Increased detrimental load possibly caused by a transposon in a local population of
Drosophila melanogaster

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ABSTRACT
An extremely large amount of detrimental load was found in the Osaka population of D. melanogaster, although the frequency of lethal-carrying second chromosomes (Q) was not different from the average of Japanese populations (Q=0.199 and D : L=2.04). Tests for male recombination and sterility tests for the P–M dysgenic system indicated that there is a putative transposon (tentatively named MY factor) which induces non-lethal deleterious mutations at a rate about 140 times higher than that of recessive lethal mutations per second chromosome in the M cytoplasm, and the frequency of individuals carrying the M cytoplasm was estimated to be ca. 20%. Thus, it was concluded that the extremely large detrimental load was induced by the MY factors.

1. INTRODUCTION
After Demerec (1937) detected the mu-F gene in the Florida population, several researchers investigated so-called mutator factors (Neel 1942; Mampell 1946; Ives 1950; Hinton, Ives and Evans 1952). Especially, Mampell (1946) pointed out that virus may induce mutations and Hinton et al. (1952) demonstrated that the mutator factor discovered by Ives (1950) also induced chromosome aberrations.

After these studies, conspicuous investigations on this matter were not made for about two decades. Chigusa, Mettler and Mukai (1969) have predicted the existence of a kind of mutator in the Raleigh, N.C., population, which may not be geographically isolated to a great extent from the Florida population where the hi gene (Ives 1950) was originally discovered. This prediction was borne out by the detection of mutator factors both in the Raleigh, N.C. population (Cardellino and Mukai 1975) and in the Erie, Penn., population (Yamaguchi and Mukai 1974). These mutators break the chromosomes and, as a result, recessive lethal mutations, male recombinations and chromosome aberrations occur (Yamaguchi, Cardellino and Mukai 1976). It was Kidwell (1975) who pointed out that for the induction of mutations or

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some genetic aberrations, some type of cytoplasm that interacts with the
genotype is required. Thus, Kidwell and Kidwell (1976) gave the name of
"hybrid dysgenesis" to the syndrome induced by this genotype-cytoplasm
interaction. It is most likely that in the previous experiments, the condition
for cytoplasm was satisfied, namely, all the investigations were conducted in
the M type cytoplasm (cf. Kidwell, Kidwell and Sved 1977), and that the
mutator factors would have been some types of movable genetic elements or
transposons, although they were not understood in this way when they were
studied.

In the genetic load analysis, in earlier years, the cytoplasm of multiple
inversion carrying stock (Cy/Pm stock) appears to have been of the M type.
Nevertheless, abnormal genetic loads have not been detected (cf. Greenberg
and Crow 1960). This may suggest that the frequency of active transposons
was low in the populations in earlier years (cf. Greenberg and Crow 1960;
Oshima and Kitagawa 1961), and that recently it has rapidly increased (cf.
Kidwell 1983).

In 1979, an unusually large magnitude of genetic load was detected in the
Osaka population of Drosophila melanogaster. This increase in genetic load
was found to be due to the detrimental load. In order to confirm this finding,
the experiment was repeated in 1981 and a similar result was obtained.
Assuming that these abnormally large genetic loads were caused by some
mutator factors (Cardellino and Mukai 1975), syndrome of the mutator factors
described above and sterility in specific F1 hybrids (cf. Kidwell, Kidwell and
Sved 1977) were investigated in the present studies. If the individuals of
both the P and M types exist in the Osaka population, mutations occur at
high rates and, as a result, the genetic load becomes larger. However, it is
possible that this extremely large genetic load is spurious, namely, detrimental
mutations occurred if the M type (female) × P type (male) crosses were made
in the process of extracting these second chromosomes. The purpose of the
present studies was to clarify whether the large amount of detrimental load
actually existed in the Osaka population or if it was a spurious phenomenon
that originated in the process of the isolation and maintenance of chromo-
some lines, and it was concluded that some new type of transposon-like ele-
ment has existed in the Osaka natural population and they have caused a
large amount of detrimental load in the original population. The results will
be described in the present paper.

2. MATERIALS AND METHODS

The stocks: The following stocks were employed in the present studies:
OS-781F: These are the isofemale lines collected at Komagatani, Habikino
City, Osaka prefecture in October 1978 by S. I. Chigusa and maintained at
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18°C in the Laboratory of Population Genetics, Kyushu University.

C-160: $Cy/Pm$ ($Cy$: Curly wings, included in $In(2LR)SM1$ and $Pm$: Plum eyes carried by $In(2LR)bw^r$). This strain is a balanced lethal strain (see Mukai 1964).

cn $bw$: A standard karyotype line whose second chromosomes carry two recessive eye color mutants, $cn$ (cinnabar, 2-57.5) and $bw$ (brown, 2-104.5).

dp $cn$ $bw$: A standard karyotype second chromosome line with three recessive mutants, $dp$ (dumpy wings, 2-13.0), $cn$ and $bw$.

$\pi$: A typical P strain, which was sent to us by Professor J. F. Crow at the University of Wisconsin, U.S.A.

Canton S: A standard laboratory wild-type stock known to be the typical M strain (from Professor J. F. Crow).

**Extraction of second chromosomes and estimation of the genetic load:** One male from each isofemale line in OS-781F was crossed with five $Cy/Pm$ females and a single $F_1$ male was crossed with five $Cy/Pm$ females. In the next generation, $Cy/+$ females and males were collected and the crosses were made between them to establish the chromosome lines. The subscript $i$ indicates line number. Thus, the wild-type chromosomes that originated from a single second chromosome were balanced with the $In(2LR)SM1$ ($Cy$) chromosomes and were maintained at 18°C as a second chromosome line.

Two hundred and seventy-six independent second chromosomes were extracted from OS-781F in 1979 and 172 in 1981.

Homozygote and heterozygote viabilities were estimated by the $Cy$ method of Wallace (1956). Crosses between five $Cy/+i$ females and five $Cy/+i$ males were made with two simultaneous replications in each chromosome line in order to estimate homozygote viabilities. The viabilities of random heterozygotes were estimated in a way similar to the above, combining two successively numbered lines, i.e., $5 Cy/+i \times 5 Cy/+i+1$, to secure random combination of different chromosome lines. The last numbered line was crossed with the first numbered one. In both cases, four days after the crosses were made, all ten flies in a vial were transferred to a second vial, and after 4 days, all flies were discarded from the vial. In both vials, the first and second cultures, all emerged flies were counted at four different times to the eighteenth day after the crosses or transfers were made. $Cy$ and wild-type flies segregated in the expected ratio of 2:1. The viability is expressed as follows (Haldane 1956):

$$v = \frac{2[\text{number of wild-type flies}]}{[\text{number of } Cy \text{ flies}]+1}$$

Crosses were made at seven different times (seven sets) in 1979, and at three (three sets) in 1981.

Genetic loads were estimated using the method of Greenberg and Crow
(1960) as follows: Let A, B and C be the average viabilities of random heterozygotes, all homozygotes and non-lethal homozygotes, respectively. The total load (T) with respect to the average of the random mating population, detrimental load (D) and lethal load (L) can be estimated as follows under the assumption of the multiplicative gene action:

\[
T = \ln A - \ln B \\
D = \ln A - \ln C \\
L = \ln C - \ln B
\]

*Cytological observation*: Cytological examination of the salivary gland chromosomes was made for each chromosome line after making the cross with [cn bw]. In the present paper, the brackets indicate the phenotype within them. Salivary gland chromosomes of five cn bw/+ + larvae in the progenies were observed by staining with 1% acetic-lactic orcein.

*Test for male recombination*: Occurrence of male recombinaton was tested for isofemale lines and two of the isogenic lines (Lines 113 and 130 which were later called WY-113 and WY-130, respectively).

1. *Isofemale lines*: A single phenotypically wild-type male was sampled from each isofemale line and was crossed with several [dp cn bw] females. A single dp cn bw/+ + + male progeny that originated from each isofemale line was crossed with 5 [dp cn bw] females. Five phenotypically wild-type male progenies from this cross were crossed with 5 [dp cn bw] females. In the next generation, the occurrence of recombination in the males of the previous generation was examined.

2. *Isogenic lines*: A few Cy/+ males from each of WY-113 and WY-130 were sampled and they were crossed with 5 [dp cn bw] females. The dp cn bw/+ + + male progenies were individually crossed with 5 [dp cn bw] females and in the progenies the occurrence of recombinations in the males of the previous generation was tested.

*Test for gonadal dysgenic (GD) sterility of F1 hybrids*: Canton S and πs are typical strains of the M type and P type, respectively for both cytotype (Engels 1979) and genotype (Engels and Preston 1979). Thus, these two strains were used as the standard lines. The cytotype of an unknown strain, U, can be classified by testing the sterility of the progenies of the following crosses at 29°C:

Cross A*: U(1♀) × πs(♀ ♀)  
Cross B*: πs(♀ ♂) × U(1♀)

If the progenies of Cross A* and Cross B* are both fertile, the cytotype of
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the U strain can be classified as the P type, regardless of whether or not this strain carries the P factor. On the other hand, if the progenies of Cross A* are sterile, but those of Cross B* are fertile, the cytotype of the unknown strain can be classified as the M type.

The genotype of an unknown strain, U, can be classified by testing the sterility of the progenies of the following crosses at 29°C:

Cross A: Canton S(♀ ♂) × U(♀)
Cross B: U(♀) × Canton S(♂ ♂)

If the progenies of Cross A and Cross B are fertile, the genotype of the U strain can be classified as follows: If its cytotype is classified as M by Cross A* and Cross B*, the genotype of this strain is classified as M, while if its cytotype is classified as P, its genotype is classified as the Q type (cf, Kidwell 1979), regardless of whether or not that strain carries derivatives of the P factor.

If the progenies of Cross A are sterile, but those of Cross B are fertile, the genotype of the U strain is classified as the P type, regardless of whether or not this strain carries the P factor. Thus, it should be pointed out that the individuals of the P type do not necessarily carry the P factors. The classification is entirely phenomenological.

The material basis of the above classification of genotype and cytotype is as follows: It is hypothesized that, in the M type cytoplasm there is no inhibitor or regulator of transposase which is supposed to be encoded by the P factors (O'Hare and Rubin 1983). Thus, we are going to test in Cross A* and Cross B* whether or not the cytotype of the U strain carries the inhibitor or regulator produced by the P factors or any equivalent transposon. In Cross A and Cross B, we are going to test whether or not the genotype of the U strain produces some type of hypothetical transposase (cf. O'Hare and Rubin 1983).

The classification is necessary now that many types of transposon-like elements are being detected in natural populations of D. melanogaster (Finnegan et al. 1978; Rubin, Kidwell and Bingham 1982; Potter et al. 1980; Dawid et al. 1981; Di Nocera and Dawid 1983; McGinnis, Shermoen and Beckendorf 1983; Mukai and Yukuhiro 1983). The above crosses were made as follows: Parents were placed in vials and kept at 29°C for seven days, an interval that includes the entire temperature-sensitive period in the progeny for sterility (Engels and Preston 1979). Then, they were removed and all progenies that emerged by the eleventh day after making the cross were transferred to fresh vials with pieces of paper towel at 25°C. After four more days, each female to be tested was placed in a vial or a single cell of a 96-cell tissue culture plate described by Engels and Preston (1979). After three days, each female was
scored as either sterile if no eggs were produced or fertile if one or more eggs were produced.

Accumulation of mutations and estimation of genetic load: This experiment was performed in order to examine whether deleterious mutations frequently occur or not when the second chromosomes from the Osaka population combine with the M cytotype for some generations.

(1) Line used: Isogenic line WY–113 was used in this experiment. Originally, this line was of the standard karyotype, but when the salivary gland chromosomes were examined for this line sometime after establishment, a unique inversion was segregating. Thus, it is expected that some kind of mutator or transposon-like element is carried by this line. Therefore, WY–113 was used for the accumulation experiment of mutations. This line classified as Q in the experiment described below was used.

(2) Accumulation of mutations: The method similar to Mukai (1964) and Mukai, Chigusa, Mettler and Crow (1972) was used in order to accumulate mutations. A single Cy/+ male from the original isogenic line WY–113 was sampled and multiplied by making a cross with Cy/Pm females. In the next generation, Cy/+ or Pm/+ males were individually crossed with Cy/Pm females and 50 second chromosome lines were established as Cy/Pm (5♀♀) × Cy/+, or Pm/+, (1♂). In the next generation, each chromosome line was duplicated and 100 lines of Cy/Pm × Cy/+, or Pm/+, (i=1–100) were established. In the following generations, the Cy/Pm (5♀♀) × Cy/+, or Pm/+, (1♂) cross was continued. For insurance, Cy/Pm (5♀♀) × Cy/+, or Pm/+, (5♂♂) cross was also made, and it was used when the above single male cross was not successful. By this mating scheme, spontaneous mutations were accumulated on these second chromosomes for five generations. After the accumulation of mutations, Cy/+, male and female flies were replicated by making crosses with each other en masse. After that, homozygous viability of each chromosome line was estimated in the same way as above. The accumulation of mutations was made at 25°C.

The control experiment was conducted as follows: During the mutation accumulation, the original line (WY–113) had been maintained in a mass culture at 18°C in order to decrease the spontaneous mutation rate, and to eliminate new spontaneous mutations. At the same time as the estimation of viabilities of experimental lines, WY–113 was expanded to 10 sublines. By making crosses between sublines (i.e., 1 × 2, 2 × 3, etc.), the control experiment was conducted in the same way as above. The mean viability of these 10 crosses was used as the control.
3. RESULTS

*Frequency of lethal-carrying chromosomes and genetic load:* Two hundred and seventy-six second chromosomes were extracted from OS-781F in 1979 and their viabilities were estimated. The distribution pattern of homozygote viability relative to the mean viability of random heterozygotes is graphically presented in Fig. 1. The frequency of lethal-carrying chromosomes was $55/276$ or $0.199\pm0.024$. The estimates of total load ($T$), detrimental load ($D$) and lethal load ($L$) are as follows:

$$
T = \ln A - \ln B = 0.656 \\
D = \ln A - \ln C = 0.440 \\
L = \ln C - \ln B = 0.216
$$

where $A = 1.0000 \pm 0.0100$, $B = 0.5192 \pm 0.0205$ and $C = 0.6441 \pm 0.0177$. The estimate of $L$ is close to the average of the previous estimates, but the $D$ value is large. Thus, the $D:L$ ratio turned out to be 2.04 (see Table 1). This estimate is much larger than the estimates of other populations (see Simmons and Crow 1977). For reference, the $D:L$ ratios of other populations are: 0.667 in the Raleigh (Mukai and Yamaguchi 1974) and 0.895 in the Florida populations (Mukai and Nagano 1983). In 1981, 172 second chromosomes were extracted from the same isofemale lines, OS-781F, and the viabilities were estimated in order to confirm the above results. The distribution pattern of viabilities relative to the mean heterozygote viability is shown in Fig. 1 together with the result of the first experiment. There is no apparent difference

![Fig. 1. Distribution patterns of homozygote and heterozygote viabilities of the Osaka and Raleigh populations. The mean viabilities of heterozygotes are assumed to be 1 in respective populations. * From MUKAI and YAMAGUCHI (1974).](image-url)
between the results of the two experiments. The estimated T, D and L are 0.646, 0.472 and 0.174, respectively. The D : L ratio is 2.72 (see Table 1). For the sake of comparison, the distribution pattern of homozygote viabilities in the Raleigh population (Mukai and Yamaguchi 1974) was shown in this Figure. The frequency of lethal-carrying chromosomes was 28/172 or 0.163±0.028. There is no significant difference between the two estimates of the frequency of lethal-carrying chromosomes ($\chi^2_{df=1}=3.52$, 0.05<P<0.10).

The frequencies of unique inversion-carrying second chromosomes were 7/276 or 0.0254±0.0095 in 1979, and 6/145 or 0.0414±0.017 in 1981. There is no significant difference between these values ($\chi^2_{df=1}=0.8149$, 0.30<P<0.40). These frequencies are similar to those of Mukai and Yamaguchi (1974) and Watanabe, Yamaguchi and Mukai (1976).

In order to test whether or not some mutator factors caused the above abnormally large detrimental load, the manifestation of syndrome of hybrid dysgenesis induced by the mutator factors was investigated. We do not know at present whether or not our “mutator” is working in the natural population, but, definitely, the P-M system works at least after the first crosses in the laboratory as long as the flies in the natural population are of the P type since the cytotype of C-160 is M. Although we tested the abnormality induced by the mutator factors in the M cytotype under the assumption that they are activated in the M cytoplasm in the same way as the P factor, it does not always mean that our mutator factors are not at work in the natural populations since there may be the M cytotype in natural populations in contrast to the result of Engels and Preston (1980).

Test for male recombination:

(1) Isofemale lines: In this experiment, we examined whether or not male recombination occurs in 78 independent second chromosomes of OS-78IF. Among them, 46 second chromosomes (46/78 or 0.590±0.056) showed male recombination under the criterion that any recombinant fly emerged in the crosses. On the average, 1888.9±504.4 (±SD) offspring individuals per cross were counted in the crosses in which male recombination did not occur. The

<table>
<thead>
<tr>
<th>Year</th>
<th>Total load (T)</th>
<th>Detrimental load (D)</th>
<th>Lethal Load (L)</th>
<th>D : L</th>
</tr>
</thead>
<tbody>
<tr>
<td>1979</td>
<td>0.656</td>
<td>0.440</td>
<td>0.216</td>
<td>2.037</td>
</tr>
<tr>
<td>1981</td>
<td>0.646</td>
<td>0.473</td>
<td>0.174</td>
<td>2.721</td>
</tr>
<tr>
<td>Accumulation experiment (5 generations)</td>
<td>1.237</td>
<td>1.154</td>
<td>0.082</td>
<td>14.031</td>
</tr>
</tbody>
</table>

Table 1. The estimates of genetic loads
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figure after ± indicates the standard deviation (but not the standard deviation of the mean). If the number of flies counted increases, definitely the frequency of chromosomes in which male recombination occurs increases. Therefore, 0.590 is a kind of minimum estimate.

A single chromosome isolated from each of OS-78IF-209 and OS-78IF-224 showed a high frequency of male recombination. Thus, we continued to count these two lines in order to detect recombinants. In OS-78IF-209, the frequency of chromosomes in which at least one male recombination occurred was 1.19% and that in OS-78IF-224 was 0.70%. These frequencies are similar to those reported by Woodruff and Thompson (1977).

(2) Isogenic lines: WY-113 and WY-130 in which unique inversions were detected were examined as to whether or not male recombination occurred in these lines. In WY-113, a total of 26,746 second chromosomes were tested. Only 4 recombinant chromosomes (2(dp), 1(dp cn) and 1(bw)) were detected. The frequency is only 0.015%. In WY-130, 15,772 second chromosomes were examined. Only two recombinant chromosomes were found (1[cn bw] and 1[bw]). The frequency is 0.013%, a very low rate. These two estimates are much lower than the average rate of male recombination estimated above. Thus, the occurrence rate of unique inversions is not necessarily correlated with the male recombination rate.

As a control, Canton S, which is known to carry a typical M type chromosome, was tested. Surprisingly, 3 chromosomes (3(dp)) out of 23,762 were recombinants and the frequency was 0.012% (see Sved et al. 1978).

Test for gonadal dysgenic (GD) sterility: Two experiments were conducted. In Experiment 1, the test was performed using 2.5 x 10 cm vials with cornmeal-molasses medium, while in Experiment 2, 96-cell tissue culture plates were used to test the sterility of daughters of male or female parents from iso-female lines.

(1) Experiment 1: In Cross A* and Cross B*, 30 iso-female lines were employed. One to 3 females (Cross A*) or males (Cross B*) from each iso-female line were employed to cross individually with π, males or females. Eight to 15 daughters were tested from a single parental female of any iso-female line to determine her cytotype. Cross B* was made as the control.

When all the results of Cross A* and Cross B* were respectively pooled, the frequency of the completely sterile daughters in Cross A* was 103/1100 or 0.0936 ± 0.0088, significantly higher than that of Cross B* (3/943 or 0.0032 ± 0.0018) (χ²(1) = 84.5, P < 0.0005). Throughout the experiments reported in this paper, when more than 20% of the F1 daughters were sterile in Cross A* and Cross A, their parents in question were defined as “potentially sterile” because the frequency of sterile daughters of any tested parent did not exceed 20% in Cross B* and Cross B (or in the control cross).
On the basis of this "20% criterion", 12 out of 72 in Cross A* and 0 out of 67 in Cross B* were "potentially sterile" on a individual parental female or male basis. The results are shown in Fig. 2(A). Thus, the cytotypes of the 30 isofemale lines were decided as follows: P type: 22/30; M type: 2/30; and coexistence of the P and M types: 6/30.

In the determination of the genotypes, Cross A and Cross B were made as follows. Thirty isofemale lines were chosen at first. These lines are the same as those employed in Crosses A* and B*. In Cross A, 2 crosses were not successful. Thus, 28 isofemale lines were used. In these crosses, 2 to 3 females (Cross B) or males (Cross A) per isofemale line were used. From each cross, 8 to 15 daughters were sampled and their sterility was individually tested as above.

![Fig. 2. Distribution patterns of fraction of sterile daughters (Experiment 1).](image-url)

(A) The results of the test for cytotype.
(B) The results of the test for genotype.
When all the results of Cross A and Cross B were respectively pooled, the frequency of the completely sterile daughters in Cross A was 21/1032 or 0.0203±0.0044, significantly higher than that of Cross B (7/1186 or 0.0059±0.0022) (χ² f=1=9.23, P<0.005). On an individual female or male basis, 2 out of 72 were “potentially sterile” in Cross A but none out of 84 was “potentially sterile” in Cross B. The results are presented in Fig. 2(B). From these results, the following was concluded: Twenty-six out of 28 were determined as Q or M since the progenies of Crosses A and B are both fertile. In 2 out of 28 iso-female lines, individuals of the P and Q types were segregating, since in each of the 2 lines the F₁ progenies of some parental males were classified as sterile but those of the others were classified as fertile.

Since the genotypes of the 2 lines described above were not able to be tested, the results of Cross A* and Cross A for the 28 isofemale lines were tabulated together in Table 2(A). From this table, it can be concluded that 18 out of 28 lines were the Q type; in 6 lines Q and M type flies coexisted; in 2 lines, P and Q type flies were segregating; 2 lines were M type.

(2) Experiment 2: The experiment was initiated with 50 isofemale lines. Although the test was completed for all lines in the determination of cytotype, it was not successful in one line for the determination of genotype. The number of parental males or females for a single isofemale line was 1 to 3, and the number of F₁ daughters tested for sterility was 9 to 16 per cross in both the crosses for the determination of cytotypes and genotypes.

In the determination of cytotype, when all the results of Cross A* and Cross B* were respectively pooled, the frequency of the completely sterile daughters in Cross A* was 127/2156 or 0.0589±0.0051. On the other hand, that in Cross B* was 1/2133 or 0.00047±0.00047. The difference between these two estimates is highly significant (χ² f₁=1=130.3, P<0.0005).

On an individual parental female or male basis, 12 out of 137 were “potentially sterile” in Cross A* using the “20% criterion”, but none of 137 was “potentially sterile” in Cross B*. Thus, 43 isofemale lines out of 50 carried the P cytotype; in 6 lines, P and M coexisted and only one line carried the M cytotype.

In the determination of genotype, when all the results of Cross A and Cross B were respectively pooled, the frequency of the completely sterile daughters in Cross A was 9/2250 or 0.0040±0.0013, while that in Cross B was 3/2213 or 0.0014±0.00078. The difference between these frequencies is not statistically significant (χ² f₁=1=2.90, 0.05<P<0.10), although the P value is close to 0.05. On an individual parental female or male basis, all tested lines (49) are fertile on the basis of the “20% criterion”. From these experimental results, it was concluded that all 49 isofemale lines tested were of the Q or M type, and no P type was detected.

Because the genotype of 1 line could not be tested, the results of Cross A*
and Cross A were presented together in Table 2(B). From this table, it can be concluded that 42 out of 49 isofemale lines were the Q type, and 1 isofemale line was the M type. In 6 isofemale lines, the Q and M type flies coexisted.

(3) Analysis of the pooled results of Experiments 1 and 2: The pooled results of Experiments 1 and 2 were also presented in Table 2(C). From this table, there are 4 types of genotype: (1) Q, (2) coexistence of P and Q, (3) coexistence of Q and M and (4) M. In order to estimate the frequency of individuals with the M cytotype when captured, the following assumptions were made: In the natural population, (1) random mating has occurred; (2) the individuals of the P and Q genotypes always have the P cytotype (Engels
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Table 3. Estimation of the frequency of individuals with the M cytotype

<table>
<thead>
<tr>
<th>Freq.</th>
<th>Offspring of isofemale l.</th>
<th>Isofemale l. when tested</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cytotype</td>
<td>Genotype</td>
</tr>
<tr>
<td>(1 - m)</td>
<td>P</td>
<td>P/P</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>P/Q</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>P/M</td>
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<tr>
<td></td>
<td>P</td>
<td>Q/P</td>
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<td></td>
<td>P</td>
<td>Q/M</td>
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<td></td>
<td>P</td>
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<tr>
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<td>M</td>
<td>M/P</td>
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<tr>
<td></td>
<td>M</td>
<td>M/Q</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>M/M</td>
</tr>
</tbody>
</table>

In addition, using the experimental data, the relationship described in Table 3 may be assumed for genotypes and cytotypes of the individuals at the times of establishment of the isofemale line and those at the time of the testing. Since no isofemale line carried the P genotype, it is possible to partition them into originally P cytotype-carrying group and M cytotype-carrying group. The latter can be separated into two groups: the M genotype-carrying group (its frequency is \(m^2\)), and the other (its frequency is \(m - m^2\)). Thus, the following logarithm likelihood expression can be obtained:

\[ L = \ln (1 - m)^2 + \ln (m - m^2)^2 + \ln (m^2)^3 \]

From \(dL/dm = 0\), \(m\) can be estimated to be 18/92 or 0.196 ± 0.050, where the standard error of \(\hat{m}\) was estimated by \(- (d^2L/dm^2)^{-1}\) (Mather 1951). Thus, it may be concluded that, in the Osaka population, at least 10% of the individuals were of the M cytotype. If in all or some strains of the Q type in the Osaka population, non-lethal deleterious mutations could be induced by the genotype-cytoplasm interaction much more frequently than lethal mutations in the M cytotype in terms of D : L ratio (cf. Simmons et al. 1980), then the large amount of D value and a large D : L ratio in that population could be explained. In order to examine this hypothesis, accumulation of mutations and the estimation of genetic load were made,
Accumulation of mutations and estimation of homozygous genetic load:
Spontaneous mutations were accumulated on the derivatives of the second chromosome (Q type) of WY-113 (Mukai and Yukuhiro 1983) in the cytoplasm of C160 (M type). Since the genotype and cytotype of C-160 [In(2LR)SM1/In(2LR)bw\textsuperscript{+}] are M, if the Q type chromosomes are dysgenic in the heterozygous condition with the M genotype in the M cytotype, the high frequency of mutations can be expected as described above. After accumulating mutations for 5 generations in the way described above, the homozygous viabilities of 58 sublines that were approximately a random sample were estimated by the Cy method.

The distribution of homozygote viabilities relative to the mean viability of the control is graphically presented in Fig. 3. When the average viability of the control is assumed to be 1.0000±0.0281, the average viability of homozygotes and the average viability of non-lethal homozygotes are 0.2904±0.0226 and 0.3152±0.0232, respectively. The homozygous genetic loads, T, D and L are estimated to be 1.237, 1.154 and 0.084, respectively using the formulas described above. The D:L ratio turns out to be 14.03, an astonishingly large value (Table 1). From these results, it appears that mildly deleterious and semi-lethal mutations occurred at an extremely high rate on the second chromosomes from WY-113 in the M cytotype. Thus, these results support our hypothesis that the abnormally large detrimental load, D, in the Osaka population came from mildly deleterious and semi-lethal mutations induced by the “mutator factors” in the Q type genomes in the M cytoplasm. Moreover, in this experiment, the accumulation of mutations was made through the males, which might indicate that our “mutator” is different from the I-R system (cf. Picard et al, 1978). This element may be a kind of transposon.
According to in situ hybridization test of salivary gland chromosomes and Southern blotting, the present factor is different from the factors of the P family (unpublished data of O. Yamaguchi and T. Yamazaki, respectively). A preliminary report with respect to the nature of this mutator or transposon has been published (Mukai and Yukuhiro 1983). According to their analysis, the mildly deleterious mutation rate in WY-113 second chromosomes in the M cytotype was 1.95/second chromosome/generation. This value is approximately 15 times larger than the previous estimate (0.14: Mukai 1964; 0.17: Mukai et al. 1972).

A small scale experiment was conducted using the second chromosome of WY-130 and the cytoplasm of C-160. The results are: \( T = 0.5504 \), \( D = 0.4823 \) and \( L = 0.0681 \), after accumulating mutations in the same way as above. Thus, the \( D : L \) ratio turned out to be 7.09. This ratio is smaller than the above estimate but much larger than the previous one (0.97: Mukai and Yamazaki 1968; 0.95: Mukai et al. 1972).

4. DISCUSSION

Nature of the mutator factor: In the present experiment, we found a mutator factor which may be a kind of transposon. This putative transposon begins excisions from the chromosomes in the M cytotype. Thus, it may be assumed that this transposon encodes some type of transposase (cf. O'Hare and Rubin 1983). At present, it can not be answered whether or not this putative transposon produces inhibitor or regulator of the transposase as probably do the P factors, but we assume it to be so (cf. O'Hare and Rubin 1983). This test is now underway. We tentatively call this putative transposon the MY factor.

Large detrimental load due to the MY factor: In the Osaka population, an unusually large detrimental homozygous load was detected. In addition, an important finding was obtained in the present experiment: the individuals of the M cytotype exist although the frequency is not high (ca. 20\%). Thus, mutations would have been induced in the Osaka population at a high frequency, since there is a mutator factor, MY factor, in this population and this factor induces non-lethal viability mutations at a high rate as shown above. Indeed, it has been shown by accumulating mutations in the second chromosomes of WY-113 in the M cytotype (Mukai and Yukuhiro 1983) that the ratio of mildly deleterious mutation rate (ca. 1.95/second chromosome/generation) to lethal mutation rate (ca. 0.014/second chromosome/generation) is extremely high (140 times higher). Occurrence of viability mutations at a high frequency in the Osaka population may be supported by the following finding: more than 50\% of the individuals showed male recombination. Thus, the large D:L
ratio estimated in the Osaka population can be explained. It should be stressed here again that the putative transposon detected in the present experiment is different from the P factor detected by Engels and Preston (1979).

These mutations seem to be induced by the insertion or excisions of the putative transposons. Therefore, it appears that this element was much more frequently inserted in the regions where deleterious mutations occur (mainly in the non-coding region) (cf. Mukai and Cockerham 1977) than the regions where lethal mutations occur (mainly in the coding region). It is expected from the finding of Judd, Shen and Kaufman (1972) that the length of the non-coding region is about twenty times longer than that of the coding region. Thus, even if the insertions of the present element randomly occur, such phenomena might be expected (Mukai and Yukuhiro 1983).

Population-genetical consideration: As has been shown in the present experiment, many mutations are induced by the coexistence of the M cytotype and the genomes of the Q (or P) type. It is generally accepted that lethal mutations are heterozygously harmful (Crow and Temin 1964; Mukai and Yamaguchi 1974) and not important for the adaptation of organisms. Although there is still controversy with respect the neutrality of protein polymorphisms, most population geneticists now accept that they are selectively neutral or nearly neutral (see Kimura 1968; Ohta 1973; Mukai, Tachida and Ichinose 1980; Mukai et al. 1982). However, it should be pointed out that these selectively neutral mutations are only a small fraction of mutations at the structural loci. The remainder are harmful mutations (Kimura 1983). Thus, in general, the invasion of some types of transposons are disadvantageous to the population. However, there is a possibility that the population, under some conditions, gains beneficial mutations that occur in non-coding regions. Such mutations appear to have a role for the control of the action of the structural genes (Mukai and Cockerham 1977; Mukai, Harada and Yoshimaru 1984). For example, Mukai, Harada and Yoshimaru (1984) found that specific activity of alcohol dehydrogenase (Adh) in D. melanogaster was modified by mutations that were most probably induced in the non-coding region by some type of transposons. Furthermore, using the sublines derived from WY-113 (they are the same sublines employed in the viability test of accumulated mutations), it has been shown that the mutations probably induced in the non-coding regions by the MY factors modified the specific activities of Adh (Harada, Yukuhiro and Mukai 1984). In this case, mutations were accumulated only for 5 generations. These findings suggest the significance of transposon-like elements in adaptive evolution of organisms.

Recently, the D/L ratios of the Japanese D. melanogaster populations increase (unpublished results of T. Mukai et al.). This phenomenon might have originated from high mutability due to coexistence of the M cytotype and the
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genomes of the Q type in the populations. Thus, it appears that the Osaka population is in a transient stage from M to Q (cf. Kidwell 1979). If the whole population becomes the Q type, the mutation rate must return to normal rate. It is important to study the adaptive significance of transposons for the populations.

Finally, it should be noted that, although a large amount of detrimental load must have been induced, some fraction of this load may have been induced in the process of isolating second chromosomes by the Cy method, since the cytotype of Cy/Pm stock is M. It is necessary to measure genetic load in the native cytoplasm.

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