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1989年から，放射線影響研究所の業績報告書は，従来の日英両文を併記した方式では発行しない．主要な報告書については，今後も日英両文で印刷するが，それぞれ別に発行する．内容が高度に専門的であり，一般の関心が少ないと思われる報告書については英文のみとし，日本文の要約を添付する．

これにより，広島・長崎の原爆電離放射線被曝の人体に及ぼす晩発性生物学的影響に関する最近の知見を今までよりも速やかにお知らせできることと思う．

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キイロショウジョウバエの酵素座位における自然突然変異率の推定§

Estimating Spontaneous Mutation Rates at Enzyme Loci in *Drosophila melanogaster*

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要　約

本実験の目的は，キイロショウジョウバエを用いてヌル（電気泳動）型突然変異とバンド（電気泳動）型突然変異の信頼度の高い自然突然変異率及び両者の比を推定することである．

1本の致死第2染色体（*l* で略記）と1本の *In*（*2LR*）*SM1*，*Cy* 染色体（*Cy* で略記）を起源とする1雌×1雄交配系統を500育成し，各系統で1雌×1雄の交配を続け，自然突然変異を第2染色体に蓄積した．同時に交配の失敗に備えて同じ1雌×1雄の子孫から5雌×5雄の交配を行い，前者が不成功のときにのみこの子孫を次の世代の親として用いた．上と同じ実験区を別に2区作り，総計1,500系統に突然変異を蓄積した．適当な世代数が経過した後，各系統より1匹の *Cy*/*l* の雄をサンプルし，これを特定の標識遺伝子をもつ *Pm*/（標識遺伝子）雌に交配する．その子孫の（標識遺伝子）/*l* では目標の7酵素遺伝子座（*ADH*，*GPDH*，*GOT*，*MDH*，*HEX-C*，*DIP*，*AMY*）についてヘテロ接合になっているので，これらの7遺伝子座でバンド型ないしはヌル型突然変異が起これば，これを電気泳動法により発見できる．この方法により突然変異を蓄積し，自然突然変異率を推定した．

突然変異の蓄積世代数は最初の2実験区では約120～130世代，最後の区で約90世代であった．蓄積された総量は，1,620,826遺伝子座×世代である．4回（最初の2実験区）ないしは3回（第3の実験区）突然変異のテストが行われたが，バンド型突然変異は発見できなかった．最後のテストでは，44のヌル型突然変異が発見された．バンド型突然変異率（μ_B）の上限の95%信頼限界は 2.28×10^{-6}/（遺伝子座・世代）となる．ヌル型突然変異率（μ_n）は 2.71×10^{-5}/

§本報告にはこの要約以外に訳文はない．

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(遺伝子座・世代)となり，95％信頼限界は，$1.97 \times 10^{-5} < \mu_n < 3.64 \times 10^{-5}$／(遺伝子座・世代)となる．

以上の主要な発見のほかにヌル型突然変異について次の結果が得られた．1) 1区(KC:勝沼起源)において *l* 染色体でヌル型突然変異率が *Cy* 染色体よりも有意に高い値を示した．2) 3区間のヌル型突然変異率について有意差が発見された．3) ヌル型突然変異について，異なった7遺伝子座間に有意差が発見された．4) ヌル型突然変異率は時と共に増減したようである．

これらの事実は，ヌル型突然変異が主としてトランスポゾンによって誘発されたと考えればよく説明できる．事実，実験終了後すべての系統が *P*，*I*，*hobo* エレメントをもっていることが in situ hybridization 法によって明らかにされた．

本実験ではバンド型突然変異は発見できなかったが，本実験前の蓄積実験の結果と有意差がなかったので，これと本実験の結果をプールして $4/(4,732,424) = 8.4523 \times 10^{-7}$／(遺伝子座・世代数)となり，その95％信頼限界は，$2.30 \times 10^{-7} < \mu_B < 2.17 \times 10^{-6}$／(遺伝子座・世代)となった．ヌル型突然変異率とバンド型突然変異率の比は約30となるが，この比はトランスポゾンの種類と頻度により変化すると考えられる．

Technical Report Series

Estimating Spontaneous Mutation Rates at Enzyme Loci in *Drosophila melanogaster*[§]

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Summary

Spontaneous mutations were accumulated for 1,620,826 allele-generations on chromosomes that originated from six stem second chromosomes of *Drosophila melanogaster*. Only null-electromorph mutations were detected. Band-electromorph mutations were not found. The average rate of null-electromorph mutations was 2.71×10^{-5} per locus per generation. The 95% confidence interval (μ_n) was $1.97 \times 10^{-5} < \mu_n < 3.64 \times 10^{-5}$ per locus per generation. The upper 95% confidence limit of the band-electromorph mutation rate (μ_B) was 2.28×10^{-6} per locus per generation. It appeared that null mutations were induced by movable genetic elements and that the mutation rates were different from chromosome to chromosome.

Introduction

In this experiment, we hoped to reliably estimate the rates of null mutation and band-morph mutation and their ratio of these two mutation types at enzyme loci of *D. melanogaster*, in order to compare these data with those for humans that had been previously obtained at our institute. In this report, any mutation causing bands in zymograms to disappear is defined as a null-electromorph mutation and any mutation causing the mobility of bands in zymograms to change is defined as a band-electromorph mutation. The former term is abbreviated to null mutation and the latter to band-morph mutation.

In a preliminary experiment conducted by the principal investigator,[1] the second chromosome of *D. melanogaster* have been used. After accumulating spontaneous mutations in second chromosomes, mutations were screened at five enzyme loci: α-glycerol-3-phosphate dehydrogenase (*αGPDH*), malate dehydrogenase (*MDH*), alcohol dehydrogenase (*ADH*), hexokinase-C (*HEX-C*), and α-

[§]**The complete text of this report will not be available in Japanese.**

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amylase (*AMY*). The null mutation rate and band-morph mutation rate were estimated to be 1.03×10^{-5} and 1.81×10^{-6} per locus per generation, respectively, after accumulating mutations for 1,658,308 allele-generations. Unfortunately, test strains of flies contained a mutator which induced chromosome breaks, causing high lethal mutation rates.[2] This mutator was later demonstrated to consist of *hobo* and *I* elements.[3] So it was assumed that the observed null mutation rate was overestimated. Thus, the results obtained by Mukai and Cockerham[1] were not considered useful for estimating the ratio of null mutation to band-morph mutations. Therefore, we planned a similar experiment to that of Mukai and Cockerham[1] using a mutator-free strain of *D. melanogaster*.

Materials and Methods

Using the methods described in Mukai and Cockerham,[1] we accumulated spontaneous mutations. Four stem chromosomes were used: two *In(2LR)SM1(Cy)* chromosomes and two second chromosomes carrying independent recessive lethal genes, which were isolated from an isogenic line originating from the Kaduna stock, *l(A)* and *l(B)*. (The *In(2LR)SM1(Cy)*-carrying chromosome will be abbreviated as *Cy*.) A single male, which was a heterozygote 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 as *Pm*.) To establish chromosome lines from the progenies, *Cy*/*l(A)* males and females were collected and as many single-pair matings inter se were made as possible. For the first generation 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 a 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 mutations in both the *Cy* and *l(A)* chromosomes under conditions of very low natural selection pressure without collecting virgin flies. Furthermore, this method uncovered any contamination from external sources, since such contamination would result in the appearance of phenotypically wild-type flies. Following the above procedure, 500 additional lines were initiated using a single *Cy* chromosome and the other single lethal-carrying chromosome, *l(B)*.

During this experiment, the importance of the combination of cytotype and genotype for the operation of mutator factors[4] became clear. Unfortunately, the cytoplasm from the above two sets of experiments was of different origin. Thus, as a control, another set of experiments was initiated using the cytoplasm and chromosomes that originated from the Katsunuma population, following the same procedure as previously described. The *Cy* chromosome and *l(C)*-carrying second chromosome were employed.

To test for the periodic occurrence of mutations, the following procedure was followed. In this test, chromosomes on which mutations were accumulated are expressed as Ⓒy and Ⓛ. 1) A large number of *Pm*/tester flies were produced by mating *Cy*/tester flies with *Cy*/*Pm* flies. 2) *Pm*/tester flies were mated with Ⓒy/Ⓛ flies. 3) Ⓒy/tester flies and Ⓛ/tester flies were selected from the progeny of these crosses, and they were screened for mutations at the following seven loci employing starch gel and acrylamide gel electrophoreses: Glutamate oxaloacetate transaminase *(GOT)*, *αGPDH, MDH, ADH,* dipeptidase *(DLP), HEX-C,* and *AMY.*

The electrophoretic mobility genotypes of Ⓒy, Ⓛ, and tester chromosomes for these seven loci are as follows:

	Ⓒy	Ⓛ	*tester*
ADH	*F*	*F*	*S*
αGPDH	*F*	*F*	*S*
GOT	*S*	*S*	*F*
MDH	*S*	*S*	*F*
HEX-C	*S*	*S*	*F*
DIP	*F*	*F*	*S*
AMY	Amy^1	Amy^1	$Amy^{2,3}$

Thus, using this method, band-morph mutations, as well as null mutations, can be detected at the same time. Moreover, since the method produces large numbers of flies of the same genotype, we were easily able to confirm a new type of mutation by repeat testing.

In the course of the accumulation experiments, deleterious mutations accumulated in many lines and occasionally some lines were lost. In such cases, these missing lines were each replaced by a neighboring line number. These replacements were all recorded and the number of accumulated generations was counted considering these replacements.

For our purposes, the experiments using *l(A), l(B),* and *l(C)* chromosomes were called KA, KR, and KC, respectively. In the KA and KR series, tests for mutations were conducted four times. These four tests were initiated in 1983, in 1984, in 1984 or 1985, and in 1986 or 1987. The maximum number of accumulated generations was 127. Initiated in 1985, early in 1987, and late in 1987, testing for the KC series was performed three times. The maximum number of accumulated generations was 93. All experiments were terminated at the end of September 1988.

In scoring the accumulated generations and mutations, the following procedure was employed. When one line continued to completion, the results of the final test (the determination of mutant or original) and the generation number at

that time were used as the final result. When a line was lost but a replacement was not introduced before the final test, the result of the last test was used. When a replacement line was introduced, the results of the final experiment were used.

After the third test of KA and KR and after the second test of KC, many lines were lost in a laboratory accident. In this case, they were not replaced, and the previous test was considered to be the final test.

In each test, some lines could not be tested due to difficulty in obtaining appropriate materials.

Results

The final results are shown in Table 1. No band-morph mutations were detected, although 1,620,826 allele-generations were accumulated and tested. However, 44 null mutations were found. From these results, the 95% confidence limits of the band-morph mutation rate and the null mutation rate became $\mu_B < 2.28 \times 10^{-6}$ and $1.97 \times 10^{-5} < \mu_n < 3.64 \times 10^{-5}$ per locus per generation, respectively. A detailed analysis was made as follows:

Table 1. The accumulation of spontaneous mutations in *Drosophila melanogaster*

	KA series		KR series		KC series		
Enzyme	*Cy*	*l*	*Cy*	*l*	*Cy*	*l*	Total
ADH	41698 0	42205 2	39429 1	39381 0	32522 0	33365 3	228600 6
αGPDH	41477 1	42028 3	39052 5	39328 1	32306 4	33134 11	227325 25
GOT	41746 0	42254 0	39383 1	39402 0	32658 0	33360 1	228803 2
MDH	45071 0	45299 0	38276 1	38506 0	32060 0	33150 1	232362 2
HEX-C	45379 0	45834 0	38974 0	38872 3	32145 0	33271 0	234475 3
DIP	45531 0	45885 1	39000 1	38913 1	32051 0	33234 2	234614 5
AMY	44109 0	44203 1	39203 0	38860 0	33655 0	34617 0	234647 1
Total	305011 1	307708 7	273317 9	273262 5	227397 4	234131 18	1620826 44

For each enzyme the upper numbers are the allele-generations and the lower numbers are the null mutants recovered.

Mutation rates for *Cy* and *l* chromosomes

Each set has two types of chromosomes (*Cy* and *l*). If a mutator exists in either of the two types of chromosome, a difference may exist in their mutation rates. Thus, mutation rates for each chromosome type within each set, KA, KR, or KC, were analyzed. A significant difference between the two chromosome types was detected for KA and KC, the results of which are summarized in Table 2. Since *l*(KA) and *l*(KR) are from the Kaduna population, the results for the KA and KR sets were pooled, after which *l* and *Cy* chromosomes types were analyzed for heterogeneity in the null mutation rates. The heterogeneity $\chi^2_{df=1} = 0.1728$ was not significant ($0.6 < P < 0.7$). The rate was 1.7291×10^{-5} for the *Cy* chromosomes and 2.0655×10^{-5} for *l* chromosomes. Thus, it may be concluded that, especially in the KC set (using materials from the Katsunuma population), the null mutation rate was significantly higher in the *l* chromosomes than in the *Cy* chromosomes.

Table 2. Comparison of the null mutation rate between *Cy* and *l*-carrying chromosomes within experimental sets

Series	*Cy*	*l*	$\chi^2_{df=1}$	Total number of allele·generation	Pooled mutation rate
KA	0.3279×10^{-5}	2.2749×10^{-5}	4.45*	612719	1.3057×10^{-5}
KR	3.2929×10^{-5}	1.8297×10^{-5}	1.14	546579	2.5614
KC	1.7590×10^{-5}	7.6880×10^{-5}	8.51**	461528	4.7668

* Significant at the 5% level

** Significant at the 0.5% level

Null mutation rates among different sets of the experiment

The results for *l* and *Cy* chromosomes were pooled within sets and then were compared. First, the heterogeneity among KA, KR, and KC was tested. $\chi^2_{df=2}$ was 7.84, which was significant at the 5% level. The mutation rates of KA, KR, KC, and for the pooled results were 1.3056, 2.5614, 4.7668, and 2.7147×10^{-5} per locus per generation, respectively.

Comparing the null mutation rates of different loci

Although there is heterogeneity between the *Cy*- and *l*-carrying chromosomes with respect to null mutation rates, results for the two types of chromosome were pooled, and heterogeneity in null mutation rates was tested after pooling the results of all sets. Table 3 is a 2×7 contingency table for these pooled sets. The heterogeneity was $\chi^2_{df=69.85}$, which is highly significant. From Table 3, it can be seen that the null mutation rate was extremely high at the *αGPDH* locus (10.9975×10^{-5} per locus per generation) and extremely low at the *AMY* locus (0.4262×10^{-5} per locus per generation). The low rate could be expected because the *AMY* locus is duplicated.

Table 3. Heterogeneity test for null mutation rates among seven second chromosome loci of *D. melanogaster*

Phenotype	*ADH*	*αGPDH*	*GOT*	*MDH*	*HEX-C*	*DIP*	*AMY*	Total
+	228594	227300	228801	232360	234472	234609	234646	1620782
Null	6	25	2	2	3	5	1	44
Total	228600	227325	228803	232362	234475	234614	234647	1620826
Mutation rate ($\times 10^5$)	2.6247	10.9975	0.8741	0.8607	1.2795	2.1312	0.4262	2.7147

Does the null mutation rate change with time?

As described above, the null mutation rates were estimated at four different times for the KA and KR series and at three different times for the KC series. In each series, mutation rates were tested for heterogeneity with respect to the different times at which mutation rate estimates were made. These results were as follows:

$$\text{KA: } \chi^2_{df=3} = 8.47, \text{ KR: } \chi^2_{df=3} = 3.60, \text{ and KC: } \chi^2_{df=2} = 3.20$$

The value for KA was significant, and the differential mutation rate tendencies of KA and KR were similar to each other. For example, mutation rates of KA and KR were high in Period III and low in Period I. Thus, these two sets were pooled and tested. These results, shown in Table 4, indicate that the null mutation rates changed with time. It should be noted here that the total number of null mutants in Table 4 is not always the same as the components of the null mutant number. This is due to the fact that mutation accumulation lines were maintained by $Cy/l \times Cy/l$ crosses. From the progeny, a single *Cy* chromosome and a single *l*-carrying chromosome were sampled and tested. Even if one of the sampled progeny chromosomes carries a null mutation, this would not necessarily indicate that the null mutant would be fixed in *l*-carrying chromosomes or in *Cy* chromosomes of the line. When a null mutation was carried by one of the two *l*-carrying (of *Cy*) chromosomes of the parents ($Cy/n\ l \times Cy/l$), about 50% of the progeny received this mutation and the others were null mutation-free. Thus, it was possible to select a single null mutation-carrying individual for the test and two null mutation-free individuals for maintaining the line. In such a case, a null mutation-carrying line changed to a null mutation-free line, though this line was once classified as a line carrying a null mutation. Of course, when the line carrying a null mutation became extinct and its replacement was made by a null mutation-free line, the line became null mutation-free. However, it should be noted that the reverse of the above situation also may have occurred, although its probability would be expected to be very low.

Table 4. Test for heterogeneity in the null mutation rate among the tests conducted at different times (KA and KR series pooled)

	Period				
Phenotype	I	II	III	IV	Total
+	297496	214493	287673	359610	1159272
Null	2	3	13	8	26
Total	297498	214496	287686	359618	1159298
Mutation rate $\times 10^5$	0.6723	1.3986	4.5188	2.2246	2.2427

$\chi^2_{df=3} = 10.60$ $\quad 0.01 < P < 0.05$

Table 5. The number of three types of movable genetic elements (*P, I,* and *hobo*) in the KC, KA and KR genomes

Elements	*P*	*I*	*hobo*
KA	25–30	15–18	~30
KR	10	21–22	~50
KC	18–28	15	~15

Discussion

Movable genetic elements and the null mutation rate

The present experiment was initiated assuming that KA and KR second chromosomes did not carry mutators or movable genetic elements, since the Kaduna laboratory population had been maintained for many years (personal communication from Dr. Brian Clarke). When we started our experiment, it was generally believed that stocks maintained in the laboratory for many years did not carry mutator factors, especially the *P* element.

On 26 April 1989, we obtained the data presented in Table 5 after examining two lines from each of the KA, KR, and KC groups using the in situ hybridization method. To our surprise, all lines carried *P, I,* and *hobo* elements. It is questionable whether all of these elements were in an active state during the accumulation experiment, but at least some may have been active and may have increased the null mutation rates. On the basis of the above findings and related speculation, it is possible to explain the present experimental results as follows:

1) In the KC series, the null mutation rate of *l*-carrying chromosomes was higher than in the *Cy* chromosomes, although in the pooled data of the KA and KR series this difference was not detected. These phenomena could be due to the difference in distribution of movable genetic elements among the lethal-carrying chromosomes.

2) Null mutation rates differed significantly between the KA and KR series versus the KC series, possibly due to the difference in activity of movable genetic elements.

3) Strong heterogeneity in null mutation rates was observed among the loci investigated. This may have been partly dependent upon gene size. 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, null mutations could be induced. As stated above, it was expected that the null mutation rate at the *AMY* locus would be low since it is duplicated.

4) In the pooled data, heterogeneity in null mutation rates was detected during the testing periods. Without relating this phenomenon to change in the activity of movable genetic elements, changes over time in the null mutation rates are difficult to explain.

Estimates of null mutation rates and band-morph mutation rates

In the present experiment, the null mutation rate (μ_n) has been estimated to be 44/1,620,826 or 2.71×10^{-5} per locus per generation. The 95% confidence interval is as follows:

$$1.97 \times 10^{-5} < \mu_n < 3.64 \times 10^{-5} \quad .$$

In the previous experiment, μ_n was estimated to be 1.03×10^{-5} per locus per generation.[1] This estimate was significantly lower than the value obtained above, possibly due to different numbers of active movable genetic elements in the genomes.

Unfortunately, no band-morph mutations appeared. However, the upper 95% confidence limit can be calculated from the present experimental result. The estimate is $\mu_B < 2.28 \times 10^{-6}$ per locus per generation. Since the previous estimate[5] is included in the above limit, the 95% confidence interval on the basis of the pooled data due to 4,732,424 allele-generations was calculated to be $2.30 \times 10^{-7} < \mu_B < 2.17 \times 10^{-6}$ per locus per generation.

Here, it should be stated that movable genetic elements did not appear to increase the frequency of the base-pair changes. If this is the case, then this may be the best estimate of the band-morph mutation rate to date.

Ratio of the null mutation rate to the band-morph mutation rate

The null mutation rate was estimated to be 2.71×10^{-5} per locus per generation. By pooling the present result with that of the previous results,[5] the band-morph mutation rate was estimated to be 8.45×10^{-7} per locus per generation. The null-to-band morph ratio is therefore about 30. However, this estimate is probably not fixed, since the null mutation rate is greatly influenced by the number and type of movable genetic elements in the genome.

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