A STUDY OF SPONTANEOUS MUTATION RATES AT TEN LOCI DETECTABLE BY STARCH GEL ELECTROPHORESIS IN *DROSOPHILA MELANOGASTER*[1]

YOSHIKO N. TOBARI[2] AND KEN-ICHI KOJIMA[3]

Department of Zoology, The University of Texas at Austin, Austin, Texas 78712

Manuscript received August 23, 1971
Revised copy received November 21, 1971

ABSTRACT

Spontaneous mutation rates at ten allozyme loci on chromosomes II and III of *Drosophila melanogaster* were studied. Over the three and a half years study, one α-GPD mutation and two different IDH mutations were obtained. The α-GPD mutation was inherited in the Mendelian fashion, as expected. The two IDH mutations were peculiar in that the band of new types appeared only in females. In males, only the original bands were stained, and the positions where mutant alleles' bands should be present were blank. Both IDH mutant homozygotes appeared as null allele homozygotes, while in females clear-cut single bands were present.——The rates of spontaneous mutation varied greatly. Eight loci studied (MDH, ADH, EST-6, APH, EST-C, ODH, XDH, AO) did not give any germ-line mutation. The average germ-line mutation rate over all ten loci was estimated as 4.5×10^{-6}. This rate is considerably smaller than that for sex-linked recessive visible mutations (MULLER, VALENCIA and VALENCIA 1950), but it is somewhat less than autosomal recessive visible mutations (GLASS and RITTERHOFF 1956).

EXTENSIVE survey studies of electrophoretically detectable allelic differences have been reported by the single fly assay technique of JOHNSON (1966) in several Drosophila species; HUBBY and LEWONTIN (1966) using *Drosophila pseudoobscura*; STONE *et al.* (1968) using *D. ananassae;* and KOJIMA, GILLESPIE and TOBARI (1970) using *D. melanogaster*, *D. simulans*, *D. affinis* and *D. athabasca*. In these studies, it became apparent that many loci were segregating for two to seven, or in some cases, more alleles. The pattern of allele distributions greatly varied from one locus to another. However, there were some loci where the mutation-selection balance mechanism might be the cause of maintaining multiple allelic situations, provided mutation occurs relatively frequently, say at the order of 10^{-4}, or even 10^{-5} (STONE *et al.* 1968). Thus, a study of mutation rate for this class of genes seemed to be a worthwhile task.

[1] This work was supported by AEC AT-(40-1)-3681 and USPHS Grant GM 15769 to K. Kojima at the University of Texas at Austin.

[2] Permanent address: Department of Biology, Tokyo Metropolitan University, Setagaya-ku, Tokyo, Japan.

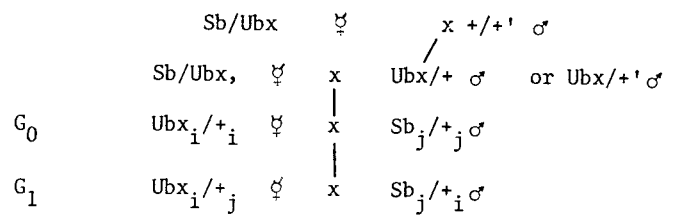
[3] Deceased November 14, 1971.

The passing of Dr. KOJIMA is a serious loss to the science of Genetics. His numerous papers, due to their originality and conceptual freshness have had a strong influence on the recent developments of population genetics, and will ensure him a lasting memory in the annals of our science. Editor.

MATERIALS AND METHODS

There were three series of studies, one using a homozygous line with respect to α-glycerophosphate dehydrogenase (α-GPD), malate dehydrogenase-1 (MDH), and alcohol dehydrogenase (ADH) on the Chromosome II; the second using a single third chromosome extracted from the Canton-S (Series A); and the third using a single third chromosome extracted from the Swedish-c stock (Series B). The loci studied in the last two series were isocitrate dehydrogenase (IDH), esterase-6 (EST-6), adult alkaline phosphatase (APH), esterase-C (EST-C), octanol dehydrogenase (ODH), xanthine dehydrogenase (XDH), and aldehyde oxidase (AO).

The method of extracting one chromosome III from Canton-S and that from Swedish-c was analogous to the *Cy/Pm* method (e.g. see WALLACE 1968, page 34). In the place of *Cy/Pm*, a third chromosome balancer line, Sb/Ubx^{130}, was used. To make sure of this procedure, the following diagram may be helpful. Hereafter the Ubx^{130} chromosome will be referred to by *Ubx* for the sake of simplicity.



where ♀ stands for virgin female.

It is important to note that $+_i$ and $+_j$ were one identical chromosome (either + or +') before generation G_0. However, after the G_0 generation, they will diverge gradually by accumulating mutations at an increasing number of loci. In any mating, the chromosome containing *Sb* was used in the male side only. To simplify the notations, these two chromosomes will be denoted as *Sb* and *Ubx*. One of the reasons for choosing these two chromosomes was that they contained the same alleles as those in Canton-S and Swedish-c with respect to all loci studied. This feature made detection of mutants (occuring as heterozygotes) much easier. The choice of multiple homozygous lines should also make easy to spot a potential contamination. For this reason, three potentially usable loci were not included.

For each line, three pair matings of the above type were made in three separate vials to avoid losing the lines. However, whenever loss inevitably occurred in existing vials of a given line, an extra vial of a successful cross (producing Ubx, Sb, + and Sb/Ubx phenotypes) was substituted. The above scheme of line maintenance is most efficient in that all detectable mutations on all four chromosomes ($+_i$, $+_j$, Sb_j, Ubx_i) can be identified for each line. An average of 101 lines were maintained through generation 28 for Series A and 152 lines for Series B. Thus, the total number of allelic generations per locus was $(28 \times 101 \times 4) = 11,312$ for Series A, whereas the corresponding number in Series B was $(28 \times 152 \times 4) = 17,024$.

After generation 28, the above method of maintaining Series A and Series B, in addition to the chromosome II study, became too laborious for the number of personnel available. Therefore, a simpler but less efficient method was introduced. (This method was similar to Mukai's line-maintaining method, MUKAI 1964). First, the third chromosomes of the original Canton-S and Swedish-c were replaced by those of the Sb/Ubx stock initially used. These two new sets of Sb/Ubx lines were mass-cultured in many replicates, and they were spaced in time so that numerous Sb/Ubx virgins could be collected at any time. The line-maintaining crosses were either *Sb/Ubx* ♀ × *Ubx/+* ♂ or *Sb/Ubx* ♀ × *Sb/+* ♂. This method allows easy maintenance of one + chromosome per line, per generation. This method was applied to Series A from generation 29 to 73, and to Series B from generation 29 to 74. The average number of lines maintained in Series A was 102 per generation, and the corresponding number in Series B was 143. The number of allelic generations per locus was 4,590 for Series A, and the corresponding number for Series B was 6,578. These numbers are actual counts which include some extra alleles

tested in sublines once in a while. Therefore, they are slightly over $102 \times 44 = 4488$ and $143 \times 45 = 6435$.

At generation 73 in Series A and generation 74 in Series B, the line maintenance method was changed to the previous system of $Ubx/+_i$ ♀ × $Sb/+_j$ ♂ for the last four generations before the termination of Series A and Series B. This switchback was accomplished by using the chromosomes in the lines maintained. From a Sb/Ubx ♀ × $Ubx/+_k$ ♂, three $Sb/+_k$ ♂ ♂ sons were saved, and from a Sb/Ubx ♀ × $Sb/+_h$ ♂, three $Ubx/+_h$ ♀ ♀ daughters were collected. As in the case of the previous diagram and the explanations accompanying it, chromosomes $+_k$ will diverge again. They were mated to make three $Ubx/+$ ♀ × $Sb/+$ ♂ lines. The numbers of allelic generations per locus in this stage were 8616 for Series A and 9832 for Series B.

Thus, the total number of allelic generations/locus was 24,518 for Series A and 33,434 for Series B. If the Series are combined, the total number of allelic generations/locus is 57,952.

The method of maintaining second chromosomes was quite different. From the single initial chromosome II extracted, about ten small mass cultures (essentially full- and half-sib matings) were initiated, and they were staggered in time to facilitate later collections. Whenever the availability of time and equipment was foreseen, one hundred virgin females and one hundred males were extracted from one or two of these mass cultures, and were pair mated. This set of 200 flies represented the check individuals, and this set was called generation G_0. Subsequent to egg laying, these individuals were assayed electrophoretically to insure the absence of electrophoretic variants at the three loci studied. (There were no variants in 33 such tests conducted over a three-year period, so that any variants occurring in the progeny generation G_1 could be assumed to be new mutants.)

From each of the 100 pair matings, five virgin females and five males were extracted, and were pair mated. After they produced progeny, all 1000 G_1 flies were assayed. If there were no variants, all progeny (the G_2 generation) were discarded. If variants were found in the G_1 generation, then G_2 flies of the variant parents were employed to conduct genetic tests. In 33 such tests, there was some variation in the number of lines tested. Consequently, the number of G_1 flies actually tested [according to the plan, the number is $(5 ♀ ♀ + 5 ♂ ♂) \times 100 \times 33$] was 44,040 instead of 33,000. Thus, the number of alleles tested was 88,080 per locus in the second chromosome study.

The three methods of maintaining genetic stocks described above had one important feature in common. All three methods allowed the distinction between genuine mutations and possible contaminants. The possibility of contamination was avoided by careful control of parental genotypes to be expected in each of the first two mating methods. For the third method, it was possible to rub out all but newly arising mutations by discarding any progeny whose parents expressed a mutant. No evidence of contamination was encountered in any of the three studies.

RESULTS

From the types of mutation rate studies described, three kinds of mutational phenomena may be observed: gametic mutation, somatic mutation, and germ-line cluster mutation. The first is characterized by an individual of original homozygous genotype which gives rise to a single mutant heterozygous progeny, which in turn shows more or less regular Mendelian segregation. The second is characterized by a homozygous individual which gives rise to a single mutant heterozygote progeny, which, in turn, gives progeny only of the original homozygous genotype. The third is characterized by a homozygous individual which gives rise to several mutant heterozygote progeny (from one mutational event in the reproductive process) each of which produces progeny in normal Mendelian ratios. One expects to detect this last class only in the chromosome II study. However, the net effetc of gametic mutation and germ-line cluster mutation are the same and should be counted as one mutational event.

In the entire study, one gametic mutation was found at the α-GPD locus and two at the IDH locus. The latter two are different electrophoretic mobilities. No gametic mutation was detected at the other eight loci. The α-GPD mutant behaved just as expected for a Mendelian mutant allele in subsequent tests. The α-GPD mutant allele was denoted as "*S*" as opposed to the original "*F*". This mutant is being kept in the heterozygous condition in the form of $Cy/+_s$, where the *Cy* chromosome carries an *F* allele and the chromosome carrying *S* is denoted as $+_s$. The heterozygotes show a three-banded pattern when the gel is run for a long period of time, indicating that the enzyme is normally a dimer.

The faster IDH mutant is denoted as *F*, and the other IDH mutant as *M*, because the allele in Swedish-c was the slowest in its allozyme's mobility (*S* allele). Thus, there are three alleles, *F*, *M*, and *S*, in the IDH study. As one might expect, *M* and *F* occurred in different lines. However, both IDH mutants exhibited strange behavior which baffled us for several months. Both mutants occurred in Swedish-c chromosomes (Series B), but their heterozygotes with the Swedish-c chromosome, *F/S* and *M/S*, showed only the slow band in males. In other words, the *M* and *F* bands *do not stain in males*. This peculiar sex-limited expression was discovered when the original line-maintaining method $Ubx/+_1 ♀ \times Sb/+_2 ♂$, was reinstituted. In one of the lines, $Ubx/+_2$ females and their $+_1/+_2$ sisters were all double banded, but none of their male sibs appeared to be double banded. Only the original S band appeared in males. When this particular *M* chromosome was made homozygous, the females were single banded at the *M* position, but the males did not stain at all or sometimes only as a faint streak line at the *M* position. That is to say, the males of mutant homozygotes looked as if they were null for this locus.

The *F* mutant was also sex limited as mentioned above. The females of *F/F* had a single band which migrated significantly more than the single band of *M/M* females. The *M/F* genotype in females appeared as a double band at the positions expected, but the males of the same genotype did not stain. The *F/S* females showed another strange phenomenon, namely a three-banded system. Among all the IDH genotypes, *F/S* females are the only ones with such a hybrid band. Thus, in summary, *F/F*, *M/M*, *S/S* females, and *F/S*, *M/S*, *S/S* males are single banded, all males showing only the *S* band; *F/M* and *M/S* females are double banded; *F/S* females are triple banded; *F/F*, *F/M* and *M/M* males show no bands.

All the zymograms of α-GPD and IDH mutant phenotypes are presented in Figure 1. The relative positions of the bands are approximately correct within each system.

Table 1-a shows the individual mutation rates for each locus and the average rate for the 10 loci. The latter is approximately 4.5×10^{-6}. However, it appears that the heterogeneity of mutation rates is considerable. At the IDH locus, the rate is 3.4×10^{-5}; at the α-GPD locus, the rate is 1.1×10^{-5}. Mutation rates at all other loci are so low that no mutation was detected in this study. The rate, 3.4×10^{-5}, is evenly divided into that for two different mutants, *F* and *M*. Thus, for a given type, the rate is 1.7×10^{-5}; this means that the mutation rate at the

TABLE 1

	Chromosome II			Chromosome III						
a: GERM-LINE MUTATIONS										
Loci	*α-GPD*	*ADH*	*MDH*	*IDH*	*EST-6*	*EST-C*	*ODH*	*XDH*	*APH*	*AO*
Mutations	1	0	0	2	0	0	0	0	0	0
Rates	1/88,080	0	0	2/57,952	0	0	0	0	0	0
Overall pooled rate = 3/669,904 = 4.5×10^{-6}										
b: BODY MOSAICS										
Loci	*α-GPD*	*ADH*	*MDH*	*IDH*	*EST-6*	*EST-C*	*ODH*	*XDH*	*APH*	*AO*
Mutations	0	0	0	0	0	1	1	0	3	0
Rates	0	0	0*	0	0	1/57,952	1/57,952	0	3/57,952	0

* 88,080 alleles per locus.

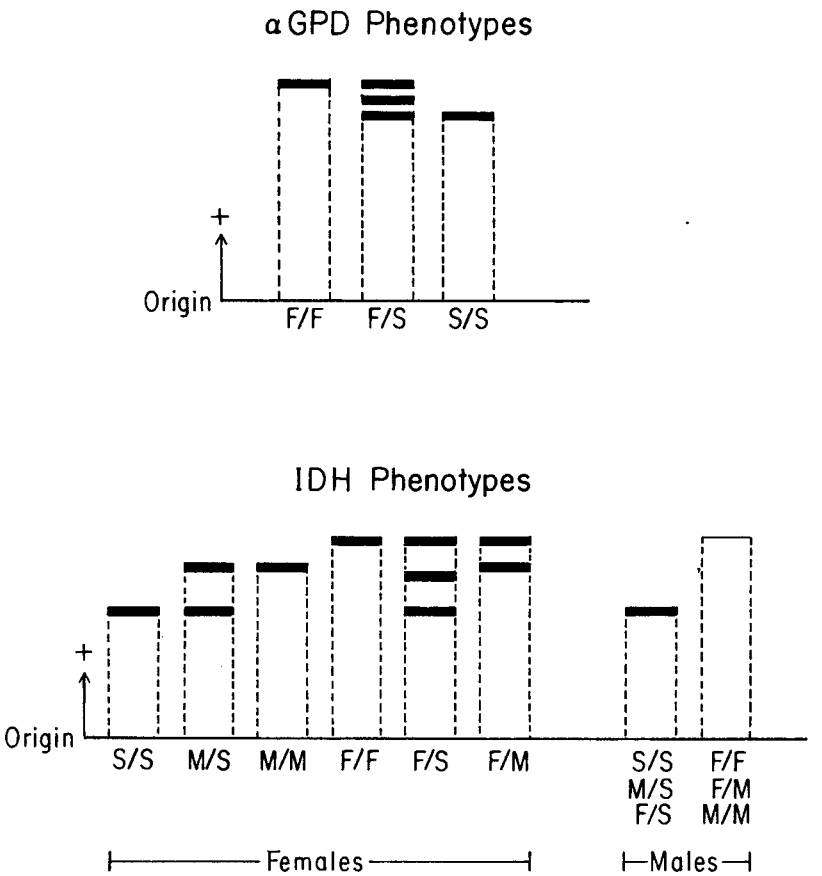


FIGURE 1.—Top: Zymograms of *F/F*, *F/S* and *S/S* at the α-GPD locus. The *S* allele is a mutant. Bottom: Zymograms of *S/S*, *M/S*, *M/M*, *F/F*, *F/S* and *F/M* in females and those in males. The *M* and *F* alleles are mutants.

IDH locus is approximately 1.5 times the rate at the α-GPD locus for a given type mutation.

Table 1-b gives the number of somatic body mosaics (the parent was unmistakably classified as a mutant heterozygote, but none of his or her progeny had the mutant alleles). The rate is considerably higher for this kind of "mutation." The Est-C body mosaic was double banded. The higher band appeared to be the one found in nature in low frequency. The APH mosaics (all 3 mosaics had different zymograms) were not identifiable with known *melanogaster* variants. In the case of body mosaics, only clear cut zymograms were counted so that the rate may be an underestimate. For example, there were a few cases of null-like mosaics at the APH locus and ODH locus. But being body mosaics, it was felt that they should be ignored from the count.

DISCUSSION

The discovery of *sex-limited* expression of IDH mutants in zymograms cannot be explained at this stage. Nevertheless, this is a real phenomenon. In an independent study of mutation induction by heat shock, PHILLIP SWARTZ, in KOJIMA's laboratory, has produced two IDH mutants which show the same peculiarity. A full account of his results will be published in the near future. At this point it is impossible to propose a solid explanation for this phenomenon.

MUKAI (1970) reported his preliminary mutation rate at the ADH locus. His figure was one mutant in about 8.6×10^4, while it is zero in about 8.8×10^4 in this study. However, his preliminary overall figure on three loci on the second chromosome is 4×10^{-6}, which is extremely similar to the overall estimate obtained in this study. Whether this is just a coincidence or not, cannot be decided at this time. Whenever such a small value as a mutation rate becomes a subject, it is very difficult to obtain a reliable figure even with a great amount of effort and time.

Finally, seven of the eight loci showing no mutation are usually polymorphic in nature, and mutation-selection balance, therefore, seems an unlikely mechanism for the maintenance of natural variants at these loci. Low frequency alleles at the α-GPD and IDH loci could be accounted for by the mutation-selection balance. However, no effort was made to identify new mutants of this study with naturally existing low frequency alleles.

The authors thank Mrs. M. WING, Mrs. M. WILSON, Mrs. G. PHILLIPS and Dr. S. L. HUANG for their technical assistance, and Dr. PETER SMOUSE for the preparation of the manuscript.

LITERATURE CITED

GLASS, B. and R. K. RITTERHOFF, 1956 Spontaneous mutation rates at specific loci in Drosophila males and females. Science **124**: 314–315.

HUBBY, J. L. and R. C. LEWONTIN, 1966 A molecular approach to the studies of genic heterozygosity in natural populations. I. The number of alleles in different loci in *Drosophila pseudoobscura*. Genetics **54**: 577–594.

JOHNSON, F. M., 1966 Rapid single fly homogenization for the investigation of Drosophila isozymes. Drosophila Inform. Serv. **41**: 193.

KOJIMA, K., J. GILLESPIE and Y. N. TOBARI, 1970 A profile of Drosophila species' enzymes assayed by electrophoresis. I. Number of alleles, heterozygosities, and linkage disequilibrium in glucose-metabolizing systems and some other enzymes. Biochem. Genet. **4**: 627–637.

MUKAI, T., 1964 The genetic structure of natural populations of *Drosophila melanogaster*. I. Spontaneous mutation rate of polygenes controlling viability. Genetics **50**: 1–19. ——, 1970 Spontaneous mutation rates of isozyme genes in *Drosophila melanogaster*. Drosophila Inform. Serv. **45**: 99.

MULLER, H. J., J. I. VALENCIA and R. M. VALENCIA, 1950 The frequency of spontaneous mutations at individual loci in Drosophila. Genetics **35**: 125–216.

STONE, W. S., M. R. WHEELER, F. M. JOHNSON and K. KOJIMA, 1968 Genetic variation in natural island populations of members of the *Drosophila nasuta* and *Drosophila ananassae* subgroups. Proc. Nat. Acad. Sci. **59**: 102–109.

WALLACE, B., 1968 *Topics in Population Genetics*. W. W. Norton Publishing Co. New York.