SPONTANEOUS ALLOZYME MUTATIONS IN *DROSOPHILA MELANOGASTER*: RATE OF OCCURRENCE AND NATURE OF THE MUTANTS[1]

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ABSTRACT

After additional generations of accumulation of allozyme mutants, the 1,000 lines of MUKAI and COCKERHAM (1977) were again screened for the same five loci (*α-Gpdh*, *cMdh*, *Adh*, *Hex-C* and *α-Amy*), as well as for two new loci (*Got-2* and *Dip-A*). Based on 3,111,598 allele generations: (1) the average mutation rate to new mobility variants with normal function was estimated to be 1.28×10^{-6}, and (2) the average mutation rate to null alleles was estimated to be 3.86×10^{-6}. A qualitative analysis of the nulls provided evidence that most of the mutants recovered are due to base substitutions. No apparent correlation was observed between structural gene size and mutation rate.

ALTHOUGH allozyme variation has been identified at many loci in numerous species (LEWONTIN 1974), little is known about the rates at which allozymic variation arises. While several earlier small studies (MUKAI 1970; TOBARI and KOJIMA 1972) attempted to determine rates of occurrence of mobility variants, the largest study was reported by MUKAI and COCKERHAM (1977). They determined the band-morph (mobility) and null mutation rates to be 1.81×10^{-6} and 1.03×10^{-5}, respectively, based on 1.66 million allele generations at five chromosome *2* loci in one thousand lines of *Drosophila melanogaster*. Mutation accumulation was continued in these lines; between generations 211 and 224, they were again screened for the previous five loci and two additional loci, after which mutation accumulation was discontinued and the lines were maintained in mass culture. We report here the mutation rate estimates from that screen together with an analysis of the qualitative nature of the null mutants recovered.

MATERIALS AND METHODS

The detailed description of the derivation and maintenance of the lines has been previously reported (MUKAI and COCKERHAM 1977). In brief, mutations were accumulated in two sets of balanced-lethal chromosome lines: 500 *SM1*, *Cy/l(AW)* and 500 *SM1*, *Cy/l(JH)*. Each set of

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500 lines had been originally derived from an single pair mating. The loci that were rescreened were: *α-Gpdh, cMdh* (*Mdh*), *Adh*, *Hex* and *α-Amy*. The two loci screened for the first time were: Glutamate-oxaloacetate transaminase (*Got-2*, 3.0, E.C. 2.6.1.1.) and Dipeptidase A (*Dip-A*, 55.2, E.C. 3.4. .). Electrophoresis and staining were as in MUKAI and COCKERHAM (1977), VOELKER and LANGLEY (1978) and LANGLEY, ITO and VOELKER (1974).

For the screening of most loci, several males from each line were crossed to females of a balanced lethal [the lethal was unrelated to *l(AW)* and *l(JH)*] tester strain of the following composition:

$$\frac{SM1,\ Roi\ Got\text{-}2^{S}\ \alpha\text{-}Gpdh^{F}\ cMdh^{S}\ Adh^{F}\ Dip\text{-}A^{F}\ Hex\text{-}C^{S}\ \alpha\text{-}Amy^{1}}{+_{\text{lethal}}\quad Got\text{-}2^{F}\ \alpha\text{-}Gpdh^{S}\ cMdh^{F}\ Adh^{S}\ Dip\text{-}A^{S}\ Hex\text{-}C^{F}\ \alpha\text{-}Amy^{2,3}}$$

Phenotypically Curly and wild-type flies were scored to detect mutants on *SM1*, *Cy* and *l(AW or JH)* (the two series of lethal-bearing "wild type") chromosomes, respectively. Heterozygous loci in the mutation lines (*α-Gpdh*, *Adh* and *α-Amy* in AW lines; *α-Gpdh* in JH lines) were screened by electrophoresis of flies from the stock lines. Putative mobility and null mutants were retested and stocks were established of all confirmed mutants.

The qualitative nature of the nulls was determined as follows: (1) the salivary gland chromosomes were examined for the presence of aberrations; (2) where possible, the nulls were crossed to known cytological deficiencies for the respective loci [*Df(2L)J69^{L}H56^{R},y+* for *Got-2*; *Df(2L) GpdhA, dp* for *α-Gpdh*] to determine whether the null might be a deficiency including adjacent lethal loci and to determine whether the null exhibited any residual staining activity; and (3) where possible, the nulls were examined for the production of heterodimers when heterozygous with a normal allele having a mobility different from that from which the null arose. Formation of a heterodimer or residual mutant homodimer activity in a null/deficiency heterozygote is *prima facie* evidence that the mutant is CRM-positive.

RESULTS AND ANALYSES

The results of the electrophoretic screening of the 1,000 lines are shown in Table 1. Four mobility variants were recovered in the *l(AW)* lines: two at *cMdh*, one at *Hex-C* and one at *α-Amy*. The mutants are the same ones as previously reported by MUKAI and COCKERHAM (1977), who verified their new mobilities and documented their being newly arisen variants. The two *cMdh* variants are probably of the same origin, with the recurrence being due to the replacement of a lost line by a sister line (see DISCUSSION). The mutation rate to new mobility forms (μ_M), thus, is $4/3{,}111{,}598 = 1.28 \times 10^{-6}$, assuming that *α-Amy* is duplicated on the *l(AW)* (*α-Amy^{1,6}*), but not on the *Cy* or *l(JH)* (*α-Amy^{1}*) chromosomes (reviewed in YARDLEY 1978). (See also Table 1, footnote †.)

Twelve null alleles were recovered: one at *Got-2*, seven at *α-Gpdh* and four at *Hex-C*. Again, assuming that *α-Amy* is a duplicated locus only on the *l(AW)* chromosome, the null mutation rate (μ_N) is $12/3{,}111{,}598 = 3.86 \times 10^{-6}$ (see also Table 1, footnote ‡).

Among the nulls recovered, two *α-Gpdh* nulls (JH 253 and JH254) and the four *Hex-C* nulls (JH 301, JH 302, JH 303 and JH 309) are probably derived from a single mutational event at each locus. The observations that they occurred on the same chromosome, (*Cy vs. l(JH)*), are qualitatively identical insofar as our analyses extend, and were found in adjacent or closely numbered lines taken together with the procedure for reestablishing a lost line from an adjacent or

TABLE 1

Results of screening for allozyme mutants

	LOCUS						
	Got-2	*α-Gpdh*	*cMdh*	*Adh*	*Dip-A*	*Hex-C*	*α-Amy*
Chromosome genotype							
SM1, Cy	S	F	S	F	F	S	1
l(AW)	S	S	S	S	F	S	1,6
l(JH)	S	S	S	F	F	S	1
Number of mobility mutants							
SM1, Cy/l(AW)	0/0	0/0	0/2	0/0	0/0	1/0	0/1
SM1, Cy/l(JH)	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Number of null mutants							
SM1, Cy/l(AW)	0/0	1/1	0/0	0/0	0/0	0/0	0/0
SM1, Cy/l(JH)	0/1	4/1	0/0	0/0	0/0	4/0	0/0
Allele generations							
SM1, Cy (AW series)	108,736	106,204	108,736	106,204	108,736	108,736	106,144*
l(AW)	108,673	106,204	108,673	106,204	108,673	108,673	213,906*
SM1, Cy (JH series)	107,282	106,023	107,282	107,282	107,282	107,282	106,643*
l(JH)	107,248	106,023	107,248	107,248	107,248	107,248	105,757*
Per locus μ_M ($\times 10^{-6}$)	0.00	0.00	4.63	0.00	0.00	2.31	1.88
Per locus μ_N ($\times 10^{-6}$)	2.31	16.49	0.00	0.00	0.00	9.26	0.00

Total allele generations = 3,111,598

Mobility mutation rate (weighted mean) = μ_M = 4/3,111,598† = 1.28×10^{-6}

Null mutation rate (weighted mean) = μ_N = 12/3,111,598‡ = 3.86×10^{-6}

Total mutation rate (weighted mean) = μ_T = 16/3,111598§ = 5.14×10^{-6}

* These values assume that *α-Amy* is duplicated on *l(AW)* but not on *SM1, Cy* or *l(JH)*.

† If *α-Amy* is also duplicated on the *l(JH)* and *SM1, Cy* chromosomes, these extra loci are counted for a total of 3,430,142 allele generations, which gives a mutation rate of 1.17×10^{-6}.

‡ If *α-Amy* is also duplicated on the *l(JH)* and *SM1, Cy* chromosomes, they cannot be used to detect null mutations; thus the total of 2,793,054 allele generations observed gives a null mutation rate of 4.30×10^{-6}.

§ If *α-Amy* is duplicated on all chromosomes scored in this study, $\mu_T = 5.46 \times 10^{-6}$.

nearby line suggest a single mutation at each locus. Unfortunately, gaps in the record keeping of line reestablishment do not permit a resolution of the question. The effect that the repeated recovery of the same mutant will have on the mutation rate estimate will be addressed in the DISCUSSION.

The total spontaneous allozyme mutation rate in these lines is, thus, $\mu_T = \mu_M + \mu_N = 16/3,111,598 = 5.14 \times 10^{-6}$, assuming that *α-Amy* is duplicated only on the *l(AW)* chromosome (see Table 1, footnote §). Also noteworthy is the apparent high mutability of *α-Gpdh* to null alleles ($\mu_N = 1.65 \times 10^{-5}$).

The results of qualitative studies of the nulls are given in Table 2. Consistent with the above reasoning, we will assume that eight nulls (AW 338, 409; JH 385, 149, 151, 231, 253, 301) are of independent mutational origin. First, none of the nulls is associated with a cytogenetically detectable aberration; thus, the nulls cannot be attributable to position effect variegation inactivating the allozyme allele.

Second, null/deficiency heterozygotes survive for the *Got-2* and *α-Gpdh* nulls (no cytogenetic deficiency is available for Hex-C). *Df(2L)GdhA* includes a

TABLE 2

Characterization of null alleles recovered

Null	Chromosomal aberration	Viability of null/deficiency	Formation of heterodimer	Mobility change
Got-2				
JH 385†	—	+	—	?
α-Gpdh¶				
JH 149†	—	+	+	+
JH 151*	—	+	+	—
JH 231*	—	+	+§	—
JH 253*	—	+	+	—
JH 254*	—	+	+	—
AW 338*	—	+	—‖	—
AW 409†	—	+	+§,‖	—
Hex-C				
JH 301*	—	n.t.	n.a.‡	?
JH 302*	—	n.t.	n.a.‡	?
JH 303*	—	n.t.	n.a.‡	?
JH 309*	—	n.t.	n.a.‡	?

n.a. = not applicable; n.t. = not tested; ? = not determinable with techniques used here.
* Null recovered on *Cy* [In(2L)SM1, Cy] chromosome.
† Null recovered on standard sequence chromosome.
‡ Not applicable; HEX-C is functional as a monomer.
§ Sometimes shows weak heterodimer activity when reared at 25° or 29°.
‖ Null homozygote (AW 409) and null/deficiency heterozygote sometimes shows very weak activity in mutant homodimer position.
¶ Analyses of the *α-Gpdh* nulls by rocket electrophoresis (G. C. BEWLEY) and two-dimensional electrophoresis (C. Y. LEE) have also shown these mutants to be CRM-positive. The results of these more detailed studies will appear in a separate paper.

number of lethal loci, several of which are closely linked to *α-Gpdh* (RACINE and VOELKER, unpublished results; M. KOTARSKI, personal communication), although *α-Gpdh* null homozygotes survive (O'BRIEN and MACINTYRE 1972). *Df(2L) J65^{L}H56^{R}, y^{+}* includes *Got-2* and an unknown number of recessive lethals; *Got-2* null *JH 385* is homozygous viable when separated from *l(JH)*. Moreover, it is well known that most genetically and/or cytogenetically detectable deficiencies in Drosophila are associated with recessive lethality (LINDSLEY and GRELL 1968). That the *Got-2* and *α-Gpdh* nulls are not suggests that the mutants involve some intralocus event.

Third, each of the *α-Gpdh* (but not *Got-2*) nulls can produce a functional heterodimer when heterozygous with a normal allele of different mobility from that from which the null arose or weak homodimer in *AW 338*, and the mobilities of the *JH 151, JH 231, JH 253 (or 4), AW 338* and *AW 409* mutant proteins are identical to those produced by the nonmutant allele from which the null arose (as judged from the electrophoretic mobility of the null/normal heterodimer or mutant homodimer). This suggests that the null proteins have only minor abnormalities such as one or, at most, a few noncharge-change amino acid substitution(s) that preclude normal stability and function. In the case of *JH*

149, the same could be true except that the amino acid substitution involved a charge change.

Taken together, these considerations indicate that the null mutants result from point mutations rather than chromosomal aberrations. The observations (1) that, in starch gel electrophoresis, the null proteins behave in a quasi-normal manner in heterodimers with normal enzyme molecules and/or form mutant homodimers, and (2) the occurrence of the mutants with altered mobility and normal function (*e.g.*, the mobility variants) suggest that the mutational events that gave rise to the mutants recovered here were base substitutions rather than frameshift mutations. Frameshifts would be expected to result in premature termination or in the alteration of a number of amino acids, which would produce a substantially greater change in the properties of the gene product.

DISCUSSION

In this study, the spontaneous mutation rate at seven chromosome *2* allozyme loci was determined to be $5.14 \times 10^{-6} (= \mu_T)$, of which $1.28 \times 10^{-6} (= \mu_M)$ is attributable to the occurrence of new mobility variants and 3.86×10^{-6} $(= \mu_N)$ to the occurrence of newly arisen null alleles. These values compare with 1.81×10^{-6} (= their μ_B = our μ_M) and 0.65×10^{-6} (= μ_N corrected for one reported *Adh* null that may have been a gene conversion, a double recombinant or a mutation) obtained by MUKAI and COCKERHAM (1977) for five of the seven loci studied in the same lines that were screened in the present study.

The lower estimates in the present study may be attributable to several factors. First, the two loci screened only in this study (*Got-2* and *Dip-A*) yielded only one null between them, suggesting that they may be less mutable than the other five loci. Second, a number of the nulls reported by MUKAI and COCKERHAM (1977) were no longer present at the time of the present screen. These included three *α-Gpdh* nulls, six *cMdh* and one *Adh* null. The failure to recover a null could be due to one or more of the following: (1) the line containing the null may have been segregating at the time of the previous screen and ultimately became fixed for the normal allele; (2) the line containing the null may have been lost and replaced with a null-free line (although the incomplete records do not document this); or (3) the null may have reverted to a functional allele. This latter possibility has not been documented for nulls at these loci, but has been for visible mutant loci in these (SCOBIE and SCHAFFER, in preparation), as well as other, lines in which the same or a similar mutator factor exists (GOLUBOVSKY, IVANOV and GREEN 1977; GREEN 1977).

Our reason for not including in the calculation of the null mutation rates the earlier reported nulls (which were not recovered in this screen) is that, at any one point in time, the best estimate of the frequency of mutations that have arisen is the frequency that exists at that time, whether fixed or segregating. First, the nulls detected only in the earlier screen may have been segregating and ultimately lost. Although half of the segregating nulls will be missed [since only two (one *Cy* and one *l*) of the four (*Cy/l* female and *Cy/l* male) chromosomes

in any line are tested], this method provides an unbiased estimate of the null frequency. This assumes that null/normal heterozygotes have no reduction in fitness. While this may not be absolutely true, the observations that null homozygotes and/or null/deficiency heterozygotes for all loci screened here (except *α-Amy*, for which nulls and deficiencies are unknown) survive and are fertile would suggest that a null/normal [*Cy/l(AW)* or *l(JH)* in the balanced lethal system used here] would have nearly normal fitness. (If this is not so, then our estimates would be minimum estimates for the null mutation rate.) Second, the number of mutants present at any one time depends on the loss and reestablishment of lines. A mutant-bearing line that is lost may be reestablished from an adjacent or nearby nonmutant line. The frequency of this is expected to be offset by the number of times an adjacent line containing a mutant was used to reestablish a nonmutant line that had been lost.

The method of lost-line replacement mentioned above, while not biasing the estimates of the mutation rates, does affect variance estimates. This leads to difficulties in attempts to determine whether there are significant differences between mutation rates at different loci. Thus, we have not attemped such calculations, except for the case to be described below.

The only locus for which there is a suggestion of a higher mutation rate is *α-Gpdh*. Even there, the difference between *α-Gpdh* and either *Dip-A* or *Adh* (at which no mutants were recovered) is of borderline significance ($P \sim 0.05$, using the tables of KASTENBAUM and BOWMAN 1970). This borderline significance could be attributable to sampling error or underlying biological causation. We think the latter is more likely for the following reasons: (1) RACINE, LANGLEY and VOELKER (in preparation), have found *α-Gpdh* to be the most mutable when low dose-rate (8.7 r/hr) ^{137}Cs γ irradiation was applied to the same seven loci (*e.g.*, in the "tester" strain detailed in MATERIALS AND METHODS); and (2) O'BRIEN and MACINTYRE (1972) and M. KOTARSKI (personal communication) have recovered *α-Gpdh* nulls with frequencies of one per 200 and one per 100, respectively, in EMS-treated chromosomes. Thus, the present finding of a relatively high mutability for *α-Gpdh* is consistent with previous observations.

A factor that may have influenced the mutation rates reported here is the known presence of a mutator factor in these lines. SCOBIE and SCHAFFER (in preparation) have observed that several chromosome *2* recessive visible loci in these lines have accumulated mutants, with some mutants being revertible, *à la* GOLUBOVSKY, IVANOV and GREEN (1977) and GREEN (1977), who observed a mutator factor that induced high frequencies (approaching 10^{-3}) of sometimes unstable mutants at several *X*-chromosome recessive visible loci. Moreover, YAMAGUCHI and MUKAI (1974) found 26A-B, the approximate cytogenetic location of *α-Gpdh*, to be the most frequent interval (36 of 225 breakpoints) involved in the rearrangements that they detected in these lines. Yet, none of the nulls recovered here was associated with a detectable aberration. The mutation rate for the most mutable locus reported here (*α-Gpdh* $= 16.5 \times 10^{-6}$) was not nearly as high as that observed by GOLUBOVSKY, IVANOV and GREEN (1977)

and GREEN (1977). Thus, although the possibility exists, we do not have direct evidence that the mutation rates at any of the loci studied here were affected by the mutator factor.

As mentioned above, the method of lost-line replacement frustrates attempts to detect interlocus differences in mutation rates. Nevertheless, it is of interest to determine whether there is any relationship between structural gene size and the mutation rates observed. The subunit molecular-weight estimates for the enzymes studied here are as follows: GOT-2: ca 50,000 (A. J. LEIGH BROWN, personal communication); α-GPDH: 32,000 (COLLIER, SULLIVAN and MACINTYRE 1976); cMDH: 30,000 (O'BRIEN 1973); ADH: 25,000 (SCHWARTZ *et al.* 1975); DIP-A: 60,000 (VOELKER and LANGLEY 1978); HEX-C: ca. 35,000 (A. J. LEIGH BROWN, personal communication); and α-AMY: 54,5000 (DOANE *et al.* 1975). The ranking according to subunit molecular weight is: *Dip-A*, *α-Amy*, *Got-2*, *Hex-C*, *α-Gpdh*, *cMdh*, *Adh*, while the mutability ranking is *α-Gpdh*, *Hex-C*, *cMdh*, *Got2*, *α-Amy*, *Dip-A* = *Adh*. There is no apparent correlation between structural gene size and higher mutability. Whether the mutabilities observed here reflect general properties of these loci or whether their behaviors are peculiar to a mutator factor is not known. Such could be the case, judging from the differential mutabilities of a number of visible loci in the lines screened in the present study (SCOBIE and SCHAFFER, in preparation), and the differential mutabilities of various *X*-chromosome visible loci in the presence of a similar mutator factor (GREEN 1977).

In summary, the evidence strongly suggests that, whatever factors may have influenced their origins, the mutants recovered here are point mutations of a base-substitution type. The most thoroughly analyzed nulls occurred at *α-Gpdh*, where none were associated with a visible cytogenetic aberration and all produced a protein sufficiently normal to form a mutant homodimer or a heterodimer with a normal protein. These properties make it unlikely that deficiencies or frameshifts are the types of mutants observed here.

The purpose for which the present study was initiated was to determine the spontaneous mutation rates at allozyme loci in order that reliable and valid estimates for that parameter be available for calculations of the dynamics of natural populations. It is ironic that a mutator factor was probably introduced into the present study when the *l(AW)* and *l(JH)* chromosomes, both from a wild-type cage population, were incorporated into the scheme so that mutation rates could be determined in chromosomes that, we hoped, would be reflective of the situation in nature. The results obtained in this study may accurately estimate the spontanious allozyme mutation rates in nature. However, inasmuch as there was a mutator factor known to be present, we lack total confidence that we have accurately estimated that parameter.

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