Autosomal recessive lethal mutations in two 
mutator stocks of Drosophila ananassae

Muneo MATSUDA

Department of Biology, School of Medicine, Kyorin University
6-20-2 Shinkawa, Mitaka, Tokyo 181, Japan

(Received 7 September 1991)

ABSTRACT

The frequency of recessive lethals in the 2nd chromosome was examined in two 
mutator stocks of Drosophila ananassae, ca and ca; px. They are characterized 
respectively by possessing an extrachromosomal clastogenic mutator in males, 
and by the retrotransposon "tom", which induces Om mutability only in females. 
The frequencies of recessive lethal mutations in the 2nd chromosome among 
progenies from males and females of the ca; px stock are 0.35 and 0.34 percent, 
respectively. Similarity of these frequencies indicates that tom does not induce 
recessive lethals in females. In contrast to the ca; px stock, the frequency of 
recessive lethals in males of the ca mutator stock was estimated to be 1.54 
percent for the 2nd chromosome. No visible mutants except Minutes were 
recovered. Some recessive lethals derived from ca stock males were associated 
with chromosomal rearrangements. Being consistent with its high rate of Minute 
mutation it was demonstrated that the ca clastogenic mutator also induced 
recessive lethals.

1. INTRODUCTION

Based on his frequent recovery of spontaneous visible mutants, Kikkawa (1938) 
suggested that Drosophila ananassae may be characterized by high mutability. 
Kikkawa (1938) also found a translocation in a Ryukyu wild stock and this has 
been followed by frequent reports of rearrangements such as reciprocal translocations, 
pericentric inversions or deficiencies that are not ordinarily reported to 
cr occur or persist in natural populations of other Drosophila (Dobzhansky and 
Dreyfus, 1943; Freire-Maia, 1961; Futch, 1966; Ray-Chaudhuri and Jha, 1966; 
Nirmala and Krishnamurthy, 1970, 1972; Sreerama and Krishnamurthy, 1970; 
Singh et al., 1971; Tomimura, personal communication). In laboratory stocks, 
bri and pc marker stocks, two distinct, dominant mutators linked to the 3rd 
chromosome were diagnosed along with specific chromosomal (bri) or extrachromosomal (pc) suppressors. These cryptic mutability of the stocks were unco 
erved only in outcrosses. In the ca marker stock, an extrachromosomally trans 
mittted clastogenic mutator responsible for chromosome damage in sperm was 
found to be accompanied by a genetic (or cytotypic) suppressor system. A ca; px
derivative of the ca stock, synthesized by outcrossing with a px stock, lacks the
clastogenic mutator, but is characterized by hypermutability in which dominant
eye morphology (Om) mutants are recovered almost exclusively. These mutants
have been attributed to insertion of a retrotransposon named tom (Shrimpton et
al., 1986; Tanda et al., 1988 and 1989).

The ca mutator system was defined primarily by assaying the frequencies of
dominant visible, recessive lethal Minutes bristle mutations, which were often
associated with deficiencies. Direct cytological screens for chromosome rear-
rangements among larvae as well as the incidence of dominant lethality among
embryos confirmed the clastogenic effect of the ca mutator on paternal chromo-
somes introduced into suppressor-free egg cytoplasm (Hinton, 1981). The muta-
tions of the ca mutator system appear among the progeny of outcrossed ca males.
Om mutations originate exclusively in the gametes of ca ; px females, being
invariably free of rearrangements. The frequency of Minutes was not significant-
ly elevated in this stock (Hinton, 1984). There is, none the less, at least one
feature common to the two systems in that the extrachromosomal mutator
induced breakages and the retrotransposon induced Om mutations are restricted
to euchromatic chromosome segments.

In view of the foregoing comparisons of these mutator stocks that share their
genetic background, it is of interest to compare them with respect to another
measure of mutability, namely recessive lethals.

2. MATERIALS AND METHODS

The simplest and most common method of detecting recessive lethal mutations
in D. melanogaster is to recover them as heterozygotes over an X chromosome
balancer. In D. ananassae, there is no suitable balancer for the X chromosome,
so recessive lethals must be detected on the 2nd chromosome. From a natural
population in Papua New Guinea, Futch (1968) isolated and described a multiple
inverted 2nd chromosome, designated Ins(2L+2R) NG2 and abbreviated NG2.
This chromosome effectively eliminates recombination throughout the 2nd
chromosome in standard(+) / NG2 flies. NG2 carries at least one recessive lethal
itself. Two NG2 balancer stocks were used: NG2 eD/ rs and NG2/ca M(2)665.
eD is ebony dominant (body color) and rs is a recessive rose eye color mutant with
cause recessive female-sterile located near the tip of 2R. ca is claret eye color
and M(2)665 is the Minutes bristle mutant in 2L. Both the ca ; px and ca mutator
stocks carried standard sequences in the X, 2nd and 3rd chromosomes.

Outcrosses of males from the ca stock, or of males and females from the ca ; px
stock, to flies from the rs/NG2 eD stock produced F1 flies which were scored
systematically for Minutes and casually for any other dominant visible mutants.
Among the F1 progeny, non-Minutes F1 females of the ca*/NG2 eD genotype
were collected and mated singly to ca M(2)665/NG2 males to produce ca*/NG2
flies sharing identical ca* chromosomes. Upon inbreeding ca*/NG2 daughters and sons of the same F₁ females, the presence or absence of claret (ca*/ca*) progeny revealed the absence or presence of a recessive lethal in that ca* chromosome. Each ca* chromosome identified with lethal was maintained in a ca* l/NG2 stock until completion of complementation tests identifying allelic lethals. In addition, polytene chromosomes of larvae from some lethal stocks were examined for any associated rearrangements.

Stocks and experimental matings were cultured in 25×95 mm shell vials containing 10 ml of medium composed of corn meal, brewers' yeast, molasses, agar and mold inhibitor. Experimental matings routinely used virgin adults aged three to four days and maintained at 25°C.

3. RESULTS

Only two Minutes appeared among the 6796 progeny of the ca ; px stock males (Table 1). Among 868 ca* chromosomes isolated via F₁ females with ca ; px father and tested for homozygous viability, six bore recessive lethals that fell into 5 complementation groups (Table 2). Progeny from the ca ; px stock females, like their male cohorts, produced only two Minutes, and no Om mutants among 4210 F₁ progeny scored. This result is not unexpected given the reported Om fre-

<table>
<thead>
<tr>
<th>Parents</th>
<th>Female</th>
<th>Male</th>
<th>No. of progeny counted</th>
<th>No. of Minutes observed</th>
<th>Minutes frequency per chromosome</th>
</tr>
</thead>
<tbody>
<tr>
<td>NG₂ e₀ × ca</td>
<td></td>
<td></td>
<td>7374</td>
<td>26</td>
<td>0.00353</td>
</tr>
<tr>
<td>NG₂ e₀ × ca ; px</td>
<td></td>
<td></td>
<td>6769</td>
<td>2</td>
<td>0.00039</td>
</tr>
<tr>
<td>ca ; px × NG₂ e₀</td>
<td></td>
<td></td>
<td>4210</td>
<td>2</td>
<td>0.00048</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parents</th>
<th>Female</th>
<th>Male</th>
<th>No. of chromosomes isolated</th>
<th>No. of chromosomes with recessive lethals</th>
<th>No. of complementation groups of recessive lethals</th>
<th>2-linked recessive lethals frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>NG₂ e₀ × ca</td>
<td></td>
<td></td>
<td>988</td>
<td>29</td>
<td>15</td>
<td>0.0154 (15/974)</td>
</tr>
<tr>
<td>NG₂ e₀ × ca ; px</td>
<td></td>
<td></td>
<td>868</td>
<td>6</td>
<td>5</td>
<td>0.00035 (3/865)</td>
</tr>
<tr>
<td>ca ; px × NG₂ e₀</td>
<td></td>
<td></td>
<td>590</td>
<td>10</td>
<td>4</td>
<td>0.00034 (2/582)</td>
</tr>
</tbody>
</table>

* see text
frequency of $2 \times 10^4$ (Hinton 1984). A total of 590 $ca^*$ chromosomes analyzed yielded ten recessive lethals, that defined four complementation groups. Two of the complementation groups derived from $ca$; $px$ stock males were allelic to those forward the recessive lethals recovered from $ca$; $px$ stock females. The mutations in these two complementation groups are considered to be pre-existing lethals in the $ca$; $px$ stock, and these were omitted them for the calculations of the frequencies. The estimated frequencies of recessive lethal in the 2nd chromosome are 0.35 (3/865) and 0.34 (2/582) percent for chromosomes derived from outcrosses of $ca$; $px$ stock males and females, respectively. None of the lethals recovered from outcrosses of $ca$; $px$ males or females were examined cytologically for chromosome rearrangements. However, all the five lethals showed independent assortment of the 2nd and 3rd chromosomes in testcrosses of $ca^*$/NG2; +/ $px$ males to $ca$; $px$ females.

From outcrosses of $ca$ stock males to $rs$/NG2 $e^D$ females, examination of 7374 progeny uncovered 26 putative Minutes mutations (Table 1). This frequency (0.0035) is lower than the estimated frequency (0.0104) by Hinton (1981, 1984) but statistically nonsignificant. From $F_1$ $ca^*$/NG2 $e^D$ females, 988 $ca^*$ chromosomes were assayed and 29 carried recessive lethals. Complementation tests among these 29 lethals revealed 15 complementation groups (Table 2). Three lethal loci were represented by clusters of two, four, and eleven mutations each. When such clusters may reflect amplification of pre-meiotic lethals occurring in the parental males' germ line, the frequency of recessive lethals is 1.54 percent per chromosome (15/974). Among the 15 lethal loci, five were associated with chromosomal aberrations including two different pericentric and one paracentric inversions, one deficiency and one X:2 translocation. All breakpoints were in euchromatin. Thus the frequency of chromosomal aberrations identified by recessive lethals was 0.51(5/974) percent.

4. DISCUSSION

$ca$; $px$ stock

The $ca$; $px$ stock was derived from the cross between $px$ females and $ca$ males (Hinton, 1984). Therefore extrachromosomal mutator elements of the $ca$ stock were not transmitted to the new synthesized stock. The $px$ stock is silent for Minutes mutation (Hinton, 1981). Both sexes of the $ca$; $px$ stock still have the property of Minutes mutability equivalent to that of the $px$ original stock, whose Minutes frequency is 0.0006 (Hinton, 1981). This value is comparable to the data of this experiment. The $ca$; $px$ stock is characterized by the Om hypermutability system in which occur at high rate spontaneously eye morphology mutations, Om (Hinton, 1984). Om mutants are semi-dominant and arise exclusively in oocytes. They map to at least 25 loci (Hinton, 1984, 1988) in the X, 2nd and 3rd chromosomes. They are associated with the insertion of the tom retrotransposon
Recessive lethal mutations in mutator stocks

(Shrimpton et al., 1986, Matsubayashi et al., 1986). No Om mutation was
detected in the F\textsubscript{1} generation bore in the present experiment, because of the small
progeny number scored. In an unrelated experiment, 3 Om mutations were
recovered among 3333 progeny of ca; px stock. Therefore I considered the ca;
px stock still has an Om hypermutability system. Minutes and other dominant
visible mutations could seldom be found in ca; px stock (Hinton, 1984; present
data). The present data shows that the frequency of the 2nd chromosomes
carrying recessive lethals is not significantly different between females (0.35%)
and males (0.34%) of the ca; px stock. This is in exquisite contrast to Om
mutability restricted to females (Hinton, 1984). The range of the lethal frequen-
cy in the 2nd chromosome, 0.34% to 0.35%, is comparable to the recessive lethal
mutation rates (0.57% in males and 0.72% in females) in the 3rd chromosome of D.
melanogaster (Wallace, 1968). The 2nd chromosome of D. ananassae corre-
ponds to the 3rd chromosome of D. melanogaster (Paterson and Stone, 1954).
Om mutation rate/generation/locus can be estimated to be 8.0×10\textsuperscript{-6} by dividing
the occurrence rate, 2×10\textsuperscript{-4}, by the number of Om loci, 25. If tom insertion
induces recessive lethal mutations as well as Om mutations throughout the
genome in the ca; px females, an excess of 1.6% (8.0×10\textsuperscript{-6}×2000 loci) or 8 to 9
mutations over what are recovered in the ca; px males would be expected for the
2nd chromosome in which 2000 loci are assumed. The number of loci on the 2nd
chromosome was assumed by the hypothesis of one gene and one polytene band
(Judd et al., 1972). In the present experiment only 0.34%, 2 lethals among 582
chromosomes were found. Despite the fact that tom elements inserts into the
target sequence TATAT (Tanda et al., 1988) which should be present in many
sites in the genome and many tom elements found at sites other than Om loci
(Shrimpton et al., 1986; Tanda et al., 1988), the present results indicate that the
Om-tom mutability may not have a significant role in the production of recessive
lethal mutations or dominant visible mutations except Om mutations. Southern
blot analyses of spontaneous complete revertants of Om(1D)9 and Om(2D)63
revealed that all tom insertions were lost without DNA rearrangements (Tanda et
al., 1989; Matsubayashi et al., 1991). These findings and the present results
indicate that tom transposition may not induce chromosome breakages, in contrast
to the ca mutator.

ca stock

The ca stock has an extrachromosomal elastogenic mutator which produced high
frequencies of chromosomal rearrangements associated with dominant lethals or
visible Minute mutations among the progeny from outcrossed ca males or the F\textsubscript{1}
sons of outcrossed ca females (Hinton 1979, 1981). The high frequency (1.54%) of
recessive lethals in the 2nd chromosome was found in the progeny from ca
mutator stock males in this study. Clusters at three lethal loci were found among
15 lethal complementation groups. Although these clusters may be amplification
of pre-meiotic lethals, it is possible that such clusters may reflect pre-existing lethals segregating in the ca stock. These two possibilities can hardly be distinguished. However, high frequency of recessive lethals found in the present experiment indicates that ca mutator males have many recessive lethals and/or newly arisen lesions in the sperms which could not be repaired in the eggs of balancer females. This result is consistent with the previous reports of Minute mutability induced by the ca extrachromosomal mutator (Hinton, 1979, 1981).

Hinton (1979, 1981) showed that ca extrachromosomal mutator produced 2.6% chromosome breakages among 639 progeny. In the present data, high frequency (33.3%) of rearrangements was found among chromosomes carrying recessive lethals derived from ca males, supporting the previous reports (Hinton, 1979, 1981). In Drosophila melanogaster, a high occurrence rate of chromosomal rearrangement has been found among progeny of the so-called dysgenic F₁. Engels and Preston (1984) reported that the majority of breaks induced by P elements were intrachromosomal two-break events. Another transposable element, hobo, has been found to mediate the rearrangements restricted to a single chromosome arm in Uc (Unstable X chromosome) stock of D. melanogaster (Lim, 1988; Blackman and Gelbart, 1989), although it is still unclear whether hobo produces only intrachromosomal aberrations. In contrast to these systems in D. melanogaster, chromosomal rearrangements induced by ca-mutator are characterized as follows, 1) breakage events were generated during spermatogenesis of the ca inbred stock, 2) both inter- and intra chromosome rearrangements were observed, 3) their breakpoints were located in euchromatin region, and 4) chromosomal lesions carried in sperm of ca males were efficiently repaired by suppressor in fertilized eggs derived from ca stock females (Hinton, 1981).

This work was suggested by, and initiated in the laboratory of, Claude W. Hinton, Department of Biology, The College of Wooster, Ohio, USA. The author expresses Drs. C. W. Hinton, C. H. Langley for their kind support throughout this work. I am grateful to Drs. C. H. Langley, M. M. Green and Y. N. Tobari for reading the manuscript and making numerous useful suggestions.

REFERENCES


