GENETIC CONTROL OF Adh EXPRESSION IN DROSOPHILA MELANOGASTER

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ABSTRACT

Natural variants displaying different levels of expression of the gene for alcohol dehydrogenase (Adh) were subjected to genetic mapping experiments. The strains studied carry one of the two common electrophoretic forms of the enzyme. The difference among Adh-fast strains appears to be due to multiple loci with trans-acting effects. Differences among Adh-slow strains are due to modifiers or quantitative sites located very close to the structural gene (less than 0.05 map unit) or part of it. The modifiers detected in the AdhS strains seem to operate only on the structural allele in the cis-position.—A modifier that affects the ratio of ADH levels in larvae and adults was also detected in the AdhS strains. This modifier is also closely linked to Adh and is cis-acting.

For the study of genetic regulation in Drosophila melanogaster, molecular methods designed to assess DNA sequences directly have become predominant. Despite the possibilities opened by in vitro mutagenesis (Shortle, Maio and Nathans 1981) and transformation (Rubin and Spradling 1982), judiciously chosen and well-characterized mutations, detectable by their phenotype expression, will continue to be an important source of material for molecular analyses.

Enzyme production is subject to genetic regulation in Drosophila, and natural variants have been used successfully to approach the questions of quantitative and developmental control of gene expression (Chovnick et al. 1976; Abraham and Doane 1978; Dickinson and Carson 1979). To identify natural variants affecting production of alcohol dehydrogenase (ADH) and several other enzymes, we screened 100 isogenic, second and third chromosome, substitution lines to measure the diversity in enzyme production (Laurie-Ahlberg et al. 1980). In D. melanogaster, two main forms of ADH are distinguishable electrophoretically, ADH-fast and ADH-slow (Grell, Jacobson and Murphy 1965); our studies showed that, for each form, practically all variation in enzyme activity can be accounted for by variation in the amount of serologically detectable ADH (24 lines tested) (Maroni et al. 1982).

Several second chromosome lines were selected for further studies reported in this paper. These include three ADH-fast lines, among which two different enzymes levels are found, and three ADH-slow lines, each of which expresses a
different level of ADH. In this report we show that (1) the difference in enzyme level between any pair of ADH-fast chromosomes appears to be due to multiple loci with trans-acting effects, whereas (2) in the ADH slow lines the quantitative sites are tightly linked to and cis-dominant over Adh. We also present evidence that one of the slow lines has abnormally low levels of ADH in the third larval instar, an apparent stage-specific effect that is also determined by a closely linked, cis-dominant genetic element.

MATERIALS AND METHODS

D. melanogaster stocks and mutations

The stocks used in construction of the recombinant chromosomes for this study are briefly described here. Further description of the mutants and rearrangements can be found in Figure 1 and in the work of LINDSLEY and GRELL (1968).

Isogenic wild-type strains: Several second chromosome substitution lines (coisogenic for the X and third chromosomes) from our earlier survey work (Laurie-Ahlberg et al. 1980; Maroni et al. 1982) were selected for further study. Here we refer to seven of the lines with single letter abbreviations: WI09 = A, NC16 = C, R142 = F, Ho-R = U, KA13 = V, KA21 = W and KA25 = X. These abbreviations are the same as in Maroni et al. (1982) but are not consistent with those used in Laurie-Ahlberg et al. (1982). The lines A, C, F, KA15, RI09 and Rl36 carry a structural gene, AdhF, responsible for the production of alcohol dehydrogenase with slow electrophoretic mobility; U, V, W, X and R123 carry an AdhF allele and produce an enzyme of fast mobility.

Isogenic marker strains: Two multiply marked second chromosomes were extracted into the same isogenic background as the wild-type second chromosomes. These are apl with the markers al dp b AdhF pr c px sp and bel with markers bel el AdhF rd pr cn.

A deficiency stock, Df(2L) 64j,pr: This chromosome lacks approximately 2 map units of chromosome 2L, a segment that includes Adh.

A second chromosome balancer, In(2LR)CyO AdhF: This carries an Adh-null allele (obtained from W. Sofer, Rutgers University, New Brunswick, New Jersey).

Lethal mutations, l(2L)GM2 and l(2L)GM11. These will be abbreviated l2 and l11, respectively. They are among several ethyl methanesulfonate mutations induced in our laboratory and selected against the deficiency Df(2L) 64j,pr. They were chosen for their proximity to Adh. Lethal l2 maps 0.2 unit to the left of Adh and displays the wing-blister phenotype in the rare homo-or hemizygous survivors; l11 maps 0.25 unit to the right of Adh (Figure 2 and Table 3). Complementation tests carried out in M. Ashburner's laboratory (Cambridge, England) showed that l2 is in the complementation group brl and l11 in brl2 (Ashburner, Aaron and Tsubota 1982).

With the strains described, genetic recombination was used to isolate stocks carrying the following chromosomes: 12AdhF (ADH); AdhF pr; AdhS (ADH); pr and AdhS pr; the Adh superscripts indicate whether the allele is slow or fast, and the letter in parentheses designates the strain of origin for the Adh allele.

Substitution of marked chromosome segments

To localize the genetic elements causing an enzyme level difference between a pair of wild-type second chromosomes, isogenic recombinant lines were constructed in which a segment of a wild-type chromosome was replaced by the corresponding segment from one of the multiply marked chromosomes (apl or bel). The X and third chromosomes remained coisogenic throughout this process. This approach was used so that a number of recombinant types from several different lines could be compared simultaneously and so that replicated mass homogenates rather than single-fly assays could be used.

From progeny of females al dp b AdhF pr c px sp/+ + + + AdhF + + + (in which AdhF represents an allele from one of the wild type strains), three types of recombinants were selected (see Table 1). Individual recombinant chromosomes were extracted into the isogenic background to establish from one to three sublines of each recombinant type.

Similarly, progeny from bel AdhF rd pr cn/+ + + + AdhF + + + females were screened for recombinants...
in the interval b-el and rd-pr (Figure 1). Three sublines of each of the following types were established: b + Adh' + ++ and ++ Adh' + pr cn.

Recombination in the 12-11 interval

To recover chromosomes that had undergone recombination in the vicinity of Adh, the crosses represented in Figure 2 were carried out. In generation 2, the majority of surviving flies are Cy; non-Cy zygotes, carrying Df[2L]64j,pr, survive only if the maternal second chromosome is a recombination product with the normal alleles for the two lethals. Males of the latter type were crossed to Df[2L]64j,pr/CyO Adh* females. From among the progeny, non-Cy males, i.e., those carrying the deficient and recombinant chromosomes, were collected in groups of three to five; they were aged from 6–8 days, then frozen and stored at −70°. Extracts from these samples were subsequently assayed for ADH activity and electrophoretic mobility. Since the deficiency chromosome does not contribute to the production of ADH, the measurements characterize the recombinant chromosome. That chromosome can be perpetuated, if desired, through the Cy sibs.

Enzyme assays

Conventional spectrophotometry: The rearing and aging of Drosophila, preparation of samples and spectrophotometric enzyme assays are described by Maroni et al. (1982; experiments 4a–d), except for the experiments summarized in Table 1 for which details are given by Stam and Laurie-Ahlberg (1982). One unit of ADH activity is defined as that amount that reduces 1 nm of nicotinamide adenine dinucleotide/min. The values reported always represent units per individual.

Gel scanning: In some cases, ADH activity was measured after polyacrylamide gel electrophoresis of extracts. This involves staining the enzyme present in gels using nitro blue tetrazolium (NBT) (Maroni 1978). ADH present in the gel reduces NBT to a deposit of formazan which can be measured spectrophotometrically at 600 nm using a gel scanner.

The absorbance of reduced NBT is proportional to the amount of extract loaded on a gel under appropriate conditions of electrophoresis and staining: we found that, when less than 25 units of ADH (determined by conventional spectrophotometry as about ¼ of the activity obtained from a single high-activity male) are loaded in a gel and when the gel is gently shaken during staining, deposition of formazan in the region of the enzyme increases linearly for at least 30 min of staining. When staining is allowed to proceed for 20 min, the resulting stain intensity is proportional to the amount of extract loaded up to 1 fly-equivalent or more per gel. Therefore, quantitative assays on gels were standardized by using an amount of extract equal to ¼ of a fly per gel and by staining for 20 min.

For gel scanning assays, 4- to 5-day-old males were used; neither the flies nor the extracts were frozen. Extracts to be compared were always matched as closely as possible: flies were grown

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**Figure 1.**—Top, map of the Adh region. Bottom, Genotype of females from which the two types of recombinants were obtained.
Figure 2.—Mating scheme used to recover chromosomes in which crossing over had occurred in the immediate vicinity of Adh, \(t^2-t^{11}\) interval. \(Adh^{50}\) stands for any of the three Adh-slow alleles used.

Figure 3.—Plots of polyacrylamide gel scans. A, Migration of the fast and slow isozymes in homo- and heterozygotes. B, Example of scans from which data were actually collected: solid line, \(Adh^{P5}/Adh^{50}\); broken line, \(Adh^{P5}/Adh^{50}\).

Concurrently and extracts prepared simultaneously; gels were run in the same electrophoresis tank. Two gels were run for each extract, and the results were averaged.

Absorbance measurements (600 nm) were made 12–24 hr after staining and fixing the gels. A Gilford spectrophotometer and gel scanner were used. The scanner was fitted with a 0.1-mm aperture, and the scanning speed was set at 0.5 cm/min. The spectrophotometer output was fed into a laboratory microcomputer which averaged data over 1-sec intervals and stored the averaged values. At the end of each gel scan, a plot of absorbance vs. time (from the beginning of the scan) was displayed on a video monitor, which allowed visual selection of an appropriate baseline and limits for integration. The computer was programmed to perform numerical integration of the plots, and the area of each plot segment was expressed as absorbance times seconds. One unit of area is defined as 1 enzyme unit.

\(ADH\) is a dimer; in heterozygotes \(Adh^7/Adh^8\), which produce both fast and slow electrophoretic
variants of the subunits, three dimeric forms are present: FF, FS and SS. We wished to determine
the proportion of the various dimeric forms contributing to total ADH activity in different hetero-
zygotes. This determination is complicated by the fact that binding of a ligand (Schwartz, O'Don-
nell and Sofer 1979) produces two distinguishable isozymes for each dimeric form; the alternative
isozymes are designated by the numbers 3 and 5. For example the FF dimer may be either isozyme
3, (FF)$_3$ or isozyme 5, (FF)$_5$. The alternative isozyme forms differ in abundance, with form 5 being
responsible for most of the activity. Figure 3 shows the migration of the isozymes with which we are
concerned. Notice that (FF)$_3$ and (SS)$_3$ appear in the same region (region III) of the gels; therefore,
in heterozygotes, both (FF)$_3$ and (SS)$_3$ contribute to peak III. Since isozyme 3 usually accounts for
less than 20% of the total enzymatic activity for any given electromorph under the condition of our
experiments (data not shown), the proportion of (SS)$_3$ in this region of the gel is minor. Moreover,
the specific value of the ratio (ADH)$_3$/ (ADH)$_5$ seems to depend more on the particular batch of
larvae than on their genotype. Nonetheless, a correction was made based on the premise that in a
heterozygote the ratios (FF)$_3$/ (FF)$_5$, (SS)$_3$/ (SS)$_5$ and (FS)$_3$/ (FS)$_5$ would be similar. This correction
involves allocating the activity in peak III between (SS)$_3$ and (FF)$_5$ in such a way as to minimize the
difference among the ratios. (Details, as well as a Basic program to perform the computations are
available on request.)

Once the contributions of each dimer form was obtained, the activity corresponding to each
monomer F and S was calculated by dividing the activity in the mixed dimer, FS, into equal parts
and adding to each the activity in the corresponding pure dimers FF and SS. This is, again, an
approximation, but we expect that it does not introduce too large an error (see, for instance,
McDonald, Anderson and Santos 1980).

RESULTS

Localization of quantitative modifiers: To localize genetic modifiers affecting ADH
production, wild-type chromosomes were recombined with either the apl or the
bel second chromosome (chromosomes carrying multiple visible markers), and
the ADH activity in the resulting recombinants was measured.

The results of experiments using the apl chromosome are shown in Table 1. The difference in ADH levels between two lines carrying different wild-type
second chromosomes is given (column labeled “Original lines”); in 17 of the 19
cases shown, ADH levels differ significantly between the two. When similar
comparisons were made after substitution of segments of apl (columns labeled
“Recombinant sublines”), the results differ depending on whether originally slow
or originally fast lines are in question. For lines that carried the slow allozyme
on the wild-type chromosome, the recombinant results consistently show that
genes responsible for ADH activity differences are localized on the left arm,
where Adh is located; i.e., recombinant sublines of the type + + + + · c px sp
having the intact left arm of the wild-type chromosomes show significantly
different ADH activity levels in 11 of 13 cases, whereas the other two recombi-
nant types fail to show any significant differences in enzyme levels. In contrast,
for lines carrying Adh$^F$ on the original wild-type chromosome, activity differences are dependent on factors in both arms; i.e., none of the three recombinant types preserves a significant difference in enzyme levels. Furthermore, epistatic inter-
actions seem to be an important component of the effects detected for the fast
lines, since the differences that may be present in the + + + + · c px sp and al
dp b pr · + + + recombinant sublines are not additive to yield a difference similar
to that between the original lines.

In a second set of experiments, a finer localization of the genetic elements
TABLE 1  
Comparison of ADH levels in second chromosome lines

<table>
<thead>
<tr>
<th>Lines compared</th>
<th>Original lines</th>
<th>Recombinant sublines</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+ + + + + + + +</td>
<td>+ + + + - c px sp al dp b pr + + + +</td>
</tr>
<tr>
<td></td>
<td>Fast allozyme</td>
<td>Fast allozyme</td>
</tr>
<tr>
<td>U, V</td>
<td>-5.15*</td>
<td>-0.28</td>
</tr>
<tr>
<td>U, W</td>
<td>-4.50*</td>
<td></td>
</tr>
<tr>
<td>U, R123</td>
<td>-4.13*</td>
<td>0.48</td>
</tr>
<tr>
<td>V, X</td>
<td>3.99*</td>
<td>2.41</td>
</tr>
<tr>
<td>W, X</td>
<td>3.34*</td>
<td></td>
</tr>
<tr>
<td>X, R123</td>
<td>-2.97*</td>
<td>-1.65</td>
</tr>
</tbody>
</table>

|                | Slow allozyme  | Slow allozyme        | Fast allozyme  | Fast allozyme  |
| KA15, A        | 2.75*          | 2.12*                | 0.59           |                |
| KA15, C        | -0.89          | -3.77*               | 1.33           |                |
| KA15, R109     | 2.04*          | 0.26                 | 1.14           |                |
| KA15, R136     | 3.46*          | 1.39                 | 2.08           |                |
| KA15, F        | 5.87*          | 5.26*                | 1.76           |                |
| A, C,          | -3.64*         | -5.89*               | 1.92           | 0.42           |
| A, R109        | -0.71          | -1.87*               | 1.73           |                |
| A, F           | 3.12*          | 3.14*                | 2.35           | -0.92          |
| C, R109        | 2.93*          | 4.02*                | 0.19           |                |
| C, R136        | 4.35*          | 5.07*                | 0.76           | -1.34          |
| C, F           | 6.76*          | 9.03*                | 0.44           |                |
| R109, F        | 3.83*          | 5.00*                | 0.62           |                |
| R136, F        | 2.41*          | 3.96*                | 0.32           |                |

* Included in this table are cases in which a significant difference in enzyme production was noted upon comparison of original lines and/or recombinant sublines. An attempt was made to generate both right arm (+ + + + + - c px sp) and left arm (al dp b pr + + + +) substitutions for each of the wild-type chromosome lines; this was successful except in the case of two of the right arm substitutions for lines originally carrying the fast allozyme. In addition, we obtained substitutions of the b-pr region in A, C, F, U, V, W and X.

* Average difference between the lines or sublines compared (first member minus second member of each pair). From one to three independent isolates of each recombinant chromosome subline were sampled in a design with four blocks; there were two replicates from each subline per block. Fast and slow line data were analyzed separately. The range of activity among slow lines was 4.14-10.90, and among Fast lines it was 10.16-16.47. These values are lower than others given in this paper because ethanol was used as a substrate.

These differences in mean activity are significantly different from zero with P < 0.05 according to the multiple comparison procedure described by Gabriel (1978).

 responsible for the quantitative differences in ADH levels among the strains A, C, F (slow) and V, W, X (fast) was attempted by generating recombinants with bel (Figure 1). Three substrains of each of the two types of recombinants (b or pr cn) were sampled for measurements of activity level. The results are shown in Figure 4.

Consistent with the result from apl recombinants, Figure 4 (top panel) shows that, for the fast lines, the original differences between strains are considerably reduced or eliminated by replacement of chromosomal segments distant from the structural gene. Replacement of either the left (b recombinants) or right (pr
FIGURE 4.—Top, Mean ADH units per fly in each of five or six independent recombinants for each of the three fast lines V, W and X and the three original lines themselves (identified with a plus sign). The bars represent averages of values from six extracts with five to ten flies each; the vertical tic marks on top of the bars are the standard error of those means. The data were treated to an analysis of variance and Duncan's multiple range test. The horizontal lines above the bars encompass substrains that are not significantly different from one another; asterisks identify those substrains with ADH levels significantly different from the corresponding original strain. The level of significance used was 95%. Bottom, Similar representation of data from the three slow lines, A, C and F.

cn recombinant) arm leads to a reduction in the ADH levels in substrains derived from V and W, whereas substitution of the left arm results in increased levels in substrains derived from X. These observations again suggest that differences among fast chromosomes are polygenic.
For Adh\textsuperscript{S} strains, segmental replacements of the type diagrammed in Figure 1 appear to have proportionally smaller effects than for Adh\textsuperscript{F} strains, as noted for the apl recombinants. The differences among A-, C- and F-derived substrains (either b recombinants or pr cn recombinants) remain approximately as large as they were among the original A, C and F strains (Figure 4, bottom panel). These results indicate that the quantitative differences in the three original slow strains are the result of genetic elements close to, or part of, the structural gene.

Developmental regulation of Adh expression is suggested by comparison of the levels of ADH accumulation in larvae and adults of certain strains. For example, in one set of experiments, 23 strains showed a range of larval ADH levels from 67 to 110\% of the adult level; but in another strain, F, larval activity was only 22\% of the adult value (MARONI et al. 1982). Strain F has consistently shown very low ADH levels in larval stages, so some of the recombinants discussed before were tested both in larval and adult stages. The results are shown in Table 2. In this set of experiments the larva to adult ratio is approximately 1.0 for strain A and 0.37 for strain F, and these values are not significantly different in the substrains carrying a substitution for the distal chromosome segments. Thus, the unusually low larval level of ADH in strain F is determined by element(s) located within the b-pr interval.

Mapping of quantitative modifiers closely linked to Adh: To map more accurately the quantitative sites that determine the ADH levels in the slow strains, a flanking lethals recombination test was done as shown in Figure 2. The three slow chromosomes were tested against the fast strain U. The numbers of progeny screened and recombinants tested are shown in Table 3. The ranges of ADH levels displayed by these recombinants and the mean values for the different Adh alleles while they were in the lethal-bearing chromosomes are given in Table 4. It appears that the amount of ADH produced by these recombinants is comparable to that produced by the corresponding alleles in the original strains (Figure 4) or in the lethal-bearing substrains (taking into account that the values in Table 4 represent the activity of a single allele per genome). In other words, the genetic elements responsible for the differences among the slow strains were not separated from the electrophoretic site by the recombinational events. If we assume that only two of the three crosses in Table 3 might yield detectable crossovers

<table>
<thead>
<tr>
<th>Strain</th>
<th>b Recombinants</th>
<th>Original strains</th>
<th>pr cn Recombinants</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Larvae</td>
<td>22 ± 1</td>
<td>24 ± 2</td>
<td>27 ± 1</td>
</tr>
<tr>
<td>Adults</td>
<td>20 ± 1</td>
<td>24 ± 1</td>
<td>25 ± 0.4</td>
</tr>
<tr>
<td>F</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Larvae</td>
<td>4.1 ± 0.3</td>
<td>4.6 ± 0.4</td>
<td>4.5 ± 0.4</td>
</tr>
<tr>
<td>Adults</td>
<td>10 ± 0.3</td>
<td>12 ± 0.5</td>
<td>11 ± 0.5</td>
</tr>
</tbody>
</table>

Six extracts were assayed twice each. Values are mean ADH units ± S.E.M.
TABLE 3
Recombinants within the I^2-1'^1 interval*

<table>
<thead>
<tr>
<th>Cross no.</th>
<th>Maternal genotype</th>
<th>No. of G2 zygotes</th>
<th>No. of Adh^5 recombinants^</th>
<th>No. of Adh^5 recombinants^</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>l^2 Adh^F(0)+ +</td>
<td>36,000</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>+ Adh^S0 1'^1 pr</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>l^2 Adh^F(0)+ +</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>+ Adh^S0 1'^1 pr</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>l^2 Adh^F(0)+ +</td>
<td>94,000</td>
<td>15</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>+ Adh^S0 1'^1 pr</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>l^2 Adh^F(0)+ +</td>
<td>32,000</td>
<td>11</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>+ Adh^S0 1'^1 pr</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* From Figure 2.

These numbers indicate the progeny-tested male recombinants from each cross. The map distances I^2-Adh and Adh-1'^1 were calculated from these data taking into account that 75% of putative, Cy^* survivors turned out to be, upon progeny testing, true recombinants and that we were unable to test 24 Cy^* survivors.

TABLE 4
ADH levels in recombinants within the I^2-1'^1 interval^*

<table>
<thead>
<tr>
<th>l^2-Bearing strain</th>
<th>Range of values for recombinants</th>
<th>l'^1-Bearing strain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fast</td>
<td>Slow</td>
</tr>
<tr>
<td>Cross no.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>l^2 Adh^F(0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G0 O Adh^a8</td>
<td>Fast</td>
<td>Slow</td>
</tr>
<tr>
<td>1</td>
<td>l^2 Adh^F(0)</td>
<td>23–32</td>
</tr>
<tr>
<td></td>
<td>Adh^F0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Df64j</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>28.8 ± 2.5</td>
<td>26–39</td>
</tr>
<tr>
<td></td>
<td>Df64j</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>22–34</td>
<td>4.1–5.4</td>
</tr>
<tr>
<td></td>
<td>Df64j</td>
<td></td>
</tr>
</tbody>
</table>

* From cross in Figure 2; the values are averages of four to six extracts ± 95% confidence limit.

For the l^2-bearing strain the value given is an average of eight extracts from the three crosses ± 95% confidence limit.

Adh^S0 represent Adh^S0 for cross 1, Adh^S0 for cross 2 and Adh^S0 for cross 3.

between the quantitative and electrophoretic sites, then, the upper boundary of the 95% confidence interval of the distance between the two sites is 0.05 map unit (Stevens 1942).

Tests of cis-dominance: To test whether the differences in ADH levels were determined by cis- or trans-acting genetic elements, ADH activity was measured in heterozygotes obtained by crossing Adh^5 strains C (high activity) or F (low activity) with Adh^F strains W (high activity) or X (low activity). Table 5 shows activity values determined by scanning electrophoretic gels (e.g., Figure 3); the activity values contributed by each dimeric form, FF, FS and SS, as well as the activity estimated for each monomeric class are given.

Comparison between heterozygotes that carry the same Adh^F second chromosome but different Adh^S second chromosomes (i.e., between Adh^F0/Adh^S0 and Adh^F0/Adh^S1) in Table 5, column 1, or between Adh^F0/Adh^S0 and Adh^F0/Adh^S0 in Table 5, column 2) indicates the existence of differences in cis-acting quantitative elements in strains C and F: we note that the amount of fast monomer is about the same in the compared heterozygotes, but the amount of slow monomer is much lower in the heterozygotes carrying an Adh^S0 chromosome.
**TABLE 5**

<table>
<thead>
<tr>
<th>Slow allele</th>
<th>Fast allele</th>
<th>Adh^{F(W)}</th>
<th>Adh^{F(D)}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F-F</td>
<td>F-S</td>
<td>S-S</td>
</tr>
<tr>
<td>Adh^{S(C)}</td>
<td>5.5 ± 1</td>
<td>9.9 ± 2</td>
<td>11 ± 3</td>
</tr>
<tr>
<td></td>
<td>(10.5 ± 3)</td>
<td>(15.8 ± 4)</td>
<td></td>
</tr>
<tr>
<td>Adh^{S(F)}</td>
<td>8.3 ± 2</td>
<td>6.4 ± 1</td>
<td>4.1 ± 2</td>
</tr>
<tr>
<td></td>
<td>(11.6 ± 2)</td>
<td>(7.3 ± 2)</td>
<td></td>
</tr>
</tbody>
</table>

ADH is expressed in units of area from gel scanning plots ± 95% confidence interval. F-F, F-S, S-S are the three dimer types. The numbers in parentheses represent the two monomers, fast on the left and slow on the right. Each average is derived from 12 measurements in six different extracts.

The ratio of slow monomer between strains C and F is approximately 2 in these heterozygotes, whereas it is closer to 4 when homozygous individuals are compared (Figure 4). We do not know whether this difference is due to the method used to estimate monomer levels in heterozygotes or whether it is due to interactions between the fast and slow alleles.

The heterozygote comparisons also suggest that there is some form of trans-acting effect of the elements controlling the quantitative differences between W and X. This conclusion is prompted by comparisons between heterozygotes carrying the same Adh^{S} chromosome but different Adh^{F} chromosome (compare values in row 1 or in row 2 in Table 5); little or no activity differences are apparent for either fast or slow monomers.

Finally, a similar test of cis- vs. trans-action was performed for the putative sites controlling differences in larval and adult production (Table 6). In comparing Adh^{F(W)}/Adh^{S(C)} larvae and adults we see that both the fast and slow monomers are in approximately equivalent amounts in the two stages; this is in agreement with our results that show strain W or strain C larvae and adults have similar levels of ADH (Maroni et al. 1982). The same comparison applied to Adh^{F(W)}/Adh^{S(F)}, however, shows that the amount of fast monomer is comparable in the two stages but that the amount of slow monomer is much lower in larvae than in adults. This indicates that the unusually low larvae to adult ADH activity ratio observed for strain F can be accounted for by the action of a cis-acting genetic element.

**DISCUSSION**

With the experiments presented here we have attempted to probe the relationship between Adh and some genetic elements that influence the amount of ADH accumulated in different strains of D. melanogaster.

For several strains carrying second chromosomes with Adh^{F} alleles, we previously observed a diversity of relative ADH levels in a somewhat continuous distribution from 1 to 1.7 (Maroni et al. 1982). The results of the present study show that most, if not all, of the differences between fast strains can be accounted for by distant modifiers in the second chromosome. The lack of
additivity, in particular, suggests the occurrence of polygenic modifiers with epistatic effects. Furthermore, there is no detectable cis-dominance of the elements causing fast line differences, which is expected for distant modifiers.

For chromosomes carrying the Adh² alleles the situation is different. It is apparent from our earlier work that the activity levels in 24 different strains fall into three, more or less discrete, levels of ADH related approximately as 1:2:4, with the majority of strains belonging to the intermediate class. The data presented here indicate that differences among strains with low, mid or high ADH levels (strains F, A and C, respectively) are determined by genetic elements very closely linked to or part of the structural gene Adh. At least in strains F and C (low vs. high activity) these genetic elements are cis-dominant.

As to the nature or mode of action of such elements, little can be said with certainty. They could represent changes in the primary structure of the protein such that it is made more susceptible to proteolysis and it, thus, equilibrates at lower steady state levels. Tests of sensitivity to heat and urea denaturation as well as substrate preference failed to show any indication of structural differences among the various Adh² strains (data not shown). Although we have not attempted to measure turnover rates directly, preliminary measurements of ADH-mRNA in these strains show ADH-mRNA to be proportional to ADH level (C. AQUADRO, unpublished observations). Thus, the different ADH levels in the three Adh² strains discussed here probably represent different rates of synthesis, processing or degradation of mRNA.

Even within the class of slow lines having intermediate ADH levels (A and R109), the differences are localized largely to 2L (unlike fast line differences of similar magnitude). At this point we can only speculate about the possible cause(s) of this difference between the allozymes (if, indeed, it is not simply a spurious consequence of the rather small number of fast lines investigated). One possibility is that polymorphism for In(2L)ₜ, which occurs among slow, but not fast, chromosomes, contributes to heterogeneity among slow chromosomes by maintaining a block of somewhat distant modifiers. This possibility can be eliminated in the present case since all of the lines reported in this paper have the standard sequence. Another possibility is that slow chromosomes contain a more heterogeneous class of structural alleles than fast chromosomes. KREIT-
MAN'S (1983) complete DNA sequencing of six slow and five fast alleles revealed no intraallozyme amino acid replacement substitutions and confirmed earlier protein-sequencing results that fast and slow alleles differ by a single amino acid substitution (THATCHER 1980). However, KREITMAN did find a greater sequence homogeneity among fast alleles with respect to silent site polymorphism. He suggests that the slow allele is ancestral to the fast one and has had more time to accumulate nucleotide variations, an interpretation supported by DNA sequence analysis of two sibling species of D. melanogaster, both of which have the slow allele (BODMER and M. ASHBURNER, unpublished results). Thus, our results can be interpreted to indicate that the increased nucleotide-level polymorphism in and around slow alleles leads to greater variation in the level of expression compared with fast alleles.

One of the slow strains, F, displays very low levels of ADH in adults and even lower levels in larvae: *i.e.*, it has an unusually low larva to adult ratio. This ratio is determined by an element closely linked to Adh and *cis*-dominant over it just as it is the case for the adult enzyme level. It is worth noting that the very low activity in strain F larvae is due to the reduced expression of the gene in all of the cell types in which it is normally expressed; no abnormality can be detected in the relative distribution of enzyme between larval fat body and alimentary canal or among the different sections of the alimentary canal (MARONI et al. 1982).

Restriction endonuclease mapping in the Adh region for 50 of our second chromosome isogenic lines has revealed that line F is unique in possessing an approximately 5-kb insertion 5' of the transcriptional unit, which has homology to *copia* (C. F. AQUADRO, M. M. BLAND and C. H. LANGLEY, unpublished results). The results suggest that the insertion differentially affects transcription in larvae and adults. In this context, it is important to note that larval and adult Adh transcripts differ in their initiation sites, with the adult site located 707 base pairs upstream from the larval site (BENYAJATI et al. 1983). The *copia* insertion in line F occurs in the SalI-HindIII restriction fragment located just 5' of the adult initiation site. Thus, although the insertion is located closer to the adult site, the level of larval enzyme is more severely affected. Further analysis of this variant promises to provide new information about the role of flanking sequences in transcription initiation and/or processing.

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**LITERATURE CITED**


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