7. *Estimation of Spontaneous Mutation Rates at Enzyme Loci in Drosophila melanogaster*

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The present experiment was conducted to obtain a reliable estimate of the rates of null mutations and band-morph mutations at enzyme loci of *D. melanogaster*. In this report, any mutation by which bands in zymograms disappear is defined as a null mutation and any mutation by which the mobility of bands in zymograms is changed is defined as a bandmorph mutation. Previously, a similar experiment was conducted by Mukai and Cockerham (1977) using the second chromosome of *D. melanogaster*. After accumulating spontaneous mutations at 5 enzyme loci [sn glycerol-3-phosphate dehydrogenase (GPDH), malate dehydrogenase (MDH), alcohol dehydrogenase (ADH), hexokinase-C (HEX-C), and α-amylase (AMY)], the following mutation rates were estimated: null mutation rate: 1.03×10^{-5} (Mukai and Cockerham 1977) and bandmorph mutation rate: 1.28×10^{-6} per locus per generation (Voelker, Schaffer and Mukai 1980). Unfortunately, it was demonstrated that the experimental material carried *hobo* and *I* elements, which were later proved to induce chromosome breaks at a high rate (Yamaguchi and Mukai 1974). Thus, it is reasonable to assume that the observed null mutation rate was an overestimate. Therefore, we planned an equivalent experiment to that of Mukai and Cockerham (1977) using a strain of *D. melanogaster* which was at that time considered to be mutator-free.

Materials and method. Following the method described in Mukai and Cockerham (1977), the following accumulation of spontaneous mutation experiment was performed. Six stem chromosomes were used: three *In(2LR)SM1(Cy)* chromosomes and three second chromosomes carrying independent recessive lethal genes. The latter three chromosomes were isolated from an isogenic line originating from the Kaduna stock, [*l(A)* and *l(B)*] and from the Katsunuma line, [*l(C)*]. The *In(2LR)SM1(Cy)*-carrying chromosome will be abbreviated *Cy*. A single male heterozygous for *Cy* and *l(A)*, was mated to a single C-160 [$Cy/In(2LR)bw^{V1}$] female. The $In(2LR)bw^{V1}$-carrying chromosome will be abbreviated *Pm*. To establish the chromosome lines from the progeny, *Cy/l(A)* males and females were collected and as many single-pair matings *inter se* were made as possible. For generation one and subsequent generations, only *Cy/l(A)* heterozygotes phenotypically curly winged survived because *Cy/Cy* and *l(A)/l(A)* are lethal. The number of single-pair matings was increased until 500 lines were obtained. Each line was maintained by both single-pair mating and five-pair mating. Whenever the single-pair mating was successful, its offspring were used to make a single-pair mating and 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. Thus, it was possible to accumulate mutation in both the *Cy* and *l(A)* chromosomes under conditions of very low natural selection pressure without collecting virgin flies. Furthermore, this

method uncovers any contamination from external sources since such contamination would result in the appearance of phenotypically wild-type flies. Following the above procedure, two sets of 500 additional lines each were initiated using the other two *Cy* chromosomes and two different lethal-carrying chromosomes, *l(B)* and *l(C)*. To avoid the possible effect of the P-M hybrid dysgenesis (Kidwell, Kidwell and Sved 1977), the cytoplasm of the Katsunuma population was used in the *Cy/l(C)*×*Cy/l(C)* set. The last experiment was initiated later than the first two experiments after hybrid dysgenesis was acknowledged to be a common phenomenon. For our purposes, the experiments *l(A)*, *l(B)*, and *l(C)* chromosomes were called KA, KR and KC, respectively.

These lines were tested for the periodic occurrence of mutations. In this test, chromosomes on which mutations were accumulated are expressed as *Cy* and *l*. First, it should be mentioned that 7 enzyme loci on the second chromosomes were tested: in addition to the 5 loci described above, glutamate oxaloacetate transaminase (GOT) and dipeptidase (DIP) were tested. These 7 loci were homozygous in the *Cy/l*(•) heterozygotes. Mutations were detected by the following procedure. (1) A large number of *Pm*/tester flies were produced by mating *Cy*/tester with *Cy/Pm* flies. (2) *Pm*/tester flies were mated with *Cy/l* flies. (3) *Cy*/tester and *l*/tester flies were selected from the progeny of these crosses, and were screened for mutation at the 7 loci. Without new mutation these two types of flies are heterozygous for any of the 7 loci. Thus, null mutation and bandmorph mutations could easily be detected. The details of the experimental procedure will be published later.

Results and discussion. The results are shown in Table I. The most important finding is that no bandmorph mutations were detected although 1,855,473 allele • generation (considering that the AMY locus is duplicated) were accumulated and tested. However, 44 null mutations were found. From these results, the average rate of null mutations (μ_n) can be estimated to be 2.37×10^{-5} per locus per generation and the estimate of the bandmorph mutation rate (μ_B) is 0. The 95% confidence limits of the band-morph mutation rate and the null mutation rate are:

$$\mu_B<1.99\times10^{-6} \text{ and } 1.72\times10^{-5}<\mu_n<3.18\times10^{-5}$$

per locus per generation, respectively. Detailed analysis will be published later.

In the previous experiment, μ_n was estimated to be 1.03×10^{-5} per locus per generation (Mukai and Cockerham 1977). This estimate is significantly lower than the value obtained above. (The difference may be due to the difference in the number of active movable genetic elements in the genomes.) The present experiment was initiated assuming that the KA and KR second chromosomes did not carry mutators or movable genetic elements since the Kaduna laboratory population had been maintained for many years in the laboratory (Personal communication from Professor Brian Clarke). When the present experiment was begun, it was generally believed that stocks maintained in the laboratory for many years did not carry mutator factors, especially the *P* element. The KC experiment was conducted in the native cytoplasm in order to avoid the effects of hybrid dysgenesis, even if the *P* element did exist in the original population.

At the end of the experiment, two lines from each of the KA, KR and KC groups were examined by *in situ* hybridization. To our surprise, all lines carried *P*, *I* and *hobo* elements. It is questionable whether or not all of these elements were in an active state during the accumulation experiment, but at least some of them might be expected to have been active and have increased null mutation

Table I. The accumulation of spontaneous mutations in *Drosophila melanogaster*

	KA Series		KR Series		KC Series		Sum
Enzyme	Cy	*l*	Cy	*l*	Cy	*l*	
ADH	41698	42205	39429	39381	32522	33365	228600
	0	2	1	0	0	3	6
α GPDH	41477	42028	39052	39328	32306	33134	227325
	1	3	5	1	4	11	25
GOT	41746	42254	39383	39402	32658	33360	228803
	0	0	1	0	0	1	2
MDH	45071	45299	38276	38506	32060	33150	232362
	0	0	1	0	0	1	2
HEX	45379	45834	38974	38872	32145	33271	234475
	0	0	0	3	0	0	3
DIP	45531	45885	39000	38913	32051	33234	234614
	0	1	1	1	0	2	5
AMY	44109	44203	39203	38860	33655	34617	234647*
	0	1	0	0	0	0	1
Sum	305011	307708	273317	273262	227397	234131	1855473
	1	7	9	5	4	18	44

The figure in the upper part of each square indicates the number of allele · generations and figure in the lower part shows the number of null mutants recovered. * This figure should be multiplied by 2, considering that the AMY locus is duplicated.

rates. The reasons for the speculation of the induction of null mutations by movable genetic elements are as follows:

(1) In the KC series, the null mutation rate of *l*-carrying chromosomes is higher than that of *Cy* chromosomes ($\chi^2_{df=1}=8.51$, $P<0.01$) although in the pooled data of the KA and KR series this difference was not detected. These phenomena may be due to the difference in distribution of movable genetic elements among the lethal-carrying chromosomes.

(2) Null mutation rates are significantly different between the KA and KR series versus the KC series ($\chi^2_{df=2}=7.84$, $P<0.05$). This result may be explained by the difference in activity of movable genetic elements.

(3) There was strong heterogeneity in null mutation rates among loci investigated ($\chi^2_{df=6}=69.85$, P: very small). This may be partially dependent upon the size of the genes. For example, the structure of the *Gpdh* gene is complex (cf. Kusakabe *et al.* unpublished) and it appears that the length of DNA that corresponds to the control region of this gene is long. If some movable genetic

elements were inserted in this region as well as in the coding region, null mutations could be induced. It is expected that the null mutation rate at the *AMY* locus is low since it is duplicated.

(4) The estimation of mutation rates was conducted at four different times in the KA and KR sets but only three different times for the KC set. In the pooled data of KA and KR, heterogeneity in null mutation rates was detected over the periods of testing ($\chi^2_{df=3}=10.60$, $0.01<P<0.05$). Without relating this phenomenon to change in the activity of movable genetic elements with time, changes in null mutation rates with time is difficult to explain.

The bandmorph mutation rate estimated in the previous experiment was $\hat{\mu}_B=1.28\times10^{-6}$ per locus per generation (Voelker, Schaffer and Mukai 1980), which is larger than the estimate in the present experiment (0). This result is in contrast to the null mutation rates comparison (1.03×10^{-5} vs. 2.37×10^{-5} per locus per generation). Thus, it is likely that movable genetic elements do not affect the bandmorph mutation rate. Since there is no significant difference in bandmorph mutation rate between the present and previous estimates, they were pooled. In this pooling, the results at about generation 210 (Voelker, Schaffer and Mukai 1980) was employed and the amylase locus was counted as two loci in both *Cy* and *l*-carrying chromosomes. The resulting value is as follows:

$$\hat{\mu}_B=4/5{,}285{,}615=7.5677\times10^{-7} \quad (1)$$

The 95% confidence interval turns out to be

$2.06\times10^{-7}<\mu_B<1.94\times10^{-6}$ under the assumption of a Poison distribution.

From estimate (1), the spontaneous base-pair mutation rate per year can be estimated as follows. The detection rate by electrophoresis is assumed to be 1/3 (Shaw 1965). The average number of base pairs per coding region for 4 genes out of the 7 employed in the present experiment was estimated from the molecular weights of the proteins to be 973 (Mukai and Cockerham 1977). Thus, the number of base pairs per coding region of a single gene was assumed to be 1,000. Therefore, the base-pair mutation rate (μ_{bp}) becomes:

$\hat{\mu}_{bp}=3\times7.5684\times10^{-7}\times10^{-3}=2.27\times10^{-9}$/generation.

Hence, the mutation rate per year ($\mu_{bp(y)}$) which can be used in the studies of molecular evolution is

$\hat{\mu}_{bp(y)}=2.27\times10^{-9}\times10=2.27\times10^{-8}$.

This corresponds to the synonymous substitution rate (base pair per year) under the assumption of the Neutral Theory (Kimura 1968). This result agrees very well with the actual data for of *D. melanogaster* (Baba, Takano and Mukai, unpublished). A detailed discussion will be given in another paper.

In summary it can be said (1) that the estimate of the bandmorph mutation rate at the enzyme loci was 2.27×10^{-9} per base-pair per generation and that of the null mutation rate was 2.37×10^{-5} per locus per generation, and (2) that null mutations appear to be induced by some of the movable genetic elements.

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