Developmental stage (%)		
	Larval	Pupal	Adult
<i>Adh^I/Adh^I</i> lines (10) ^a			
0.7 M GuHCl at 18 C	0.24 (9.11)	0.39 (17.34)	1.12 (20.56)
0.7 M GuHCl at 28 C	0.18 (14.70)	0.27 (17.71)	0.91 (12.67)
Heat treatment at 18 C	0.31 (15.48)	0.29 (16.57)	2.85 (54.15)
Heat treatment at 28 C	0.42 (18.67)	0.43 (26.52)	3.89 (57.93)
<i>Adh^{II}/Adh^{II}</i> lines (15) ^a			
0.7 M GuHCl at 18 C	0.39 (25.88)	0.34 (24.91)	0.63 (27.56)
0.7 M GuHCl at 28 C	0.39 (23.96)	0.34 (30.47)	0.33 (22.87)
Heat treatment at 18 C	0.21 (15.38)	0.34 (32.48)	1.14 (57.84)
Heat treatment at 28 C	0.36 (22.82)	0.43 (43.10)	1.40 (59.98)

^aActual ADH values and percentages ADH activity subsequent to treatment values by specific lines are available upon request.

Effect of GuHCl and Heat on ADH Activity

Development of Cryptic Variant Lines at 18 C. The Adh^I/Adh^I cryptic variant lines designated susceptible (flies cultured at 25 C) to GuHCl were found to be susceptible during all the developmental stages studied (Table II). Only one Adh^I/Adh^I line (Pu-b-1) found to be resistant (flies cultured at 25 C) to GuHCl was susceptible during the larval stage, intermediate during the pupal stage, but still resistant during the adult stage. All of the Adh^{II}/Adh^{II} cryptic variant lines susceptible or resistant to GuHCl maintained this property during all the developmental stages examined.

Adh^I/Adh^I cryptic variant lines found to be susceptible to heat treatment maintained this characteristic during the larval, the pupal, and the adult stages (Table II). The isochromosomal lines designated resistant were found to be susceptible during both the larval and the pupal stages but were intermediate in residual ADH activity during the adult stage. Adh^{II}/Adh^{II} lines susceptible to heat treatment were susceptible during all of the developmental stages. Adh^{II}/Adh^{II} lines raised at 25 C and found to be resistant to heat treatments were found to be susceptible during the larval stage, intermediate during the pupal stage, but still resistant during the adult stage.

Development of Cryptic Variant Lines at 28 C. The Adh^I/Adh^I and Adh^{II}/Adh^{II} lines raised at 25 C and found susceptible to GuHCl treatment continued showing this property during all the developmental stages studied (Table II). The resistant lines consistently showed intermediate levels of ADH activity when treated with GuHCl during all of the developmental stages.

Heat treatment of Adh^I/Adh^I and Adh^{II}/Adh^{II} cryptic variant lines classified as susceptible continued to be susceptible during the three developmental stages (Table II). On the contrary, the Adh^I/Adh^I and Adh^{II}/Adh^{II} lines designated resistant to heat treatment did not show this property during the early stages (larval and pupal) but were still resistant during the adult stage.

A significant increase in the coefficient of variation values after treatment of ADH, in most isochromosomal lines studied, with GuHCl or heat, was interpreted as evidence to support the discovery of additional genetic variation at a biochemical level from the isochromosomal lines having approximately the same ADH activity levels. These observations clearly indicated that there exists an enormous amount of cryptic variation between and within the Mexican strains of *D. melanogaster*.

DISCUSSION

The maintenance of both *Adh* alleles at relative higher frequencies in most of the Mexican populations of *D. melanogaster* (Pipkin *et al.*, 1976) suggested a form of heterosis at the molecular level (Kojima and Tobari, 1969). A positive

correlation between *Drosophila* ADH activity and egg-to-adult survival on alcohol-supplemented media (Thompson and Kaiser, 1977) suggested that the presence or absence of a particular gene function has a direct effect on the survival of the individual.

Highly significant differences ($P < 0.001$) were seen for developmental stages at both 18 and 28 C. This suggested that temperature was one of the factors influencing levels of ADH activity. Gionfriddo and Vigue (1978) suggested that temperature alone may not be a selective factor but may be acting in combination with other environmental factors.

Biochemical reactions are influenced by environmental factors, as evidenced by the fact that enzyme activity levels can be regulated in different ways in different situations (Rasmusson *et al.*, 1966). A survey of the literature indicated that various factors influencing ADH activity included the external source of ethanol, dosage effect, position effect, modifying genes, cofactor binding of structural flexibility, structural loci other than *Adh*, substrate, pH, developmental stage, etc. McDonald and Ayala (1978) suggested that in natural populations, ample variation exists in regulatory genes which act not by affecting the *Adh* gene product as such but rather by controlling the number of ADH molecules. McDonald and Avise (1976) suggested that at least one of the major functions in the variable levels of enzyme activity was perhaps of an environmental adaptive nature.

The cryptic variant lines exhibited modifications in terms of their susceptibility or resistance properties during various developmental stages under variable temperatures. This was indicated by the fact that the two denaturants affected various developmental stages differently at the two temperatures (Table II). These observations clearly demonstrated that the increased biochemical variation of ADH activity, which appeared to be under genetic control, had an adaptive significance for the individual during development by compensating in metabolism for changes in environmental conditions.

Therefore, the present research supports the hypothesis that enzyme polymorphisms are associated with regulatory reactions in metabolism. In addition, under variable temperatures, the cryptic variants seem to utilize differential enzyme regulatory mechanisms during development. This "cryptic variation" imparts an increased adaptive value to the population.

A question may arise as to the significance of the fact that different stages of the life cycle of *Drosophila* exhibited different responses to GuHCl and heat treatment. Several possibilities can be suggested to account for the existence of this differential response and any or all may be operative at a given time. There may indeed be cryptic variation at the ADH locus; these variants may be expressed differentially at various stages of the life cycle. At different developmental stages, there may be different proteolytic enzymes

present which degrade ADH differentially; or in different developmental stages, the concentrations of proteolytic enzyme inhibitors may differ. The relative quantity of stabilizing cofactors may vary from one developmental stage to the other. Polymorphism (genetic variation) in one or more of these classes of molecules could account for the strain differences observed in this investigation.

The question may be asked to what extent the strain variations seen in the present study are due to differences in their respective genetic backgrounds or to differences in the structural alleles (*Adh*^I or *Adh*^{II}). The isochromosomal lines used in the present study were constructed homozygous for an original chromosome 2 (*Adh*^I or *Adh*^{II}) but with the X chromosome and chromosome 3 replaced with those of a laboratory strain. This was done to provide a homozygous background for all of the isochromosomal lines. That significant changes in ADH activity were observed in the lines with similar ADH activity subsequent to treatment with heat or GuHCl suggests a change in the metabolic regulation of ADH in strains having similar laboratory third and X chromosomes but differing in the geographical origin of chromosome 2. It is conceivable, then, that these differences that affected certain isochromosomal strains were due to genes in chromosome 2 other than the *Adh* locus affecting the metabolic regulation of ADH activity.

On the other hand, it could also be hypothesized that a multiple series of alleles exists at the *Adh*^I (or *Adh*^{II}) locus, producing proteins which yield the same electrophoretic mobilities on starch gel, but specifying different transcriptional and/or translational regimes resulting in modifying the quantity and quality of mRNA or the enzyme.

The significance of the variation observed in the present study, which we termed "cryptic variation" because it was discovered only after treatment, is possibly adaptive or preadaptive in nature, imparting a greater fitness to individuals during development by compensating in metabolism for changes or possible changes in environmental conditions.

DEDICATION

This paper is dedicated to the late Dr. Sarah Bedichek Pipkin, who made her Mexican and other laboratory stocks of *D. melanogaster* available to us.

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