SPONTANEOUS MUTATIONS MODIFYING THE ACTIVITY OF ALCOHOL DEHYDROGENASE (ADH) IN *DROSOPHILA MELANOGASTER*

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Manuscript received January 26, 1983
Revised copy accepted September 1, 1983

ABSTRACT

In a marked-inversion balanced lethal system of the second chromosome of *Drosophila melanogaster*, mutations were accumulated under minimum pressure of natural selection in 1000 individual lines that originated essentially from two individuals. After about 300 generations, the specific activities of alcohol dehydrogenase of 69 randomly selected individual lines were measured with replications using four replicated vials (on 2 days—two replications per day) by observing the reduction of NAD^+ to NADH at 340 nm. Total soluble protein as the basis of standardization of enzyme activity was measured by the Lowry method for each vial. A control experiment was made immediately after the establishment of 20 individual lines from a single genotype. A significant increase in genetic variance was observed among the mutation-accumulating lines but was not detected in the control experiment. The statistical analysis of the data on the basis of the one-band/one-gene hypothesis suggests that many mutations controlling the activity of alcohol dehydrogenase occurred in regions different from the alcohol dehydrogenase locus itself, mainly in the noncoding DNA. Furthermore, it is suggested that transposon-like elements are related to the induction of these changes in alcohol dehydrogenase specific activities. Additional experimental evidence supporting this conclusion is also given.

SPECIAL attention has been paid to the mechanisms involved in the maintenance of protein polymorphisms since LEWONTIN and HUBBY (1966) found an astonishingly high level of average heterozygosity at structural loci. Although there has been much controversy between selectionists and neutralists concerning the maintenance of these protein polymorphisms, most population geneticists now accept that they are selectively nearly or completely neutral. Using *Drosophila melanogaster* populations, we have accumulated experimental results supporting the neutral theory of protein polymorphisms (KIMURA 1968; KIMURA and OHTA 1971). Most of the results are summarized in the work by MUKAI *et al.* (1982).

On the other hand, a large amount of homozygous load with respect to viability has been found in natural populations of *D. melanogaster*, and this type of genetic variability cannot be explained either by the presence of null alleles or by bandmorph mutant alleles at the structural loci (see DISCUSSION). If this is the case, the genetic load existing in *D. melanogaster* populations should be

controlled by factors located outside of the structural genes. From studies of the rates of mutations affecting viability, we have already reported that the majority of the mutant viability polygenes must be located outside structural loci (MUKAI and COCKERHAM 1977).

Under the working hypothesis that the majority of viability polygenes regulate the time of expression and amount of proteins encoded by structural genes, we have carried out an experiment with *D. melanogaster* and have obtained results supporting the hypothesis. The results are reported here.

MATERIALS AND METHODS

Establishment of lines: One thousand second chromosome lines were established using four ancestral chromosomes: two *In(2LR)SM1* chromosomes marked by the dominant gene Curly (*Cy*) and two unrelated lethal-carrying chromosomes, *l(AW)* and *l(JH)*, which were derived in 1967 from a cage population (W-1). A single male, which was a heterozygote for *In(2LR)SM1* and *l(AW)*, was mated to a single C-160 [*In(2LR)SM1/In(2LR)bw*^{V1}, which is abbreviated *Cy/Pm*] female. To establish the chromosome lines from the progeny *Cy/l* males and females were collected, and many single-pair matings were made between them. In the progeny and all subsequent generations, only *Cy/l* heterozygotes survived because *Cy/Cy* and *l/l* are lethal. The number of single-pair matings was increased, and, by generation 3, 500 lines were established (AW-1, AW-2, ..., AW-500). Each line was maintained by both a single-pair mating and a five-pair mating. Whenever the single-pair mating was successful, its offspring were used to make the single-pair mating and the five-pair mating for the next generation. When the single-pair mating was not successful, the five-pair mating was used as a substitute source of flies for the next generation. It is possible to detect any contamination from external sources using this method, because, if it occurs, phenotypically wild-type flies appear. Following the same procedure, 500 lines of the other group (JH-1, JH-2, ..., JH-500) were established with a single *In(2LR)SM1(Cy)* chromosome and a single lethal-carrying chromosome, *l(JH)* (see MUKAI and COCKERHAM 1977).

These chromosome lines were established at the end of 1967 and maintained until March, 1975, by T. MUKAI. After that H. E. SCHAFFER and C. C. LAURIE-AHLBERG maintained them. Early in 1980, about 100 lines, 50 from AW and 50 from JH, were transferred to Kyushu University. We are grateful to H. E. SCHAFFER for his help. These 100 lines were carefully chosen in order to avoid selecting duplicated lines in the process of accumulation of mutations (see MUKAI and COCKERHAM 1977). When these lines were maintained at North Carolina State University, transfers were made every 14 days. Thus, about 300 generations had passed there after the initiation of these chromosome lines. These chromosome lines were maintained at 25°.

After transfer to Kyushu University, the genetic background (*X*, *Y*, third and fourth chromosomes) of these chromosome lines was substituted by repeated backcrosses to C-160 (*Cy/Pm* stock), whose genetic background is that of an *isogenic* wild-type stock. The JH and AW lines carrying the isogenic genetic background were called JHBC and AWBC lines, respectively. After establishing these backcrossed lines, they were maintained at 18° by mass mating. The experimental crosses were made at 25°.

Measurement of specific activity of alcohol dehydrogenase (ADH): The specific activity of ADH was measured in the following way: From each chromosome line, six $Cy/l_i \times Cy/l_i$ crosses were made with five flies of each sex in vials of 2.5 × 10 cm on a culture medium without living yeast. Four of six vials were selected at random. From each of two vials, five *Cy/l* 4-day-old adult males were sampled, and from each of the remaining two vials, five *Cy/l* adult 5-day-old males were sampled. These five flies from each vial were homogenized in 0.2 ml of buffer (0.05 M Tris-HCl buffer, pH 8.5) and centrifuged. Supernatant, 0.025 ml, was used for the test of activity of ADH, which was measured by observing the reduction of NAD^+ to NADH at 340 nm. Furthermore, 0.025 ml of the supernatant from each vial was used for measuring the amount of total soluble protein according to the Lowry method (LOWRY *et al.* 1951). Thirty-two JHBC and 37 AWBC lines were used for the assays of the activity of ADH, isopropanol being used as substrate.

The control experiment was made using a single line (JHBC-292). Twenty lines were developed

from JHBC-292. In the same way, the activity of ADH and the total amount of soluble protein were measured. For the JHBC, AWBC and control experiments, the specific activities of ADH per milligram of soluble protein were measured, and these values were employed for the analyses.

RESULTS

At first, 32 JHBC lines were used for the measurements of ADH activity. These were all of the lines for which the substitution of the genetic background was successful. The measurements were made twice per vial, and their mean values were employed for the analysis. Thus, a total of four measurements (2 days × 2 vials) were used. The sequence of the measurements was randomized daily, so that the errors were randomized within and between days. An analysis of variance was made, and the result is shown in Table 1. There are significant differences between days and between lines at the 1% level. The genetic variance between lines is estimated as 0.000409 ± 0.000164 $(\text{units/mg})^2$. An identical experiment was made using 37 AWBC lines, and very similar results were obtained, namely, significant differences were observed between days and between lines at the 1% level.

It should be stressed that day × line interaction was not significant. This means that the ranking of the specific activity among lines does not change significantly on different days. The most important finding is that, in spite of the fact that these 32 or 37 *Cy*- and 32 or 37 *l*-carrying chromosomes employed in the present experiment originated from a single *Cy*- and a single *l*-carrying chromosome [*In(2LR)SM1* and *l(JH)* or *l(AW)*], respectively, significant differences were found between lines after accumulating spontaneous mutations for about 300 generations.

A control experiment was made using a single JHBC line at the same time as the JHBC experiment. From a single male of *Cy/l(JH)* of JHBC-292 20 independent lines were derived with the marked inversion stock (C-160, *Cy/Pm*) with an isogenic genetic background, and the same experiment as described before was conducted. The results are also given in Table 1. There was a significant difference only between days, and no significant difference was detected between lines.

It has been reported that ADH activity should be adjusted for total soluble protein, considering the nonproportionality between body weight and the amount of the enzyme (CLARKE *et al.* 1979). For the amount of total soluble protein in 0.025 ml of the supernatant per line, the genetic variances between lines are: $\hat{\sigma}_G^2$ = 3.8261 ± 2.5380, 2.9795 ± 1.7638, and 4.9967 ± 3.3074 μg^2, for the JHBC, AWBC and control groups, respectively. These figures are not significantly different from 0. The mean body weights are: 58.7, 53.0 and 58.3 μg, for the JHBC, AWBC and control groups, respectively. These findings suggest that the environmental conditions were well controlled in the present experiments and that the effects of differential body weight (amount of soluble protein per 0.02 ml) on the specific activity of ADH need not be considered. In fact, the correlation coefficients between the specific activity of ADH and the amount of soluble proteins are 0.07 (d.f. = 30), 0.14 (d.f. = 35) and −0.15 (d.f. = 18) for the JHBC, AWBC and control groups, respectively. (In the

TABLE 1

Analysis of variance of specific activities of ADH in D. melanogaster

Source	Sum of squares	d.f.	Mean square	*F*
(a) JHBC lines				
Days	0.021172	1	0.021172	26.02**
Lines	0.075993	31	0.002451	3.01**
Interaction	0.025219	31	0.000814	1.41
Error	0.036950	64	0.000577	
Total	0.159333	127		
Genetic variance component of lines = 0.000409 ± 0.000164 (units/mg)^2				
(b) AWBC lines				
Days	0.001937	1	0.001937	6.34*
Lines	0.042354	36	0.001176	3.85**
Interaction	0.010995	36	0.000305	1.09
Error	0.020666	74	0.000279	
Total	0.075952	147		
Genetic variance component of lines = 0.000218 ± 0.000072 (units/mg)^2				
(c) Control lines				
Days	0.020205	1	0.020205	23.64**
Lines	0.017495	19	0.000921	1.08
Interaction	0.016236	19	0.000855	0.62
Error	0.055164	40	0.001379	
Total	0.109101	79		
Genetic variance component of lines = 0.000017 ± 0.000102 (units/mg)^2				

** Significant at 1% level.
* Significant at 5% level.

control, one extremely deviant vial was disregarded.) These estimated correlation coefficients are not significantly different from 0.

From these results, it may be concluded that significant differences in specific activity of ADH have arisen between lines. This phenomenon must be caused by spontaneous mutation. The distributions of the specific activities of ADH in the JHBC, AWBC and control groups are shown in Figure 1. From this figure, it can clearly be seen that mutations affecting specific activity of ADH occurred in many lines of the JHBC and AWBC groups. The mean specific activities of the JHBC, AWBC and control groups are: 0.317, 0.242 and 0.320 *units/mg, respectively.*

ANALYSES

The genetic variances of specific activity of ADH as increased by spontaneous mutation are estimated from Table 1. The results are as follows: JHBC group, 0.000409 ± 0.000164; AWBC group, 0.000216 ± 0.000071; and control group, 0.000015 ± 0.000102 (units/mg)^2. Of course, the first two figures are significantly different from 0 as described in the analysis of variance table, but the figure for the control group is not.

These increases in genetic variance are due to spontaneous mutations that

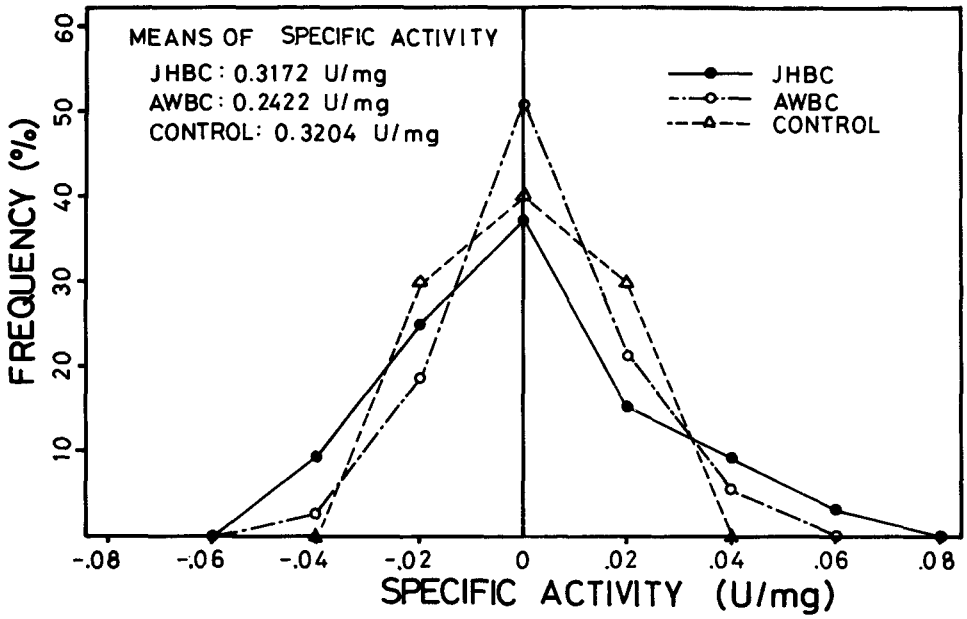

FIGURE 1.—Frequency distribution of lines with respect to specific activity of ADH.

occurred during about 300 generations. There are two possible hypotheses about the mutations: (1) The mutations occurred at the *Adh* locus in the second chromosome, and (2) mutations occurred at loci or chromosome regions other than the *Adh* locus. The second possibility includes two cases: (a) Mutations modifying ADH activity occurred only in coding regions other than those of the *Adh* locus, and (b) those mutations occurred not only in coding regions other than those of the *Adh* locus but also in noncoding regions, including introns and spacers. For the sake of convenience, structural aberrations such as small duplication, insertion, deletion, inversion and so on are included in this category.

For discussion of the possibilities, the following assumptions were made: (1) The band-morph mutation rate is 1.81×10^{-6} per locus per generation (MUKAI and COCKERHAM 1977), and the detection rate is ⅓ or higher (SHAW 1965; RAMSHAW, COYNE and LEWONTIN 1979; FUERST and FERRELL 1980); (2) the estimate of the average number of base pairs in the loci for which the band-morph mutation rate was estimated is 973 (MUKAI and COCKERHAM 1977); (3) the mutation rate to null alleles was estimated to be 10.3×10^{-6} per locus per generation for the loci having the average number of base pairs described before.

Possibility (1) may be rejected by the following calculation: The probability that the *Adh* locus obtained a mutation during 300 generations is approximately 0.0037, considering that the number of base pairs at this locus is 765 (BENYAJATI *et al.* 1981), that the estimated mutation rate (base pair change rate) is 5.58×10^{-9} per base pair per generation and that the mutation rate to null alleles is 8.10×10^{-6} per generation at this locus (*cf.* MUKAI and

COCKERHAM 1977). This may be an overestimate (see VOELKER, SCHAFFER and MUKAI 1980), and so its use is conservative. Thus, the expected number of lines carrying at least one new mutation at the *Adh* locus is: $69 \times (1 - e^{-0.0037 \times 2}) = 0.51$. Since this is less than 1, mutations at the *Adh* locus cannot explain the significant increase in genetic variance of ADH activity. In fact, no band-morph mutation was detected at the *Adh* loci in these lines.

Possibility (2a) may be examined as follows. The following parameters were defined for estimating the number of structural loci affecting the activity of ADH (x): μ = mutation rate of a structural gene per locus per generation, and T = number of generations for which spontaneous mutations were accumulated.

From comparison of the distributions of specific activities of JHBC and the control in Figure 1, the minimum frequency of lines that acquired at least one mutant modifier of ADH during 300 generations can be estimated to be about 0.25. This was estimated as the sum of the deviations of the frequencies of JHBC from the control (y) in respective classes of activity in Figure 1, when y is positive. This is surely an underestimate. We hypothesize the actual frequency to be approximately 0.50. Then, the following equations can be obtained:

$$(1 - \mu)^{2Tx} = (1 - 0.25) \text{ or } (1 - 0.5) \qquad (1)$$

in which 2 comes in because mutations can accumulate on both the *Cy*- and *l*-carrying chromosomes. In this formula, $\hat{\mu} = (10.3 + 1.81 \times 3) \times 10^{-6}$ or 15.73×10^{-6} and $T = 300$ are used. Then,

$$x = \frac{ln\ 0.5}{2T \cdot ln(1 - \mu)} = 30.5 \text{ or } 74.5.$$

It is doubtful that more than 50 structural loci in the second chromosome affect the specific activity of ADH. Therefore, it may be reasonable to assume that the specific activity of ADH is controlled by other factors in addition to the structural genes. Thus, possibility (2b) becomes plausible. Tentatively, we may call these other factors "controlling factors," including structural changes in the chromosome.

DISCUSSION

There have been two mutually contradictory hypotheses with respect to the biological role of repeated sequence, spacer or intron DNA: One states that this extragenic DNA may be related to the regulation of gene action (BRITTEN and DAVIDSON 1969; DAVIDSON and BRITTEN 1979; others), and the other considers that structural genes are, as it were, oases in a barren desert. That is to say, extragenic DNA is thought to be freely drifting junk or selfish DNA (OHNO 1972, 1981). Our experimental results appear to support the first hypothesis.

Our experimental results suggest that there are many controlling factors outside the structural gene loci. In fact, MUKAI and COCKERHAM (1977) concluded that the majority of mutant viability polygenes should be located outside

structural loci in *D. melanogaster* since the spontaneous mutation rate of viability polygenes is 0.14–0.17 per second chromosome per generation, whereas the total mutation rate at structural loci in the second chromosome was estimated to be 0.012 per generation (assuming the detection rate to be ⅛). Further evidence supporting this statement can be obtained without directly using the spontaneous mutation rate of viability polygenes, as follows. Using the second chromosome lines on which spontaneous mutations were accumulated at a minimum pressure of natural selection for about 160 generations in *D. melanogaster*, additive and dominance variances for viability were estimated to be 0.0202 ± 0.0038 and 0.00063 ± 0.00057, respectively (MUKAI 1977). The latter is negligibly small. For comparison, the corresponding values were 0.0096 ± 0.0025 and 0.00085 ± 0.00027 for the Raleigh, North Carolina, population (MUKAI *et al.* 1974). Thus, about 75 generations are necessary to accumulate spontaneous mutations that create an additive genetic variance equivalent to that in the aforementioned natural population (MUKAI 1979).

Under the null hypothesis that all the mutations affecting viability are located at structural loci, an attempt was made to predict the magnitude of additive variance due to the accumulation of spontaneous mildly deleterious mutations for 75 generations and to compare it with the actual estimate. First, the additive genetic variance due to mutations other than mildly deleterious mutations should be estimated. VOELKER *et al.* (1980) surveyed the gene frequencies of null alleles over 20 polymorphic enzyme loci that are located in the second and third chromosomes of *D. melanogaster* collected in the same natural population. The average gene frequency (q) was 0.0025. hs was estimated to be 0.0021, using the relationship $q \cong \mu/chs$, where μ is the null-allele mutation rate, hs is the product of the average degree of dominance and selection coefficient of a null allele with respect to viability in the Wrightian fitness model and c is the coefficient that makes chs the selection coefficient against heterozygote for fitness as a whole. In the present case, c was assumed to be 2 (MUKAI and YAMAGUCHI 1974; calculated from the data of KUSAKABE and MUKAI 1982), which implies that a null allele is deleterious for viability and fertility to the same extent in heterozygotes. This rate of null-allele mutations was reported to be 1.03×10^{-5} per locus per generation, although this may be an overestimate due to the kind of mutation described previously (MUKAI and COCKERHAM 1977). Thus, the additive variance of viability due to null alleles [$\sigma^2_{A(n)}$] can be estimated using the following formula: $\sigma^2_{A(n)} \cong 2 \cdot m \cdot n \cdot (\overline{sh})^2$, where m is the number of generations and n is the number of structural loci. From the one-gene/one-band hypothesis (JUDD, SHEN and KAUFMAN 1972), n is assumed to be 2200 per second chromosome. The result is $\sigma^2_{A(n)} \cong 2 \times 75 \times 2200 \times 1.03 \times 10^{-5} \times (0.0021)^2 = 0.000015$.

Since it is known (VOELKER *et al.* 1980; O'BRIEN and MACINTYRE 1978) that most null mutations are viable when homozygous, the contribution of lethal genes to the additive variance should be considered: The recessive lethal mutation rate ($\Sigma\mu_l$) has been reported to be 0.006 per second chromosome per generation for the experimental material (MUKAI *et al.* 1972), and the degree of dominance is known to be approximately 0.015 (MUKAI and YAMAGUCHI

1974). Thus, the additive genetic variance due to lethals [$\sigma^2_{A(l)}$] that accumulated during 75 generations becomes $\sigma^2_{A(l)} \cong 2 \cdot m \cdot \Sigma\mu_l \cdot (\overline{sh})^2$ or $\sigma^2_{A(l)} = 2 \times 75 \times 0.006 \times (0.015)^2 = 0.0002025$. The sum of $\sigma^2_{A(n)}$ and $\sigma^2_{A(l)}$ is 0.00022, which is only 2.3% of the total additive variance. Thus, the 97.7% of the additive variance due to newly arisen mutations (0.00958) must be due to mildly deleterious mutations. (Incidentally, the values of $\sigma^2_{A(l)}$ and $\sigma^2_{A(n)}$ in the natural population first described were estimated using the average gene frequency of null alleles, 0.0025, and the homozygous load due to lethal genes, 0.50 (MUKAI and YAMAGUCHI 1974) to be $\sigma^2_{A(l)} = 0.000225$ and $\sigma^2_{A(n)} = 0.000047$. These estimates are extremely small in comparison with the total additive variance (only 2.8%) but similar to those estimated from the mutation rates.)

The spontaneous band-morph mutation rate was estimated to be 1.81×10^{-6} per locus per generation, as described before (MUKAI and COCKERHAM 1977). Thus, the rate of nondrastic mutations at the base pair level (μ_{bp}) may be estimated to be $3 \times 1.81 \times 10^{-6}$ per locus per generation, where 3 is the inverse of the detection rate of base pair changes detected by observing the change in electromorph of soluble proteins. If it is assumed that mildly deleterious mutations are due to nondrastic mutations at the base pair level, hs is assumed to be 0.015. Indeed, h and s for newly arisen mildly deleterious mutations were estimated to be 0.43 ± 0.008 and 0.023 ± 0.027, respectively (MUKAI 1964, 1969; MUKAI *et al.* 1972). Therefore, the additive variance due to nondrastic second chromosome mutations at the structural loci [$\sigma^2_{A(m)}$] that accumulated during 75 generations can be predicted as:

$$\begin{aligned}\sigma^2_{A(m)} &\cong 2mn\hat{\mu}_{bp}(\overline{sh})^2 \\ &= 2 \times 2200 \times 75 \times (3 \times 1.81 \times 10^{-6}) \times (0.015)^2 \\ &= 0.000403 \text{ per second chromosome.}\end{aligned}$$

This value is only 4.3% of the expected value. Thus, the null hypothesis should be rejected, and it is most reasonable to assume that the majority of mildly deleterious mutations are different from mutations at the structural loci. Although there is no direct evidence, it is assumed that viability polygenes and controlling factors are of the same molecular nature. Otherwise, the total mutation rate would become too high.

There are two possibilities for these controlling factors in noncoding DNA, although they are not mutually exclusive: (1) Some structural changes in noncoding DNA affect the activity of structural genes (in the present case, *Adh*) (OHTA 1983); (2) change of base pairs in some specific noncoding DNA affects the activity of some specific structural gene or genes. The most attractive experimental result that supports possibility (1) were obtained by D. HOGNESS and W. BENDER (see MARX 1981). They analyzed the bithorax gene complex at the molecular level, which had been studied by EDWARD LEWIS. According to MARX, most mutations of the complex gene were caused by insertion or deletion of large DNA segments and not just by changes in one or a few base pairs. It is interesting that these insertion elements are transposable. Furthermore, some of the bithorax mutations (*abx*, *bx* and *Ubx*) do not appear to be

transcribed. If these types of genes (or factors) can generally be found in chromosomes and if they control the activity of structural genes, the results of our experiments can easily be explained—namely, many insertions or deletions occurred in connection with "mutators" or transposition in the JH and AW chromosomes, and these mutations controlled the specific activity of ADH. The present experimental results are consistent with this speculation. The genetic variances of the specific activity of ADH are 0.000409 ± 0.000164 $(\text{units/mg})^2$ in the JHBC group and 0.000216 ± 0.000071 $(\text{units/mg})^2$ in the AWBC group. The former is approximately twice as large as the latter, although they are not significantly different from each other. The frequencies of inversions, which might be considered to be proportional to the number of breaks of the chromosomes, are nine in 30 in the JHBC and three in 28 in the AWBC group. These frequencies were estimated by examination of salivary gland chromosomes. In the process of accumulating mutations, the salivary gland chromosomes were also examined at about generation 90, and the numbers of detectable break points were found to be 183 and 39 in the 500 JH and 500 AW chromosome lines, respectively (YAMAGUCHI and MUKAI 1974). These figures suggest that there was some type of transposon-like element such as *P* factor in the lines investigated, at least at the starting time (*cf.* YAMAGUCHI and MUKAI 1974), but these factors appear to have disappeared from the lines. Recent tests of these lines indicate that they are all the *M* type of the *P-M* system (*cf.* KIDWELL, KIDWELL and SVED 1977).

It is necessary to add the following: If polygenic viability mutations are small chromosome aberrations (small duplication, insertion, deletion, etc.), then transposon-like elements and unequal crossing over, including sister-strand exchange, may induce mutant viability polygenes (OHTA 1983). Indeed, it has been reported that spontaneous sister-strand exchange must occur in somatic cells even in males of *D. melanogaster* (TSUJI 1982).

As for the second hypothesis, we have no good suggestive evidence at present. Although it has been reported that base pair changes in promoter regions in *E. coli* cause change in the efficiency of transcription (*cf.* ROSENBERG and COURT 1979), mutations of this type alone cannot explain the high mutation rate of controlling factors relative to one specific structural gene. However, recent published results indicate that some mutations in introns are functional in mammalian cell culture (*e.g.*, BUSSLINGER, MOSCHONAS and FLAVELL 1981) and in mitochondrial DNA in yeast (DUJON 1979). Furthermore, it has been reported that there are approximately ten species of abundant and stable RNAs ranging in size from 100 to 300 nucleotides in mammalian cells in culture. Their biological role has not been clarified, but it is speculated that they are potential affectors of RNA maturation, perhaps involved in transcriptional processes or RNA processing or transport (ZIEVE 1981). These RNAs are transcribed from extragenic DNA by RNA polymerase III (OHNO 1981; DENISON *et al.* 1981).

In fact U1 RNA, an abundant small nuclear RNA, was identified in cultured *D. melanogaster* cells (MOUNT and STEITZ 1981). If there are a variety of small (nuclear) RNAs in Drosophila cells, and if they have some effects on RNA

maturation, the present experimental result may partly be explained by mutations affecting extragenic DNA.

Two possibilities for the controlling factors have been discussed in this paper, and they are not mutually exclusive. Although possibility (2) is entirely speculated on the basis of information obtained from the literature, possibility (1) is more likely to be realistic. However, its molecular mechanism is unknown. Recently, we obtained an interesting experimental result relating to possibility (1). Mildly deleterious mutations (probably equivalent to mutations of controlling factors in the present experiment) were induced by γ-ray irradiation and some chemicals *only* in the lines carrying some transposon-like element [*i.e.*, our "mutator" (see YAMAGUCHI and MUKAI 1974)], which was identified by the occurrence of male recombination (MUKAI 1982; T. TOMITA, T. TANAKA, Y. GONDO, A. KOGA, T. NAKAMA, K. KONDO and T. MUKAI, unpublished data). Further data supporting the present experimental result will be published soon.

Finally, it should be mentioned that, if the present experimental results are generally confirmed, it is mutations in the noncoding region that are important for the adaptive microevolution of organisms. Adaptive microevolution will be preceded by the change in controlling factors that modify the effects of different allelic structural genes approximately equally. This hypothesis is not contradictory to the neutral theory (KIMURA 1968; KIMURA and OHTA 1971).

We are grateful to TOMOKO OHTA and HIDENORI TACHIDA for their suggestions. This is paper no. 15 from the Laboratory of Population Genetics, Department of Biology, Kyushu University. This work was supported by United States Public Health Service research grant GM 11546 and by research grants 348003 and 56480002 from the Ministry of Education, Science, and Culture of Japan.

LITERATURE CITED

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. USA **78:** 2717–2721.

BRITTEN, R. J. and E. H. DAVIDSON, 1969 Gene regulation for higher cells: a theory. Science **165:** 349–357.

BUSSLINGER, M., N. MOSCHONAS and R. A. FLAVELL, 1981 β^+ Thalassemia: aberrant splicing results from a single point mutation in an intron. Cell **27:** 289–298.

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

DAVIDSON, E. H. and R. J. BRITTEN, 1979 Regulation of gene expression: possible role of repetitive sequences. Science **204:** 1052–1059.

DENISON, R. A., S. W. VAN ARSDELL, L. B. BERNSTEIN and A. M. WEINER, 1981 Abundant pseudogenes for small nuclear RNAs are dispersed in the human genome. Proc. Natl. Acad. Sci. USA **78:** 810–814.

DUJON, B., 1979 Mutants in a mosaic gene reveal functions for introns. Nature **282:** 777–778.

FUERST, P. A. and R. E. FERRELL, 1980 The stepwise mutation model: an experimental evaluation utilizing hemoglobin variants. Genetics **94:** 185–201.

JUDD, B. H., M. W. SHEN, and T. C. KAUFMAN, 1972 The anatomy and function of a segment of the *X* chromosome of *Drosophila melanogaster*. Genetics **71:** 139–156.

KIDWELL, M. G., J. F. KIDWELL and J. SVED, 1977 Hybrid dysgenesis in *Drosophila melanogaster*: a syndrome of aberrant traits including mutation, sterility, and male recombination. Genetics **86:** 813–833.

KIMURA, M., 1968 Evolutionary rate at the molecular level. Nature **217:** 624–626.

KIMURA, M. and T. OHTA, 1971 Protein polymorphism as a phase of molecular evolution. Nature **229:** 467–469.

KUSAKABE, S. and T. MUKAI, 1982 Maintenance mechanism of genetic variability due to viability polygenes and its implication to adaptive evolution of organisms. Jpn. J. Genet. **57:** 193–218 (in Japanese).

LEWONTIN, R. C. and J. L. HUBBY, 1966 A molecular approach to the study of genic heterozygosity in natural populations. II. Amount of variation and degree of heterozygosity in natural populations of *Drosophila melanogaster*. Genetics **54:** 595–609.

LOWRY, O. H., N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, 1951 Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193:** 265–275.

MARX, J. L., 1981 Genes that control development. Science **213:** 1485–1488.

MOUNT, S. M. and J. A. STEITZ, 1981 Sequence of U1 RNA from *Drosophila melanogaster*: implications for U1 secondary structure and possible involvement in splicing. Nucleic Acids Res. **9:** 6351–6368.

MUKAI, T., 1964 The genetic structure of natural populations of *Drosophila melanogaster*. I. Spontaneous mutation rate of polygenes controlling viability. Genetics **50:** 1–19.

MUKAI, T., 1969 The genetic structure of natural populations of *Drosophila melanogaster*. VIII. Natural selection on the degree of dominance of viability polygenes. Genetics **63:** 467–478.

MUKAI, T., 1977 Genetic variance for viability and linkage disequilibrium in natural populations of *Drosophila melanogaster*. pp. 97–112. In: *Measuring Selection in Natural Populations*, Edited by F. B. CHRISTIANSEN and T. M. FENCHEL. Springer-Verlag, New York.

MUKAI, T., 1979 Polygenic mutation. pp. 177–196. In: *Quantitative Genetic Variation*, Edited by T. N. THOMPSON, JR. and T. M. THODAY. Academic Press, New York.

MUKAI, T., 1982 What is mildly deleterious mutations?—from the studies of Drosophila. pp. 97–108. In: *Environment and Human Body*, Vol. I, Edited by I. CHUMA, S. KONDO and H. TAKEBE. University of Tokyo Press, Tokyo (in Japanese).

MUKAI, T., O. YAMAGUCHI, S. KUSAKABE, H. TACHIDA, M. MATSUDA, M. ICHINOSE and H. YOSHIMARU, 1982 Lack of balancing selection for protein polymorphisms. pp. 81–120. In: *Molecular Evolution, Protein Polymorphism and the Neutral Theory*, Edited by M. KIMURA. Japan Scientific Societies Press, Tokyo/Spring-Verlag, Berlin.

MUKAI, T., R. A. CARDELLINO, T. K. WATANABE and J. F. CROW, 1974 The genetic variance for viability and its components in a local population of *Drosophila melanogaster*. Genetics **78:** 1195–1208.

MUKAI, T., S. I. CHIGUSA, L. E. METTLER and J. F. CROW, 1972 Mutation rate and dominance of genes affecting viability in *Drosophila melanogaster*. Genetics **72:** 335–355.

MUKAI, T. and C. C. COCKERHAM, 1977 Spontaneous mutation rates at enzyme loci in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA **74:** 2514–2517.

MUKAI, T. and O. YAMAGUCHI, 1974 The genetic structure of natural populations of *Drosophila melanogaster*. XI. Genetic variability in a local population. Genetics **76:** 339–366.

O'BRIEN, S. J. and R. J. MACINTYRE, 1978 Genetics and biochemistry of enzymes and specific proteins of Drosophila. pp. 395–551. In: *Genetics and Biology of Drosophila*, Vol. 2a, Edited by M. ASHBURNER and T. R. F. WRIGHT. Academic Press, New York.

OHNO, S., 1972 So much "junk" DNA in our genome. pp. 366–370. In: *Evolution of Genetic System*, Edited by H. H. SMITH. Gordon and Breach, New York.

OHNO, S., 1981 Evolution of genes by Epimethean hindsight. Jpn. J. Hum. Genet. **26:** 97–99.

OHTA, T., 1983 On the evolution of multigene families. Theor. Pop. Biol. **23:** 216–240.

RAMSHAW, J. A. M., J. A. COYNE and R. C. LEWONTIN, 1979 The sensitivity of gel electrophoresis as a detector of genetic variation. Genetics **93:** 1019–1037.

ROSENBERG, M. and D. COURT, 1979 Regulatory sequences involved in the promotion and termination of RNA transcription. Annu. Rev. Genet. **13:** 19–53.

SHAW, C. R., 1965 Electrophoretic variation in enzymes. Science **149:** 936–943.

TSUJI, H., 1982 Presence of a base line level of sister chromatid exchanges in somatic cells of *Drosophila melanogaster in vivo* and *in vitro*. Genetics **100:** 259–278.

VOELKER, R. A., C. H. LANGLEY, A. J. L. BROWN, S. OHNISHI, B. DICKSON, E. MONTGOMERY and S. C. SMITH, 1980 Enzyme null alleles in natural populations of *Drosophila melanogaster*: frequencies in a North Carolina population. Proc. Natl. Acad. Sci. USA **77:** 1091–1095.

VOELKER, R. A., H. E. SCHAFFER and T. MUKAI, 1980 Spontaneous allozyme mutations in *Drosophila melanogaster*: rate of occurrence and nature of the mutants. Genetics **94:** 961–968.

YAMAGUCHI, O. and T. MUKAI, 1974 Variation of spontaneous occurrence rates of chromosomal aberrations in the second chromosomes of *Drosophila melanogaster*. Genetics **78:** 1209–1221.

ZIEVE, G. W., 1981 Two groups of small stable RNAs. Cell **25:** 296–297.

Corresponding editor: D. L. HARTL