# GENETIC VARIATION IN THE EXPRESSION OF *ADH* IN *DROSOPHILA MELANOGASTER*[1]

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ABSTRACT

Several chromosomes derived from natural populations have been identified that affect the expression of alcohol dehydrogenase (ADH). Second chromosomes, which also carry the structural gene *Adh*, show a great deal of polymorphism of genetic elements that determine how much enzyme protein accumulates. The level of enzyme was measured in third instar larvae, 6-to-8-day-old males and in larval fat bodies and alimentary canals. In general, activities in the different organs and stages are highly correlated with one another. One line was found, however, in which the ADH level in the fat body is more than twice the level one would expect on the basis of the activity in alimentary canal. We have also found evidence of third-chromosome elements that affect the level of ADH.

ALCOHOL dehydrogenase is one of the most intensively studied gene–enzyme systems in *Drosophila melanogaster*. Work has been done on the primary structure of the wild-type and mutant forms of the enzyme (SCHWARTZ and JÖRNVALL 1976; FLETCHER *et al.* 1978; THATCHER 1980), on a number of its physical and catalytic properties (DAY, HILLIER and CLARKE 1974; MCDONALD, ANDERSON and SANTOS 1980) and on the nature of the post-translational modification (tight binding of a carbonyl derivative) that leads to the occurrence of multiple electromorphs (SCHWARTZ, O'DONNELL and SOFER 1979). The structural gene for alcohol dehydrogenase (*Adh*) has been localized to map position 50.1 and polytene bands 35B1–35B3 on *2L* and the region surrounding it has been well characterized cytogenetically (O'DONNELL *et al.* 1975; WOODRUFF and ASHBURNER 1979a, b). Selective media have been developed that allow the isolation of ADH-negative mutants (O'DONNELL *et al.* 1975) or ADH-positive recombinants (VIGUE and SOFER 1976; MARONI 1978) and therefore permit fine structure genetic mapping. Finally, molecular characterization of the structure of the *Adh* region is now under progress and DNA sequences complementary to ADH-mRNA have been cloned (GOLDBERG 1980; BENYAJATI *et al.* 1980, 1981). This background information provides a unique opportunity to identify the various genetic and environmental factors that affect the expression of *Adh* and to investigate the molecular mechanisms by which they do so. Further-

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more, the role that ADH plays in protection against the toxic effects of environmental alcohols (DAVID *et al.* 1976) provides an opportunity to investigate the physiological effects of variants that affect ADH structure or quantity in a context that is probably relevant to the fly in nature. The ADH system is therefore also well suited to the study of evolutionary problems (see CLARKE 1975).

Here we are concerned with the extent and causes of variation of ADH activity levels among lines derived from natural populations. The first problem is to distinguish between environmental and genetic causes of variation in a quantitative, continuously-variable trait like enzyme activity. This distinction is easily made by working with isogenic lines so that genotypes can be replicated over environments, and so that standard biometrical techniques can be used to partition the variance of activity into genetic and environmental components. A second major problem is to determine what proportion of the genetic component of variation is due to variation of the DNA sequence that codes for the primary structure of the enzyme (the structural gene) and what proportion is due to variation of other sequences (modifiers). A partial solution to this problem was achieved by the constitution of our isogenic lines (LAURIE-AHLBERG *et al.* 1980). There are two such sets of lines in which either second or third chromosomes from natural populations were substituted into an isogenic background. This design permits detection of activity variants that are not linked to the structural locus of the enzyme and can therefore easily identify one class of modifier loci (as shown previously by MCDONALD and AYALA 1978). Modifiers linked to the structural locus can also be identified by recombination, even when very close (MARONI 1978).

The ultimate goals of this project are to identify the types of modifiers that are polymorphic in natural populations, to understand the range of effects that they have, relative to the effects of the structural gene, and to develop material suitable for investigating the molecular mechanisms by which the modifiers exert their effects. LAURIE-AHLBERG *et al.* (1980) reported the occurrence of a great deal of genetic variability among 50 second and 50 third chromosome isogenic substitution lines with respect to the level of ADH activity as well as several other enzymes. From these 100 lines, we selected 37 to represent the range of variability. This report is primarily a characterization of those 37 lines with particular reference to the following questions: How repeatable are the line effects on activity over time and what is their magnitude relative to the effects of random laboratory culture environments? To what extent is the variation in activity due to variation in amount of enzyme as opposed to variation in its structure? Are the line effects on activity specific for individual tissues or stages in development or are they systemic?

Some progress has already been made in addressing these questions, particularly with reference to ADH. There have, for example, been some reports on the existence of modifiers on the *X* or third chromosome that affect ADH activity (WARD 1975; BARNES and BIRLEY 1978; MCDONALD and AYALA 1978; LAURIE-AHLBERG *et al.* 1980) and in two instances, modifiers closely linked to the structural gene were found (THOMPSON, ASHBURNER and WOODRUFF 1977;

MARONI 1978). The contribution of the common allozymes to ADH activity variation has been investigated by many workers (RASMUSON *et al.* 1966; GIBSON 1970; VIGUE and JOHNSON 1973; WARD 1974; DAY, HILLIER and CLARKE 1974; MCDONALD and AYALA 1978; MCDONALD, ANDERSON and SANTOS 1980; and others), and the contribution of variation in amount of ADH protein to activity variation has also received attention (DAY, HILLIER and CLARKE 1974; LEWIS and GIBSON 1978; MCDONALD and AYALA 1978; MCDONALD, ANDERSON and SANTOS 1980). Although the tissue and stage specificity of genetic effects on ADH activity have not been investigated to our knowledge in any detail, such studies have been made with other Drosophila gene–enzyme systems. For example, tissue-specific modifiers of alpha-amylase (ABRAHAM and DOANE 1978) and aldehyde oxidase (DICKINSON 1975) have been identified in *D. melanogaster*. In this study, we investigate several aspects of ADH variation using a well-defined set of lines in which a large sample of chromosomes from natural populations are substituted into an isogenic background.

## MATERIALS AND METHODS

*Stocks*: Two sets of isogenic chromosome substitution lines were used in this study. The constitution of a line of each type is $i_1/i_1;+_2/+_2;i_3/i_3$ (referred to as a second chromosome line) and $i_1/i_1;i_2/i_2;+_3/+_3$ (a third chromosome line), where *i* refers to a chromosome from a highly inbred line (*Ho-R*) and + refers to a chromosome sampled from a natural population. The + but not the *i* chromosomes vary within a set of lines. The construction and electrophoretic analysis of these lines is described in LAURIE-AHLBERG *et al.* (1980), which also gives other detailed methods for Experiment 1.

*Assays*: The assay procedures for ADH (EC 1.1.1.1.) are described by MARONI (1978), for α-glycerophosphate dehydrogenase (GPDH, EC 1.1.1.8) by BEWLEY, RAWLS *and* LUCCHESI (1974) and for aldehyde oxidase (AOX, EC 1.2.3.1) by DICKINSON (1971). General protein concentration was estimated by the dye-binding method of BRADFORD (1976).

*Rearing conditions and sampling*: For experiments 1, 2 and 3, flies were raised on standard cornmeal–molasses medium with live yeast. The procedure for obtaining samples from the isogenic lines is to place 50 pairs of parents in a half-pint bottle for 48 hr, rear the offspring at 25°, collect them within 18 hr of emergence, age the male imagoes in groups of 15 for 8 days in vials with dry live yeast, weigh the live flies and freeze the samples at −70°.

*Experiments 2a and 2b—Repeatability of line differences*: Sixteen second and 11 third chromosome lines were sampled at two different times (2a, 2b). The second chromosome lines were sampled about three months apart (December 1978 and March 1979) and the third chromosome lines about one year apart (March 1979 and April 1980). Two replicate sets of five or ten males were collected from each of three sets of bottles ("blocks") in each experiment, except that in Experiment 2b for the third chromosome lines, three samples of males were collected from two sets of bottles on three different days.

*Experiment 3—Relationship between activity and CRM level*: Thirteen second and 11 third chromosome lines were sampled in two separate experiments with the same design. One "sample" of five males was collected from each of three sets of bottles for each line. Each sample was assayed for ADH activity and two replicate aliquots from the same homogenate were run on each of two immunoelectrophoresis gels on the same day. One sample from each line was analyzed on each assay day and each of the two gels run on each day contained an aliquot from all the lines of a given chromosome type.

Activities for second chromosome lines were adjusted by linear regression on rocket length for each allozyme × assay day separately because of heterogeneity among the regression coefficients. A single regression coefficient was used for adjustment of activities for third

chromosome lines, which was obtained by pooling sums of squares and products over assay days.

A number of dilution series for two $ADH^S$ and two $ADH^F$ lines gave an average correlation between rocket length and fraction of fly per rocket of $r = 0.98$ ($n = 12$). The correlation for rocket area (estimated as the product of rocket width and length) was very similar ($r = 0.94$). The corresponding correlations between rocket length or area and activity of the extract were 0.97 for length and 0.93 for area. The results presented here are based on rocket length but rocket area gave essentially the same results.

*Experiments 4a-d—Tissue and stage specificity of line differences*: In experiment 4a, 12 second chromosome lines were sampled at two stages of the life cycle, late third instar larvae and eight-day post-emergence males; and two tissues were dissected from the larvae, fat body and alimentary canal. These larvae were grown on standard Drosophila medium covered with a layer of yeast paste. The food was made in methylene blue 0.5% and the cultures were kept in a moist chamber to encourage larvae to leave the food for pupariation. When they stop feeding, larvae with a distinctly blue alimentary tract can be seen crawling on the glass walls. After 4 to 6 hr the color becomes noticeably paler; in 30–40 min it disappears altogether and 10–15 min later the larvae stop moving and evert the spiracles in preparation for pupariation. It is our experience that larvae with the lighter shade of blue have significantly less ADH activity than earlier ones in the wandering stage. Our samples were therefore taken from among the dark blue wandering-stage larvae.

The lines were sampled in pairs by setting up one bottle per line with approximately 150 eggs that were collected over an 8-hr period. The larvae and adults were collected, the larvae dissected and the assays performed at the same time for both members of a pair. Three replicate samples of each stage and tissue were obtained from each bottle and the bottles for each pair of lines were set up at two or three different times. Analyses of variance were performed for each pair of lines separately. Samples consisted of alimentary canal or fat bodies from two larvae; for whole individuals each sample had one larva or five adult males. Each sample was assayed for ADH, AOX, GPDH and soluble protein.

For experiment 4b, four of the ADH lines from experiment 4a were resampled by the same methods, except that each of the possible pairs was set up, collected, dissected and assayed at the same times. There were two to four sampling times per line and three replicates per sampling time.

For experiment 4c, we sampled 12 additional second chromosome lines at the late third instar and 8-day post-emergence male stages. The methods were the same as for experiment 4a except that the lines were set up, collected and assayed in two groups of six. Each group was set up as in 4a at three different sampling times; two replicate sets of larvae and flies from each bottle were collected.

In experiment 4d, 11 third chromosome lines were set up with the methods of 4a but in an unbalanced design; two to six lines were set up on a given day, but the lines set up simultaneously varied from day to day. Four of the lines were dissected at the larval stage, with three or four sampling times per line and with two replicates per sampling time. All 11 lines were assayed at the larval and adult stages with from two to six sampling times per line and with two replicates per sampling time.

## RESULTS

*Experiment 1—A large sample of chromosomes from natural populations*: To establish the range and distribution of ADH levels found in natural populations, samples of 50 second and 50 third chromosome lines were assayed for ADH activity in adult males and females. Lines were a significant source of variation in both sets, both sexes and for both raw activity (units per fly) as well as for activities that were adjusted by linear regression on live weight or protein. These results, as well as the correlations between ADH and some other

enzymes, have been published elsewhere (LAURIE-AHLBERG *et al.* 1980), but some additional details of that experiment are reported here.

The distributions of line means of raw activity in adult males are shown in Figure 1. Except for a discontinuity between ADH$^S$ and ADH$^F$ second chromosome lines, the distributions are essentially continuous and approximately normal. Overlap between ADH$^S$ and ADH$^F$ is minimal, but consistently observed. The ranges of line means within each allozyme are similar in magnitude to the difference between the two allozyme means.

It is common practice when using the activity of an enzyme as an indicator of genetic expression to "standardize" the raw enzyme measurements (units per fly) by dividing by protein content or live weight per fly to obtain "specific activity" or "units per mg live weight." The present study affords an opportunity to test the validity of such standardization and we find that forming ratios is not appropriate. Figure 2 shows only a weak relationship between the line means of ADH activity per fly and protein or live weight per fly. Thus, dividing activity by weight or protein (which has a larger coefficient of variation than activity) inflates the variance unnecessarily. In some cases the linear regression of activity on weight or protein is significant, but the intercept is far from zero, which again suggests that forming a ratio is inappropriate. In many

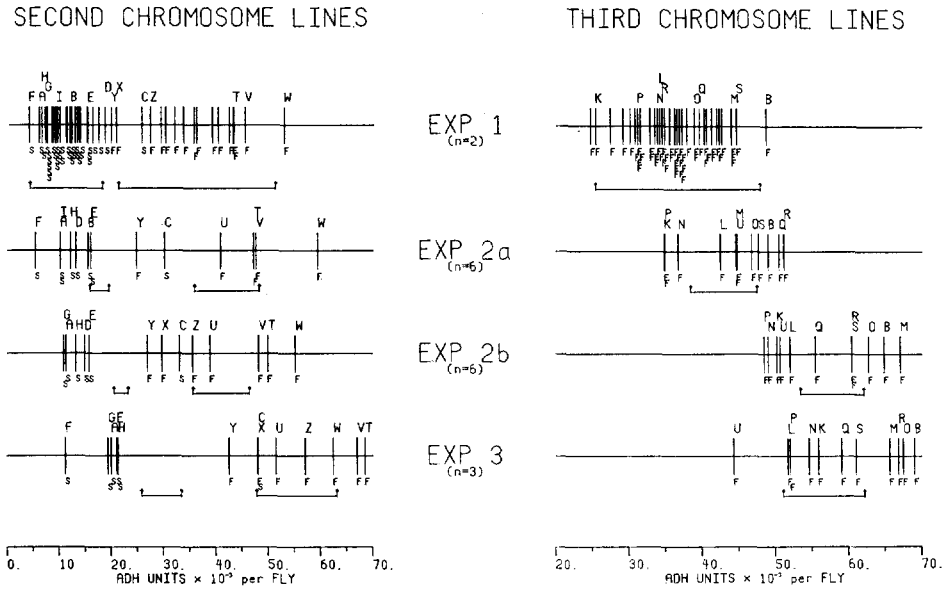


FIGURE 1.—Distributions of line means of ADH activity from four experiments. Labels of lines tested in more than one experiment appear above each distribution and the ADH allozyme type appears below. The 95% confidence intervals for the difference between any two means within an allozyme and an experiment (TUKEY's multiple comparison procedure) are indicated by the bars below each distribution. The interval for ADH$^S$ lines is on the left and that for ADH$^F$ lines is on the right. Except for Experiments 2b and 3, third chromosome, for which the flies were collected at the same time, each experiment represents independent samples collected over a two-year period. The number of observations for each mean is *n*.

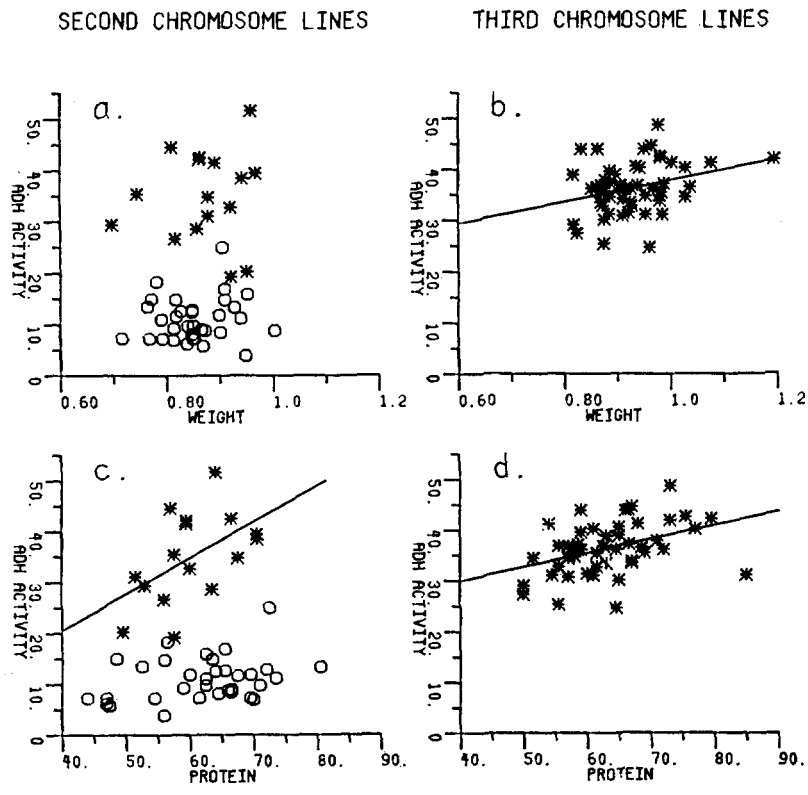


FIGURE 2.—Plots of the line means from Experiment 1. Linear regressions were performed for each allozyme separately; only those with a slope significantly greater than zero are shown. The corresponding coefficients of determination ($R^2$) are: (b) $R^2 = 0.09$, (c) $R^2 = 0.27$, (d) $R^2 = 0.16$. Each mean is based on two observations. * = $ADH^F$, 0 = $ADH^S$.

cases, the ratio of activity to weight or protein is correlated with the divisor and is therefore an ineffective method of standardization (LAURIE-AHLBERG *et al.* 1980). Therefore, we used an adjustment by linear regression for Experiment 1, but no adjustment or standardization was used for other experiments because the regressions were not significant. We find no evidence for a quadratic relationship between ADH and live weight over lines such as that observed by CLARKE *et al.* (1979) over individuals within a line.

*Experiments 2a and 2b—Repeatability of line differences*: A total of 16 second and 11 third chromosome lines were retested in two experiments in order to investigate the repeatability of the line effects. The lines were chosen from the original distributions of 50 lines in such a way as to obtain representative levels of activity. The distributions of the line means of activity units per fly are given in Figure 1 and the results of the analyses of variance are summarized in Table 1. The residual variances of the Fast and Slow allozyme lines are significantly different in these experiments so the analyses of variance were performed separately. The variance component ratio, K, is here defined as the pro-

TABLE 1

*Summary of analyses of variance of ADH units per fly for experiments 2a and 2b*

Experiment 2a

| Source | d.f. | Chromosome 2 lines: Fast allozyme F-test | $\hat{\sigma}^2$† | d.f. | Slow allozyme F-test | $\hat{\sigma}^2$ | Source | d.f. | Chromosome 3 lines: Fast allozyme F-test | $\hat{\sigma}^2$ |
|---|---|---|---|---|---|---|---|---|---|---|
| Block | 2 | ns | 4.9 | 2 | * | 1.0 | Block | 2 | ns | 0.2 |
| Line | 4 | *** | 151.1 | 7 | *** | 53.0 | Line | 10 | *** | 31.9 |
| B × L | 8 | ** | 22.2 | 14 | ** | 1.4 | B × L | 20 | ns | 2.8 |
| Error | 15 | | 8.8 | 24 | | 1.1 | Error | 33 | | 19.8 |
| Total | 29 | | | 47 | | | Total | 65 | | |

Experiment 2b

| Source | d.f. | Chromosome 2 lines: Fast allozyme F-test | $\hat{\sigma}^2$ | d.f. | Slow allozyme F-test | $\hat{\sigma}^2$ | Source | d.f. | Chromosome 3 lines: Fast allozyme F-test | $\hat{\sigma}^2$ |
|---|---|---|---|---|---|---|---|---|---|---|
| Block | 2 | ns | 3.1 | 2 | ns | 0.5 | Block | 1 | ns | 4.4 |
| Line | 6 | *** | 112.1 | 5 | *** | 63.8 | Day (B) | 4 | ** | 5.5 |
| B × L | 12 | ns | −12.4 | 10 | * | 0.7 | Line | 10 | *** | 41.6 |
| Error | 21 | | 44.8 | 18 | | 0.9 | B × L | 10 | ns | 4.5 |
| Total | 41 | | | 35 | | | Error | 40 | | 17.3 |
| | | | | | | | Total | 65 | | |

† Variance component estimates assuming all effects random.
ns $p>0.05$, * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

portion of variation within a block that is attributable to differences among the lines. The value of K is typically lower for the third chromosome lines than for the seconds and is somewhat lower for second chromosome lines with the Fast allozyme than those with the Slow allozyme (*e.g.*, K = 0.59, 0.83 and 0.95 for the thirds, Fast seconds and Slow seconds, respectively). Block effects, which represent both environmental and measurement error effects in these experiments, were quite small relative to line effects.

The high repeatability of line differences is demonstrated in Figure 1 and Table 2 by the 95% confidence interval for each experiment and by the correlation over the line means between each pair of experiments, which were performed over approximately a two-year period. For second chromosome lines, there seem to be at least three classes of activity within each allozyme, particularly the Slow lines (see also Figure 4). There may also be more than two classes of third chromosome lines, but the situation here is not as clear. Table 2 also demonstrates the higher repeatability of second as opposed to third chromosome effects.

*Experiment 3—Relationship between activity and level of ADH protein*: As in Experiments 2a–b, thirteen second and 11 third chromosome lines were sampled to investigate the relationship between activity and the level of ADH protein estimated by rocket immunoelectrophoresis. The analyses of variance of rocket length are summarized in Table 3. Lines within allozyme are a highly significant source of variation for both second and third chromosome lines with variance component ratios of K = 0.66 and K = 0.35, respectively. Allozyme is also a significant component of variation in rocket length for the second chromosome lines. Table 4 shows the corresponding analyses of activity, for which the results are very similar except that the variance component ratios are larger —K = 0.86 for second and K = 0.80 for third chromosome lines.

Figure 3 shows the relationships between the line means of rocket length, activity per fly and live weight per fly. None of the regressions of activity or rocket length on weight are significant but the regressions of activity on rocket length are each highly significant. It is clear that a large part of the activity variation is accounted for by rocket length—$R^2 = 0.99$ for $ADH^S$ and $R^2 =$

TABLE 2

*Correlations over line means from four experiments*

| Experiment pair | Second chromosome lines: Slow allozyme r | n | Second chromosome lines: Fast allozyme r | n | Third chromosome lines: Fast allozyme r | n |
|---|---|---|---|---|---|---|
| 1,2a | 0.88** | 8 | 0.99** | 4 | 0.71* | 10 |
| 1,2b | 0.87* | 6 | 0.99*** | 6 | 0.81** | 10 |
| 1,3 | 0.92* | 6 | 0.87* | 6 | 0.67* | 10 |
| 2a,2b | 0.99*** | 5 | 0.98** | 5 | 0.70* | 11 |
| 2a,3 | 0.98** | 5 | 0.81 | 5 | 0.56 | 11 |
| 2b,3 | 0.99** | 5 | 0.89** | 7 | 0.87*** | 11 |

* $p<0.05$, ** $p<0.01$, *** $p<0.001$.

TABLE 3

*Summary of analyses of variance of rocket length, experiment 3*

| Source | Second chromosome d.f. | F-test | $\hat{\sigma}^2$† | Source | Third chromosome d.f. | F-test | $\hat{\sigma}^2$ |
|---|---|---|---|---|---|---|---|
| Allozyme | 1 | * | 76.2 | Line | 10 | *** | 6.1 |
| Line(A) | 11 | *** | 47.8 | Day | 2 | ns | 13.3 |
| Day | 2 | ns | 14.8 | Gel(D) | 3 | ** | 5.3 |
| Gel(D) | 3 | ns | 1.4 | L × D | 20 | ns | 0.4 |
| A × D | 2 | ns | 2.8 | Error | 30 | | 10.9 |
| L(A) × D | 22 | *** | 14.0 | Total | 65 | | |
| A × G(D) | 3 | ** | 5.7 | | | | |
| Error | 33 | | 10.7 | | | | |
| Total | 77 | | | | | | |

† Variance component estimates for fixed (A) and random (L,D,G) effects.
ns $p>0.05$, * $p<0.05$, ** $<0.01$, *** $p<0.001$.

0.85 for $ADH^F$ in second chromosomes and $R^2 = 0.86$ for third chromosome lines. For second chromosome lines, the slopes for $ADH^S$ and $ADH^F$ are not significantly different but the intercepts are ($p < 0.001$).

The analyses of variance of the raw and rocket-length adjusted activities are summarized in Table 4 for each allozyme separately. The second chromosome

TABLE 4

*Summary of analyses of variance of raw and rocket length—adjusted ADH activity*

| Second chromosome, slow allozyme | | Raw activity | | Adjusted activity | |
|---|---|---|---|---|---|
| Source | d.f. | F-test | $\hat{\sigma}^2$† | F-test | $\hat{\sigma}^2$ |
| Line | 5 | *** | 156.7 | ns | 1.5 |
| Block | 2 | ** | 8.6 | *** | 31.5 |
| Error | 10 | | 7.7 | | 4.0 |
| Total | 17 | | | | |
| **Second chromosome, fast allozyme** | | **Raw activity** | | **Adjusted activity** | |
| Source | d.f. | F-test | $\hat{\sigma}^2$ | F-test | $\hat{\sigma}^2$ |
| Line | 6 | *** | 87.0 | ns | 0.5 |
| Block | 2 | * | 20.5 | *** | 73.7 |
| Error | 12 | | 30.0 | | 32.8 |
| Total | 20 | | | | |
| **Third chromosome** | | **Raw activity** | | **Adjusted activity** | |
| Source | d.f. | F-test | $\hat{\sigma}^2$ | F-test | $\hat{\sigma}^2$ |
| Line | 10 | *** | 57.9 | * | 7.1 |
| Block | 2 | ns | 2.0 | *** | 68.7 |
| Error | 20 | | 14.5 | | 10.0 |
| Total | 32 | | | | |

† Variance component estimates.
ns $p>0.05$, * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

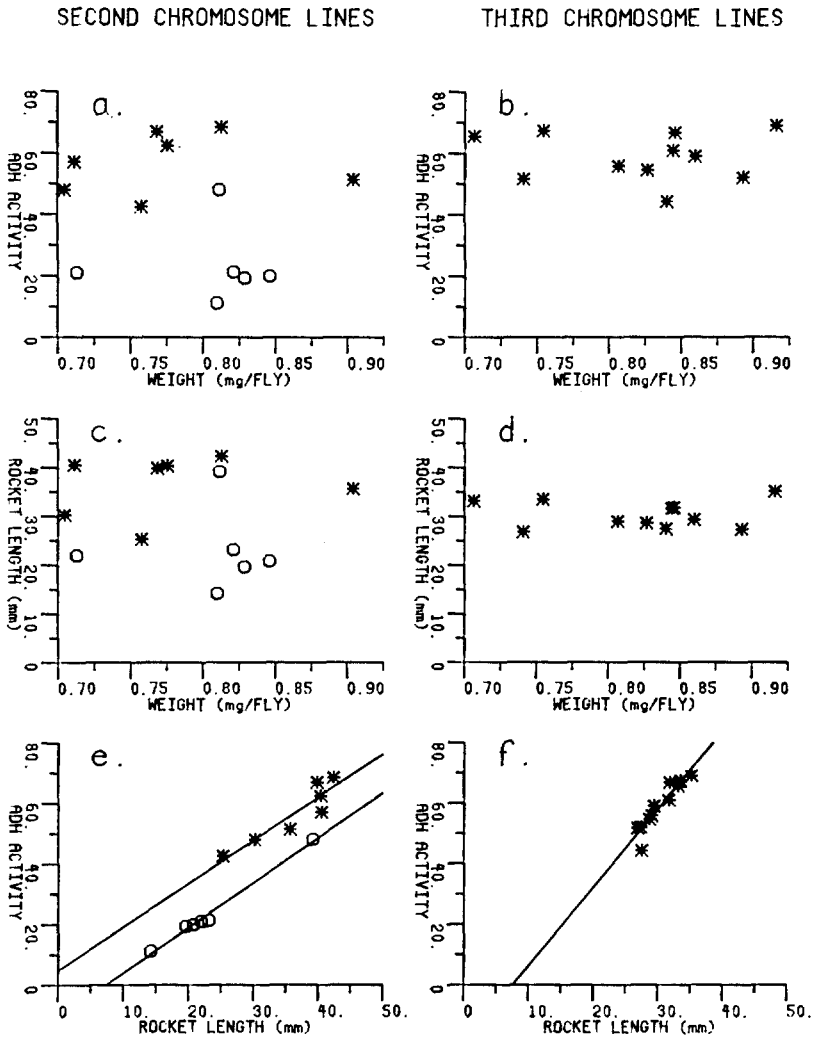


FIGURE 3.—Plots of the line means from Experiment 3. The regression of either ADH activity or rocket length on weight was not significant for either chromosome, but the regression of activity on rocket length was highly significant ($p < 0.001$) for both allozymes and chromosomes. The $R^2$ values are: (e) $ADH^F$, $R^2 = 0.85$ and $ADH^S$, $R^2 = 0.99$ and (f) $R^2 = 0.86$. Each mean is based on three observations. * = $ADH^F$, 0 = $ADH^S$.

line component loses significance after adjustment (for both allozymes). The third chromosome line component remains significant after adjustment but at a lower level and the magnitude of the line variance component is decreased considerably (*i.e.*, K decreases from 0.80 to 0.41). These results indicate that, for third chromosomes, there is a significant portion of the genetic (line) component of variation in activity that is not accounted for by variation in ADH protein (which is suggestive of structural heterogeneity). For second chromo-

somes, there is no significant within-allozyme component of activity variation that is not accounted for by variation in level of ADH protein.

*Experiment 4—Tissue and stage specificity of line differences*: The results of the experiments that were designed to investigate the stage specificity of the genetic effects on activity (Experiments 4a and 4d) are summarized in Figure 4 (a and b) for ADH and Figure 5 (b, d, and f) for GPDH, AOX and soluble protein. Although analyses of variance show significant line × stage interaction effects on ADH for both second chromosome experiments, only one line, "W," appears to be clearly different from the other lines. However, retesting lines U, X, V and W (Experiment 4b) showed that the unusually low larval/adult ADH activity ratio of line W apparent in Experiment 4a was not repeatable. The results of the other two enzymes were similar; both AOX and GPDH show significant line × stage interactions but the correlations between larval and adult activities are high (see Figure 5). In the third chromosome experiment (Figure 4d), the line × stage interaction component is significant for ADH. It appears that lines R and N have unusual larval/adult ratios for ADH but the repeatability and specificity of these effects have not been established yet. Overall the genetic effects on activity are very similar in late third instar larvae and eight-day post-emergence adult males.

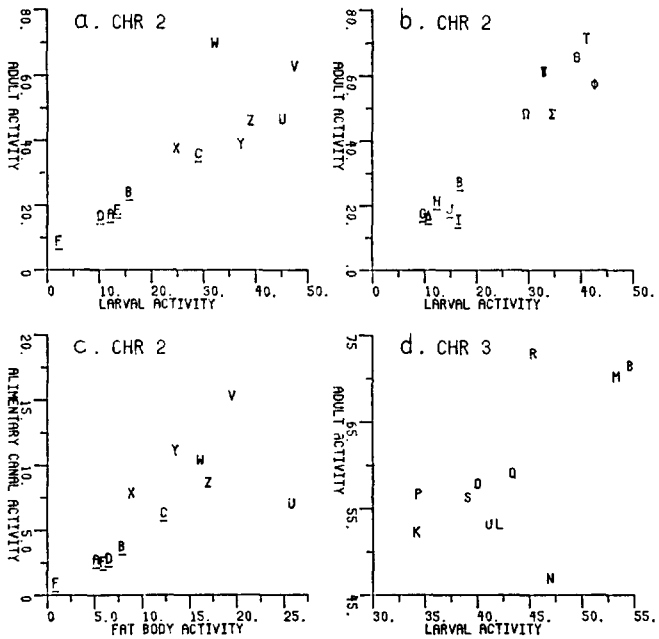


FIGURE 4.—Plots of the line means of ADH activity for Experiments 4a (plots a and c), 4c (plot b) and 4d (plot d). The correlations between the variables on the abscissa and ordinate are: (a) $r = 0.87$ ($p < 0.001$), (b) $r = 0.96$ ($p < 0.001$), (c) $r = 0.73$ ($p < 0.01$) and (d) $r = 0.62$ ($p < 0.05$). Sample sizes for each mean vary from 6 to 12 for plots a and c, from 6 to 8 for plot b and from 4 to 12 for plot d. The line labels are as in Figure 1, with $ADH^S$ lines underscored.

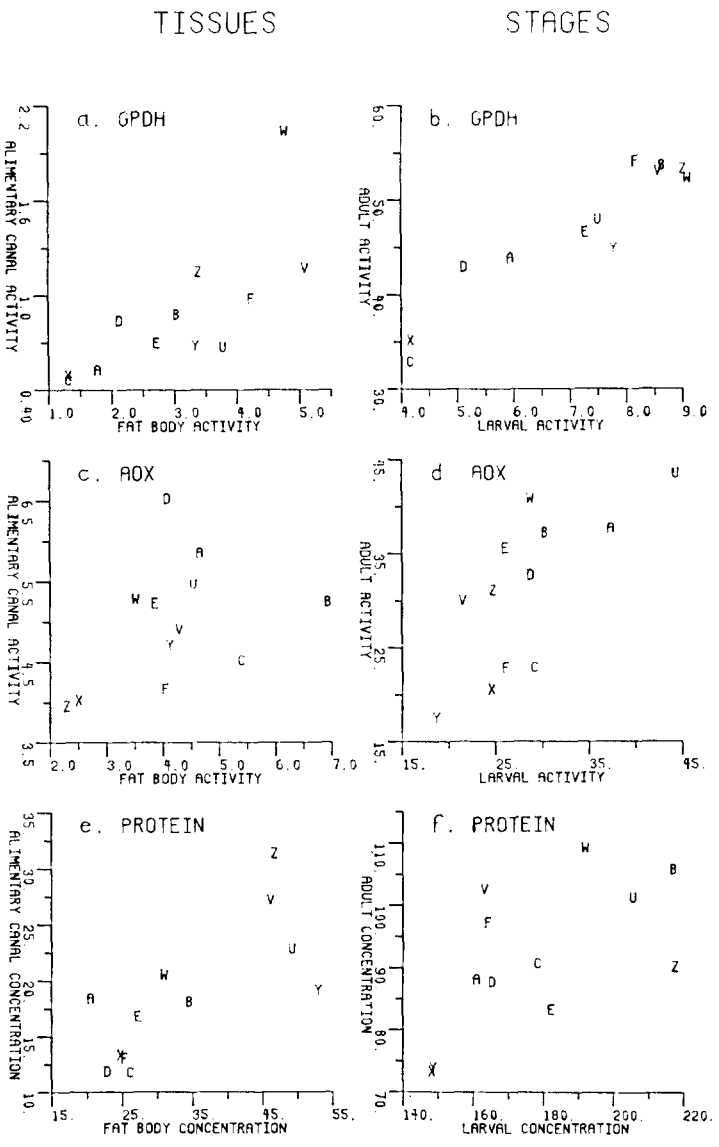


FIGURE 5.—Plots of the line means of GPDH, AOX and soluble protein for Experiment 4a. The correlations between the variables on the abscissa and ordinate are: (a) $r = 0.74$ ($p < 0.01$), (b) $r = 0.95$ ($p < 0.001$), (c) $r = 0.38$ ($p < 0.05$), (d) $r = 0.71$ ($p < 0.01$), (e) $r = 0.74$ ($p < 0.01$) and (f) $r = 0.60$ ($p < 0.05$). Sample sizes for each mean vary from 6 to 12. The line labels are as in Figure 1.

Figures 4 and 5 summarize the results of the larval dissections in Experiment 4a. Again, the correlation between ADH activities in the two tissues is strong although some pairs of lines show a significant line × tissue interaction (Figure 4c). Line U has an unusually low alimentary canal/fat body activity ratio for ADH but not for protein, AOX or α-GPDH (Figure 5a, c and e). Re-

testing of lines U, X, V and W (Experiment 4b) showed that this observation is repeatable. These results indicate a tissue-specific effect in line U that is rather specific for ADH since it is not clearly observed for AOX, GPDH or proteins. Only four third chromosome lines have been dissected (Experiment 4d), and no significant line × tissue interaction was found for ADH.

## DISCUSSION

The results reported here confirm our earlier observation of a large degree of genetic variability among second and third chromosomes from natural populations that affect ADH activity levels (LAURIE-AHLBERG *et al.* 1980). Although environmental and measurement error effects on ADH levels are often substantial and some genotype × environment interactions are observed, the direction and magnitude of differences among our isogenic lines are highly repeatable. The second chromosome lines, among which the *Adh* gene may vary, show a greater magnitude and repeatability of genetic variation than the third chromosome lines. A large part of the second versus third chromosome difference is due to the effects of the two common allozymes. The mean of $ADH^F$ second chromosome lines is more than twice as great as that for $ADH^S$ lines, a difference that is consistent in direction and, approximately, in magnitude with the observations of several other workers (*e.g.*, DAY, HILLIER and CLARKE 1974; LEWIS and GIBSON 1978; MCDONALD and AYALA 1978). Unlike most other workers, however, we find a consistent overlap between $ADH^S$ and $ADH^F$ lines, which is probably simply due to a larger sample of lines. Within each allozyme for the second chromosome lines we find at least three classes of activity. This observation is particularly striking for the $ADH^S$ lines, in which we find discontinuities between a very low activity line ("F"), a very high activity line ("C") and the rest of the $ADH^S$ lines (see Figures 1, 3 and 4). Genetic mapping of these within allozyme activity differences is in progress.

The results of Experiment 3 show that most, if not all, the within-allozyme second chromosome ADH activity variation is due to variation in amount of ADH protein. However, there is a significant genetic component to the activity variation among the third chromosome lines that is not accounted for by variation in amount of ADH protein. A similar result has been obtained for third chromosome effects on the glucose 6-phosphate and 6-phosphogluconate dehydrogenases in an experiment employing the same set of lines (LAURIE-AHLBERG *et al.* 1981). These observations are suggestive of third chromosome modifier effects on catalytic efficiency (and therefore perhaps structure) of ADH, but any conclusions must clearly await direct measurements of kinetic parameters on purified enzyme.

We find a large difference between the amounts of ADH protein estimated by rocket immunoelectrophoresis in $ADH^S$ and $ADH^F$ second chromosome lines (Table 3, Figure 3). This result is similar to those of LEWIS and GIBSON (1978) and MCDONALD, ANDERSON and SANTOS (1980), who used radial immunodiffusion; DAY, HILLIER and CLARKE (1974) did not find such a difference between allozymes using rocket immunoelectrophoresis. The difference between the in-

tercepts of the regression of activity on rocket height for $ADH^S$ and $ADH^F$ that we observe (Figure 3) could be an artifact of the immunoelectrophoresis method due to the charge difference between the allozymes. Therefore, we do not believe that our data are appropriate for drawing conclusions about the nature of the activity difference between the allozymes.

Three cell populations are involved in the production of most ADH in *D. melanogaster*: epithelial cells in the larval midgut, and larval and imaginal fat cells. The results summarized in Figure 4 show that there are strong correlations among the ADH levels found in the three cell populations. It therefore appears that most of the activity variation in natural populations is of the systemic type rather than being due to tissue-specific "switches" that control level of expression. One possible exception is the *Ho-R* line ("U" in Figure 4), the genetic background stock, which ranks highest among $ADH^F$ second chromosome lines by its level in fat body but lowest by its level in alimentary canal. Mapping and further characterization of this putative tissue-specific variant is in progress.

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## LITERATURE CITED

ABRAHAM, I. and W. W. DOANE, 1978 Genetic regulation of tissue-specific expression of *Amylase* structural genes in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. U.S.A. **75**: 4446–4450.

BARNES, B. W. and A. J. BIRLEY, 1978 Genetical variation for enzyme activity in a population of *Drosophila melanogaster*. Biochem. Genet. **11**: 155–165.

BENYAJATI, C., A. R. PLACE, D. A. POWERS and W. SOFER, 1981 Alcohol dehydrogenase gene of *Drosophila melanogaster*. Relationship of intervening sequences to functional domains in the protein. Proc. Natl. Acad. Sci. U.S.A. **78**: 2717–2721.

BENJAYATI, C., N. WANG, A. REDDY, E. WEINBERG and W. SOFER, 1980 Alcohol dehydrogenase in Drosophila: isolation and characterization of messenger RNA and cDNA clone. Nucleic Acids Res. **8**: 5649–5667.

BEWLEY, G. C., J. M. RAWLS and J. C. LUCCHESI, 1974 L-Glycerophosphate dehydrogenase in *Drosophila melanogaster*: kinetic differences and developmental differentiation of the larval and adult isozymes. J. Insect Physiol. **20**: 153–165.

BRADFORD, M. M., 1976 A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72**: 248–254.

CLARKE, B., 1975 The contributions of ecological genetics to evolutionary theory: Detecting the direct effects of selection on particular polymorphic loci. Genetics **79**: 101–113.

CLARKE, B., R. CAMFIELD, A. GALVAN and C. PITTS, 1979 Environmental factors affecting the quantity of alcohol dehydrogenase in *Drosophila melanogaster*. Nature **280**: 517–518.

DAVID, J. R., C. BOCQUET, M. F. ARENS and P. FOUILLET, 1976 Biological role of alcohol dehydrogenase in the tolerance of *Drosophila melanogaster* to aliphatic alcohols: Utilization of an ADH-null mutant. Biochem. Genet. **14**: 989–997.

DAY, T. H., P. C. HILLIER and B. CLARKE, 1974 The relative quantities and catalytic activities of enzymes produced by alleles at the alcohol dehydrogenase locus in *Drosophila melanogaster*. Biochem. Genet. **11**: 155–165.

DICKINSON, W. J., 1971 Aldehyde oxidase in *Drosophila melanogaster*: A system for genetic studies on developmental regulation. Develop. Biology **26**: 77–86. ——, 1975 A genetic locus affecting the developmental expression of an enzyme in *Drosophila melanogaster*. Develop. Biol. **42**: 131–140.

FLETCHER, THOMAS S., F. J. AYALA, D. R. THATCHER and G. K. CHAMBERS, 1978 Structural analysis of the $ADH^S$ electromorph of *Drosophila melanogaster*. Proc. Natl. Acad. Sci. U.S.A. **75**: 5609–5612.

GIBSON, J., 1970 Enzyme flexibility in *Drosophila melanogaster*. Nature **227**: 959–960.

GOLDBERG, D. A., 1980 Isolation and partial characterization of the *Drosophila* alcohol dehydrogenase gene. Proc. Natl. Acad. Sci. U.S.A. **77**: 5794–5798.

LAURIE-AHLBERG, C. C., G. MARONI, G. C. BEWLEY, J. C. LUCCHESI and B. S. WEIR, 1980 Quantitative genetic variation of enzyme activities in natural populations of *Drosophila melanogaster*. Proc. Natl. Acad. Sci. U.S.A. **77**: 1073–1077.

LAURIE-AHLBERG, C. C., J. H. WILLIAMSON, B. J. COCHRANE, A. N. WILTON and F. I. CHASALOW, 1981 Autosomal factors with correlated effects on the activities of the glucose-6-phosphate and 6-phosphogluconate dehydrogenases in *Drosophila melanogaster*. Genetics **99**: 127–150.

LEWIS, N. and J. GIBSON, 1978 Variation in amount of enzyme protein in natural populations. Biochem. Genet. **16**: 159–170.

MARONI, G., 1978 Genetic control of alcohol dehydrogenase levels in *Drosophila*. Biochem. Genet. **16**: 509–523.

MCDONALD, J. F. and F. J. AYALA, 1978 Genetic and biochemical basis of enzyme activity variation in natural populations. I. alcohol dehydrogenase in *Drosophila melanogaster*. Genetics **89**: 371–388.

MCDONALD, J. F., S. M. ANDERSON and M. SANTOS, 1980 Biochemical differences between products of the *Adh* locus in Drosophila. Genetics **95**: 1013–1022.

O'DONNELL, J., L. GERACE, F. LEISTER and W. SOFER, 1975 Chemical selection of mutants that affect alcohol dehydrogenase in Drosophila II. Use of the l-pentyne-3-ol. Genetics **79**: 78–83.

RASMUSON, B., L. R. NILSON, M. RASMUSON and E. ZEPPEZAUER, 1966 Effects of heterozygosity on alcohol dehydrogenase (ADH) activity in *Drosophila melanogaster*. Hereditas **56**: 313–316.

SCHWARTZ, M. F. and H. JÖRNVALL, 1976 Structural analysis of mutant and wild-type alcohol dehydrogenases from *Drosophila melanogaster*. Eur. J. Biochem. **68**: 159–168.

SCHWARTZ, M. F., J. O'DONNELL and W. SOFER, 1979 Origin of the multiple forms of alcohol dehydrogenase from *Drosophila melanogaster*. Arch. Biochem. Biophys. **194**: 365–378.

THATCHER, D. R., 1980 The complete amino acid sequence of three alcohol dehydrogenase alleloenzymes ($Adh^{N\text{-}11}$, $Adh^S$ and $Adh^{UF}$) from the fruitfly *Drosophila melanogaster*. Biochem. J. **187**: 875–886.

THOMPSON, J. N., M. ASHBURNER and R. C. WOODRUFF, 1977 Presumptive control mutation for alcohol dehydrogenase in *Drosophila melanogaster*. Nature **270**: 363.

VIGUE, C. L. and F. M. JOHNSON, 1973 Isozyme variability in species of the genus Drosophila. VI. Frequency-property environmental relationships of allelic alcohol dehydrogenases in *D. melanogaster*. Biochem. Genet. **9**: 213–227.

VIGUE, C. and W. SOFER, 1976 Chemical selection of mutants that affect ADH activity in *Drosophila*. III. Effects of ethanol. Biochem. Genet. **14:** 127–135.

WARD, R. D., 1974 Alcohol dehydrogenase in *Drosophila melanogaster*: Activity variation in natural populations. Biochem. Genet. **12:** 449–458. ——, 1975 Alcohol dehydrogenase activity in *Drosophila melanogaster*: a quantitative character. Genet. Res. **26:** 81–93.

WOODRUFF, R. C. and M. ASHBURNER, 1979a The genetics of a small autosomal region of *Drosophila melanogaster* containing the structural gene for alcohol dehydrogenase. I. Characterization of deficiencies and mapping of *Adh* and visible mutations. Genetics **92:** 117–132.

WOODRUFF, R. C. and M. ASHBURNER, 1979b The genetics of a small autosomal region of *Drosophila melanogaster* containing the structural gene for alcohol dehydrogenase. II. Lethal mutations in the region. Genetics **92:** 133–149.

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