Proc. Indian Acad. Sci. (Anim. Sci.), Vol. 90, Number 5, September 1981, pp. 545-552.
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# Statistical analysis of cryptic variation in *Drosophila* alcohol dehydrogenase*

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MS received 13 February 1981

**Abstract.** Statistical analysis indicated that mean alcohol dehydrogenase (ADH) activity levels within $ADH^{I}$ and $ADH^{II}$ isochromosomal lines varied significantly among the Mexican populations of *Drosophila melanogaster* used. Also as indicated by coefficient of variation values for ADH activity, the relative amount of genetic variation differed among and within the $ADH^{I}$ and $ADH^{II}$ strains examined. The quantitative nature of the differences in ADH activity supported the concept of the additive gene action of the alcohol dehydrogenase (*Adh*) locus. The manner in which our isochromosomal lines were constructed suggested that genes regulating ADH activity in *D. melanogaster* were located on the second chromosomes.

**Keywords.** *Drosophila melanogaster*; alcohol dehydrogenase; cryptic variation; statistical analysis.

## 1. Introduction

Electrophoresis does not detect all extent variation of enzymes (Lewontin and Hubby 1966) because variant proteins with the same charge and mass ratio may go undetected by this technique. Spectrophotometric analyses showed that enzyme activities varied among and within species (Avise and McDonald 1976; Birley and Barnes 1973; Hewitt *et al* 1974; Pipkin and Hewitt 1972; Ward 1974; Ward and Herbert 1972). The present study uses a statistical approach in the analysis of cryptic variation in activity for alcohol dehydrogenase ($ADH^{I}$ and $ADH^{II}$) among and within Mexican populations of *Drosophila melanogaster*.

## 2. Materials and methods

### 2.1. *Drosophila stocks*

Populations of *Drosophila melanogaster* employed in this study were collected by Dr Sarah Bedichek Pipkin from 24 locations in south-eastern Mexico during

* Dedicated to the late Dr Sarah B Pipkin of the Howard University.

ADH—Alcohol dehydrogenase enzyme.

*Adh*—Alcohol dehydrogenase locus.

the summers of 1972 and 1974, using either fruit bait traps or sweeping over fruits at roadside fruitstands (Pipkin *et al* 1973, 1976). Of those populations, the following sixteen were selected for the present investigation : Puebla (Pu), San Luis Potosi (SLP), Tahuacan (Teh), Saltillo (Sa), Actalan (At), Oaxaca (Ox), Cuatla (Cu), Orizaba (Oz), Cordoba (Co), Yanga (Ya), Palenque (Pe), Acayuacan (Ac), Aleman (Al), Coatzocoalcos (Cz), Merida (Me) and Rio Papalopan (Rio).

### 2.2. *Extraction of isochromosomal lines*

Lines homozygous for the $Adh^{I}$ or $Adh^{II}$ allele were produced by replacement of the X and the 3rd chromosomes of a wild type strain with those of laboratory strains. This procedure provided a homogeneous genetic background for all of the isochromosomal lines. The second chromosomes from wild-type individuals were maintained. The laboratory stocks used in production of these lines were : S/Cy ; D/In(3)C3x and Cy L/Pm ; Sb/In(3)C3x Ubx. The genetic locations of these mutations were described by Lindsley and Grell(1968). The procedure for the production of the isochromosomal lines was described by Pipkin *et al* (1975).

### 2.3. *Nomenclature of the isochromosomal lines*

Trinomial nomenclature was followed to designate an extracted isochromosomal line. The first symbol (e.g. Pu, SLP) represented the population to which the wild-type male belonged. The second part, a latin letter (e.g. a,b) designated the wild-type male. The last part consisted of a number (e.g. 1,2) which revealed the cross number in the $F_4$ generation. Thus the isochromosomal line Oz-d-5 means that the line came from the "fifth" cross of wild-type male "d" of the "Orizaba" population.

To determine whether an extracted line was homozygous for the $Adh^{I}$ or $Adh^{II}$ allele, sixteen flies were selected at random and were subjected individually to agar gel electrophoretic analysis by the method of Ursprung and Leone (1965).

The gels were drained of the petroleum ether and placed in the staining mixture of Ursprung and Leone (1965), except that 2-butanol was used as substrate instead of ethanol. The staining mixture consisted of $NAD^{+}$(50 mg/ml) ; Nitro Blue Tetrazolium (Dajac ; 5 mg/ml) 0·375 ml ; Tris buffer (0·5 M, pH 9·0) 37·5 ml ; 2-butanol (Fischer) 1·5 ml. Staining was completed in 20-30 min at 37° C. The gels were placed in a fixative (75 ml of 95% ethanol ; 5 ml glacial acetic acid ; 20 ml deionized water) for approximately two hrs.

### 2.4. *Preparation of homogenates*

The isochromosomal lines were cultured at 25° C. Forty freshly emerged males were chosen at random and allowed to develop for six days ; the peak activity of ADH in adults occurs on the sixth day when cultured at 25° C (Ursprung *et al* 1968). The flies were weighed on a Sartorium micro-balance and hand ground in 2 ml of 0·05 M phosphate buffer (pH 7·5). The crude extract was centrifuged at 27,000 × g for 30 min at 4° C in a Sorvall RC-2B centrifuge. The supernatant was immediately subjected to spectrophotometric analysis.

### 2.5. *Assay for native ADH activity*

In a 1 ml cuvette containing 0·95 ml of freshly prepared reaction mixture (34·5 g $NAD^+$ and 0·25 ml 2-butanol in 25 ml of 0·05M phosphate buffer, pH 7·5) and 0·05 ml of supernatant were mixed. The conversion of $NAD^+$ to NADH was followed in a Gilford recording spectrophotometer (Model 2000) with the cuvette chamber maintained by a Haake pump (Model FJ) at 30° C (Pipkin and Hewitt 1972). The reaction was allowed to proceed for 2 min. The ADH activity was expressed as nM of NADH produced/min/ml/mg live weight (Schwartz and Sofer 1976). Duplicate runs (on separate days) for each assay in triplicate were performed on fresh homogenates. An average of six readings represented the ADH activity (Miglani 1980).

### 2.6. *Statistical analysis*

Mean ($\bar{X}$), standard deviation ($s$) and coefficient of variation (CV) values for native ADH activity were calculated for each of the populations and standard statistical analysis were used to examine mean and CV differences (Sokal and Rohlf 1969).

## 3. Results

Fifty-two isochromosomal lines were extracted from 16 Mexican populations of *D. melanogaster*. Of these lines, 12 were homozygous for the $Adh^s$ allele and 40 were homozygous for $Adh^u$ allele. Homozygosity of the isochromosomal lines for $Adh^s$ or $Adh^u$ was confirmed by electrophorezing 16 females selected at random from each line. The isozymes of the lines homozygous for the $Adh^s$ allele had identical electrophoretic mobility. Also, lines homozygous for the $Adh^u$ allele were electrophoretically indistinguishable (Miglani 1980).

Native ADH activity (nM of NADH produced/ml/min/mg of live weight) levels were determined for all 12 $Adh^s/Adh^s$ table (1) and all 40 $Adh^u/Adh^u$ (table 2) isochromosomal lines. Different populations within the $Adh^s/Adh^s$ and $Adh^u$ lines showed variation in native activity levels of ADH. Only those Mexican populations in which it was possible to extract three or more isochromosomal lines were selected for conducting comparisons of mean and CV values. Puebla and Orizaba populations were selected from $Adh^s/Adh^s$ lines (table 3) and San Luis Potosi, Actalan, Oaxaca, Cordoba, Yanga and Merida populations were selected from the $Adh^u/Adh^u$ lines (table 4).

The mean native ADH activity levels for the 5 $Adh^s/Adh^s$ lines from the Puebla population were statistically higher than those for the 3 $Adh^s/Adh^s$ lines from the Orizaba population. Also, the CV value of Puebla was significantly greater than that of Orizaba (table 3).

Oaxaca and Yanga ($Adh^u/Adh^u$) populations had significantly lower mean native ADH activity levels than all other $Adh^u/Adh^u$ lines (table 4). San Luis Potosi, Oaxaca, Cardoba and Yanga populations had CV values that were significantly lower than all the other $Adh^u/Adh^u$ lines (table 4). Merida had a CV significantly higher than Yanga, Cordoba, Oaxaca and San Luis Potosi. The Yanga population had a CV that was significantly lower than that of San Luis

Table 1. Weight and native ADH activity (nM of NADH produced/ml/min/mg of live weight) for isochromosomal lines of genotype $Adh^{I}/Adh^{I}$ raised at 25° C

| Isochromosomal line | Weight (mg) per 40 males | Native ADH activity |
|---|---|---|
| Pu-a-2 | 33·00 | 4·68 |
| Pu-b-1 | 33·40 | 4·44 |
| pu-c-1 | 34·30 | 5·83 |
| Pu-d-1 | 32·20 | 5·19 |
| Pu-d-2 | 42·19 | 5·42 |
| SLP-c-1 | 32·50 | 5·59 |
| Tch-a-5 | 34·67 | 4·39 |
| Cu-a-1 | 34·05 | 4·49 |
| Oz-c-1 | 31·10 | 4·01 |
| Oz-c-5 | 31·70 | 4·10 |
| Oz-d-5 | 30·09 | 3·93 |
| Cz-d-1 | 29·90 | 4·49 |

Potosi, Actalan, Oaxaca and Cordoba. The Cordoba population had a CV significantly higher than Oaxaca and significantly lower than Actalan and San Luis Potosi. However, Actalan had a significantly higher CV than San Luis Potosi (table 4).

## 4. Discussion

The extracts from $Adh^{I}/Adh^{I}$ flies consistently exhibited greater ADH specific activities than extracts from $Adh^{II}/Adh^{II}$ flies ; the extent of the differences depended on the origin of the strains and the alcohols used as substrate (Birley and Barnes 1973 ; Day *et al* 1974a ; Gibson and Miklovich 1971 ; Ward 1974 ; Ward and Herbert 1972). The present experiments, using 2-butanol as substrate indicated that the homogenates from $Adh^{I}/Adh^{I}$ flies, on the average, had a more than 2½-fold greater ADH activity than those from $Adh^{II}/Adh^{II}$ flies. Gibson (1972) and Lewis and Gibson (1978) attributed the increased ADH activity of $Adh^{I}/Adh^{I}$ flies over $Adh^{II}/Adh^{II}$ flies to greater amounts of ADH protein. Gibson (1972) found that the $Adh^{I}/Adh^{I}$ strains contained roughly twice as many ADH molecules as $Adh^{II}/Adh^{II}$ strains. The low ADH activity in $Adh^{II}/Adh^{II}$ flies was attributed to a decrease in the quantity of ADH protein and not to any additional regulatory mechanisms associated with this locus (Day *et al* 1974b). In addition, Clarke *et al* (1979) and Daly and Clarke (1979) have stated that environmental factors could change the relative amounts of ADH protein.

ADH activities were shown to vary among and within species (Avise and McDonald 1976 ; Birley and Barnes 1973 ; Hewitt *et al* 1974 ; Pipkin and Hewitt 1972 ; Ward 1974 ; Ward and Herbert 1972). Significant differences in mean ADH activity levels and CV values for ADH activity in electrophoretically indistinguishable $Adh^{I}$ or $Adh^{II}$ lines constructed from the Mexican strains in the Mexican *Drosophila* geographical strains used (tables 3 and 4) suggested the

**Table 2.** Weight and native ADH activity (nM of NADH produced/ml/min/mg of live weight) for isochrom-somal lines of genotype $Adh^{11}/Adh^{11}$ raised at 25° C.

| Isochromosomal line | Weight (mg) per 40 males | Native ADH activity |
|---|---|---|
| SLP-a-1 | 36·10 | 2·57 |
| SLP-a-2 | 36·70 | 3·00 |
| SLP-a-3 | 36·40 | 2·58 |
| SLP-d-2 | 35·14 | 2·67 |
| SLP-d-4 | 35·00 | 1·83 |
| SLP-d-5 | 35·90 | 2·43 |
| Teh-b-6 | 31·70 | 1·67 |
| Sa-a-1 | 37·03 | 1·86 |
| At-a-1 | 34·85 | 1·62 |
| At-b-2 | 35·10 | 2·79 |
| At-b-3 | 35·80 | 2·51 |
| At-b-4 | 34·90 | 3·01 |
| Ox-a-1 | 32·40 | 1·51 |
| Ox-b-2 | 28·76 | 1·60 |
| Ox-c-4 | 31·19 | 1·56 |
| Ox-d-7 | 25·56 | 1·70 |
| Ox-c-8 | 32·15 | 1·34 |
| Oz-c-4 | 34·19 | 1·81 |
| Co-a-1 | 31·70 | 1·61 |
| Co-b-1 | 27·75 | 1·84 |
| Co-c-1 | 33·36 | 2·18 |
| Co-c-2 | 32·90 | 2·21 |
| Co-c-3 | 32·75 | 2·61 |
| Co-d-4 | 31·90 | 2·35 |
| Ya-a-1 | 33·80 | 1·44 |
| Ya-b-2 | 31·21 | 1·54 |
| Ya-c-1 | 34·80 | 1·47 |
| Ya-d-1 | 33·00 | 1·40 |
| Pe-a-4 | 28·85 | 1·86 |
| Pe-b-5 | 26·80 | 1·14 |
| Ac-a-4 | 31·10 | 2·15 |
| Ac-b-4 | 31·30 | 2·10 |
| Al-a-1 | 32·70 | 2·49 |
| Cz-b-1 | 28·90 | 2·15 |
| Cz-c-1 | 31·10 | 2·21 |
| Me-c-2 | 29·60 | 2·19 |
| Me-d-3 | 33·30 | 2·82 |
| Me-f-5 | 32·06 | 1·69 |
| Rio-b-2 | 30·50 | 2·02 |
| Rio-y-1 | 31·00 | 1·85 |

**Table 3.** Comparison of the native ADH activity mean ($\bar{X}$) and coefficient of variation (CV) values for the populations representing the $Adh^{I}/Adh^{I}$ lines.

| *Mean* ($\bar{X}$) | | | |
|---|---|---|---|
| | $\bar{X}$ = All (12)/4·7 | Pu/5·1 | Oz/4·0 |
| *All* (12) | — | | |
| *Pu* | NS | — | |
| *Oz* | NS | * | — |
| *Coefficient of variation* (*CV*) | | | |
| | CV = All (12)/13·6 | Pu/10·9 | Oz/2·2 |
| *All* (12) | — | | |
| *Pu* | ** | — | |
| Oz | *** | *** | — |

NS = Non-significant ; * = Significant $p < 0.05$ ; ** = Significant $p < 0.01$ ; *** = Significant $p < 0.001$.

**Table 4.** Comparison of native ADH activity mean ($\bar{X}$) and coefficient of variation (*CV*) values for the populations representing the $Adh^{II}/Adh^{II}$ lines.

| *Mean* ($\bar{X}$) | | | | | | | |
|---|---|---|---|---|---|---|---|
| | $\bar{X}$ = All (40)/2·0 | SLP/2·5 | At/2·5 | Ox/1·5 | Co/2·1 | Ya/1·5 | Me/2·2 |
| *All* (40) | — | | | | | | |
| *SLP* | ** | — | | | | | |
| *At* | NS | NS | — | | | | |
| *Ox* | * | *** | * | — | | | |
| *Co* | NS | NS | NS | ** | — | | |
| *Ya* | * | *** | ** | *** | NS | — | |
| *Me* | NS | NS | NS | ** | NS | ** | — |
| *Coefficient of variation* (*CV*) | | | | | | | |
| *CV* = | All (40)24·5 | SLP /15·4 | At/24·6 | Ox/8·5 | Co/16·9 | Ya/3·9 | Me/25·3 |
| *All* (40) | — | | | | | | |
| *SLP* | *** | — | | | | | |
| *At* | NS | *** | — | | | | |
| *Ox* | *** | *** | *** | — | | | |
| *Co* | *** | NS | ** | *** | — | | |
| *Ya* | *** | *** | *** | ** | *** | — | |
| *Me* | NS | *** | NS | *** | ** | *** | — |

NS = Non-significant; * = Significant $p < 0.05$ ; ** = Significant $p < 0.01$ ; *** = Significant $p < 0.001$.

existance of additional hidden genetic variation among and within these populations.

Since the activit, levels of enzymes are under genetic control (Hewitt *et al* 1974; Ward and Herbert 1972), they are probably subject to natural selection. Hewitt *et al* (1974) investigated variation in ADH activity of a number of strains of *D. melanogaster* from Japan, United States, Australia and Guyana. The influence of modifying genes in the X and third chromosomes was noted in specially constructed lines from the above populations. Since our isochromosomal lines differed for the second chromosome and had homogeneous genetic background for the X and the 3rd chromosomes, the variation observed in the ADH activity can be associated with the second chromosome. Therefore, the studies of Hewitt *et al* (1974) along with our study implicate chromosomes X, 2 and 3 in regulation of ADH activity which support the investigation of Ward (1975) which suggested that the variation in ADH activity levels in *D. melanogaster* was due to the genes located on all three major chromosomes.

Recently it was reported that the modifying genes influencing ADH activity levels in *D. melanogaster* were due to additive genetic action (Barnes and Birley 1975 ; Birley and Barnes 1973; Birley and Barnes 1975). The quantitative nature of the differences observed (tables 1 and 2) supports the additive gene concept as the basis for the regulation of ADH activity in *Drosophila*.

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