Increased Variation in ADH Enzyme Activity in Drosophila Mutation-Accumulation Experiment Is Not Due to Transposable Elements at the Adh Structural Gene

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ABSTRACT

We present here a molecular analysis of the region surrounding the structural gene encoding alcohol dehydrogenase (Adh) in 47 lines of Drosophila melanogaster that have each accumulated mutations for 300 generations. While these lines show a significant increase in variation of alcohol dehydrogenase enzyme activity compared to control lines, we found no restriction map variation in a 13-kb region including the complete Adh structural gene and roughly 5 kb of both 5′ and 3′ sequences. Thus, the rapid accumulation of ADH activity variation after 28,200 allele generations does not appear to have been due to the mobilization of transposable elements into or out of the Adh structural gene region.

QUANTITATIVE variation in phenotype is considered to be the “stuff of evolution” (LeWontin 1974). However, little progress has been made toward determining the precise genetic and molecular sources of this variation. Does the source of variation lie at the structural gene in coding sequences, in tightly linked regulatory sequences, and/or at separate modifier loci? A large number of classical spontaneous mutations at genetically well characterized loci in Drosophila melanogaster have been traced either to the insertion or imprecise excision of transposable elements within or adjacent to the transcriptional unit of the structural gene for the character, or to chromosome rearrangements associated with transposable elements in dispersed locations (e.g., Rubin 1983; Finnegar and Fawcett 1986; O’Hare 1987; Goldberg et al. 1983; Davis, Shen and Judd 1987; Lim 1988). This result has been viewed as evidence that transposable elements are the major source of variation in gene expression for adaptive evolution (e.g., Syvanen 1985; Mackay 1988), speciation (Rose and Dolittle 1983; Ginzburg, Bingham and Yoo 1984) and macroevolution (Erwin and Valentine 1984). Green (1988), however, has cautioned that these results may provide a biased view of spontaneous mutations in nature.

Here we examine the role of sequence alterations at the 13-kb Adh gene region in accounting for a significant increase in variance of ADH activity observed after 28,200 allele generations in a D. melanogaster mutation-accumulation experiment. While a level of ADH activity variation equivalent to that seen for an allozyme class in nature is observed, no evidence of transposable elements or other variants were found in the 13-kb Adh region.

MATERIALS AND METHODS

Mutation accumulation lines: Two sets of 500 lines of D. melanogaster had been established by T. Mukai that were heterozygous for a second chromosome balancer (smI marked by Cy) and a chromosome carrying a recessive lethal from a single heterozygous female (see Mukai and Cockermam 1977). One set is referred to as JH lines and the other AW referring to their chromosomes of origin. To avoid contamination, these lines were constructed as a recessive lethal-bearing chromosome balanced by the SM1 chromosome marked by Cy and carrying a recessive lethal. After an average of 300 generations, a significant increase in genetic variation was found to have accumulated among these lines for electrophoretic mobility of enzyme coding loci, chromosomal aberrations, visible mutants, and ADH and amylase enzyme activity (Mukai and Cockermam 1977; Yamaguchi and Mukai 1974; Voelker, Schaffer and Mukai 1980; Scobie and Schaffer 1982; Mukai, Harada and Yoshimaru 1984; Tachida et al. 1989). A subset of 47 of these lines previously characterized for ADH activity was available for molecular analysis.

ADH activity: Prior to determination of ADH activity the genetic backgrounds (X, Y, third and fourth chromo-
Molecular analysis of the Adh region: Genomic DNA was prepared from each line as described by Bender, Spierrer and Hogness (1983), and digested with EcoRI and SalI together or with EcoRI and HindIII together using the high and medium salt buffers, respectively, of Maniatis, Fritsch and SAMBROOK (1982) and 5 mM spermidine for 2 hr at 37°C. These digests were chosen so as to divide the Adh region into a large number of readily detectable but small fragments (see Figure 1). This should allow detection of insertion/deletion variation greater than approximately 100 bp on average judging from prior experience (Aquadro et al. 1986). Fragments were separated electrophoretically on 1.2% agarose gels using TBE buffer (Maniatis, Fritsch and SAMBROOK 1982), denatured and neutralized (Smith and Summers 1980) and blotted to charged nylon membrane (Zetabind from AMF Cuno) in 1 M ammonium acetate/0.05 M NaOH. The filters were prehybridized, hybridized and washed as described by the manufacturer (AMF Cuno). Nick translated probe DNA was the plasmid sAS1 (Goldenberg 1980) that contains an 11.5-kb genomic fragment from D. melanogaster containing the Adh structural gene approximately in the center.

RESULTS

An analysis of variance for ADH activity is presented in Table 1 for the 47 lines (27 AW and 20 JH lines; listed in Table 1 footnote). As expected from the analysis of the larger number of lines presented by Mukai, Harada and Yoshimaru (1984), both sets (AW and JH) show highly significant variation among lines, while an identical analysis of 20 replicates of a single JH line showed no significant line variance (control lines of Mukai, Harada and Yoshimaru 1984). The genetic variance component of lines is estimated to be 0.000285 ± 0.000086 and 0.000524 ± 0.000186 (units/mg)^2 for the AW and JH lines, respectively. Variation in ADH activities were continuously distributed with no particularly unusual variants contributing to a lot of the genetic variance.

The rate of accumulation of genetic variance for ADH enzyme activity is 0.000285/300 = 9.5 × 10^-7 to 0.000524/300 = 1.75 × 10^-6 per generation per line. To provide perspective to the magnitude of genetic variance that has accumulated after 300 generations, we can compare the accumulated variance to that observed for ADH in natural populations using the data of Laurie-Ahlberg et al. (1980; raw data kindly provided by C. C. Laurie). Since there is a strong bimodality of ADH activity associated with the two common allozymes (Fast vs. Slow) in natural populations, we compare the mutation-line variance to that of a single allozyme. Genetic variance of ADH activity in units comparable to the data of Mukai, Harada and Yoshimaru (1984) are 0.000659 and 0.000265 for 16 Fast and 31 Slow ADH allozyme lines, respectively, sampled from the eastern United States (Laurie-Ahlberg et al. 1980). Thus, 300 generations appears to be a sufficient period of time to accumulate a level of variation in activity among lines comparable to that observed in natural populations within an allozyme (Fast or Slow; Table 1).

A 13-kb region encompassing the Adh structural gene was surveyed for transposable elements or other major alterations via Southern blot analysis of genomic DNA (Figure 1). This region contains sequences required for normal gene expression as asayed by germline transformation (Goldberg, Posakony and Maniatis 1983; Posakony, Fischer and Maniatis 1985; Laurie-Ahlberg and Stam 1987). All sequence length variants over 100 bp in size would have been detected, thus including virtually all transposable elements (Finnegan and Fawcett 1986). We
detected no evidence of any sequence alterations of any kind among the lines (including restriction site

gains or losses).

DISCUSSION

No insertions, deletions or other sequence alterations were observed in or around the Adh structural
gene among 47 lines of D. melanogaster that had been allowed to accumulate mutations for approximately
300 generations. The 95% confidence interval for the rate of insertion by any element into the 13-kb Adh
region would thus be between 0 and 1.31 \times 10^{-4} insertions per chromosome per generation. (The upper
bound is computed as \(-\ln(0.025)/28200\), where 28200 is the total number of allele generations.) This
rate is generally consistent with previous estimates of single-element rates which range from approximately
10^{-3} to 10^{-6} per element per generation (CHARLES-
worth and Lapid 1988; Eggleston, Johnson-Schultz and Engels 1988; Charlesworth and Langley 1989; Harada, Yukuiro and Mukai 1990). For example, Eggleston, Johnson-Schultz and Engels (1988) scored insertion frequencies of 19 different families of transposable elements on D. mel-
anogaster X chromosomes by in situ hybridization. Five insertions were observed in 140 line-generations in non-P-M hybrid dysgenic crosses, and 200 insertions in 220 line-generations in dysgenic crosses. Considering that the X-chromosome comprises one ninth of the total euchromatic portion of the genome (Charlesworth and Langley 1989), we can extrapolate that 0.321 (= 45/140) and 8.18 (= 1800/220) insertions would be expected in the total euchromatic portion of the genome in nondysgenic and dysgenic crosses, respectively. By comparison, our Adh data leads to an expectation of a maximum of 1.2 insertions
when we extrapolate from our maximum rate of 1.31 \times 10^{-4} insertions into the 13-kb region surveyed and consider that the euchromatic portion of the genome of Drosophila is approximately 115,000 kb (John and Miklos 1988).

Given the lack of evidence of transposable element insertions in the Adh region of the mutation accumu-
lation lines we examined, how can the significant increase in variance among lines for ADH enzymatic activity be explained? Several hypotheses can be pro-
posed. First, the variation could be the result of base pair substitutions in or close to the Adh gene that were
undetected by our restriction site map survey. Given a mutation rate of $5.58 \times 10^{-9}$ per base pair per
generation (Mukai and Cockerham 1977), the probability of a mutation occurring in the 13 Kb Adh
region on any one chromosome is $(300 \text{ generations}) (5.58 \times 10^{-9} \text{ substitutions/bp/generation}) (13,000 \text{ bp})$
$= 0.0218$. Taking into account that each line repre-
sents two chromosomes, the expected number of lines carrying at least one new mutation in the 13-kb Adh
region is $47(1 - e^{-2\times0.0218}) = 2.0$. Thus, we expect only two base substitutions over the whole region
among the 47 lines, and our results would require each to be of large effect with one per set of lines
(AW and JH). Considering only the 765 bp that code for ADH, the expected number of lines with substi-
tutions drops to 0.12, and clearly does not explain our data. In addition, no mobility or null alleles at
Adh have been detected in larger analyses of this set of mutation accumulation lines including over
426,904 allele generations (Voelker, Schaffer and Mukai 1980). Thus, the hypothesis that base pair
substitutions at or near the Adh gene account for the accumulated variance in ADH activity seems unlikely
as has been argued by Mukai, Harada and Yoshimaru (1984).

Second, transposable elements may have inserted
into the Adh region and subsequently excited leaving
behind small but undetectable (to our methods) alter-
ations that affected expression. Only complete se-
quencing of all alleles would allow a rigorous test of
this hypothesis. There is certainly no evidence of such
events for Adh coding sequence, as discussed above.

A third explanation is that sequence alterations
(perhaps due to base pair substitutions or transposable
element insertions/deletions) in other genes could have
modified the level of ADH expression or enzyme
activity ("controlling factors" of Mukai, Harada and
Yoshimaru 1984). It is known that modifier loci can
influence ADH enzyme activities (reviewed by La-
rue-Ahlberg 1985). However, efforts to map and
characterize these modifier loci have been unsuccess-
ful due in large part to their small individual effects.
New chromosome inversions have been noted in several of these lines (Yamaguchi and Mukai 1974; T. Mukai, unpublished results). Yamaguchi and Mukai (1974) found that some of the chromosome rearrangements shared common cytological breakpoints. Furthermore, the transposable element hobo was found at the cytological breakpoints of all eleven inversions studied among 70 mutation accumulation lines by Harada, Yukuhiro and Mukai (1990). Thus, these inversions could have been mediated by recombination between homologous transposable elements in nonhomologous sites in the chromosome (e.g., Lim, Harada, Yukuhiro and Mukai 1990). Analysis of our 47 lines revealed that 3 of the 27 AW lines (11%) and 9 of 20 JH lines (45%) had new paracentric inversions on their lethal-bearing wild second chromosome. None of the inversions had breakpoints immediately at or adjacent to Adh. Analysis of variance for lines with and without inversions (Table 2) shows a significant line effect for JH lines with inversions and not for lines without. AW lines show the reverse trend with no significant line effect for inversion lines and a highly significant line effect for lines without inversions. Overall, there is not a significantly higher genetic variance component of lines with inversions versus that for lines without inversions. The results from the JH lines do support the hypothesis that changes in gene expression due to modifier loci, mediated in some cases by chromosome rearrangements, may account for some of the accumulated variance in ADH activity.

The significant result of our study is that transposable elements inserting and remaining in or immediately flanking the structural gene are not the source of the significant increase in genetic variation for ADH enzyme activity in our mutation-accumulation region in many of the same mutation accumulation rate those of a companion study of the Amylase gene inversions. Overall, there is not a significantly higher conversion of the Amy genes on the Cy balancer chromosome had lower activity, and that its replacement in the four lines by the gene from the lethal-bearing chromosome accounted for the increased amylase activities in these lines (Tachida et al. 1989). The Amy results suggest the possibility that similar gene conversion or double crossing over events between the balancer and lethal-bearing chromosomes may have contributed to the variation in ADH enzyme activity observed in the present study. The Cy balancer and lethal JH chromosomes carry the ADH-fast allele, while the lethal AW chromosomes carry ADH-slow (Voelker, Schaffer and Mukai 1980). Homozygotes for ADH-fast have, on average, ADH activities in adults that are twice the level observed in ADH-slow homozygotes (reviewed in Laurie-Ahlberg 1985). Consistent with this difference, activities for JH lines averaged 31% higher than AW lines (Mukai, Harada and Yoshimaru 1984). Only the HindIII-

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* ** *** Mean significant at 5.0, 1.0 and 0.5% levels. Lines with inversions (and breakpoints where data available) were as follows: AW lines: 17, 52 and 3470 (all 2L inversions, breakpoints not available). JH lines: 190 (28C;32D), 193 (32C;36F), 212 (2L inversion, breakpoints not available), 218 (26A;32E), 292 (32C;36E), 300 (25E;29C), 329 (32C;36E), 377 (32D;39E), 449 (25A;36D).
4.5 site appeared to differ between the balancer and lethal bearing chromosomes (see Figure 1). All lines were heterozygous for this site indicating only that no gene exchange occurred in this region upstream of Adh. Additional detailed restriction site or direct sequence data will be required to critically test the contribution of gene conversion and double crossing over to the increase in genetic variance in ADH activity in these mutation accumulation lines.

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