MUTAGENICITY OF AN EXTRACHROMOSOMAL ELEMENT DELTA FOR X CHROMOSOMES IN *DROSOPHILA MELANOGASTER*¹⁾

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The extrachromosomal element denoted by delta was found and studied in our laboratory. The main findings obtained were reported in a series of papers. The main conclusions relevant to the present report are given briefly below. 1) Delta is transmitted extrachromosomally (Minamori 1969a); however, it is associated inseparably with certain 2nd chromosome lines (Minamori 1969b, 1972). Delta-bearing 2nd chromosome lines can be induced by an alkylating mutagen, ethyl methane sulfonate (EMS), from a line carrying no delta. Moreover, the gene responsible for delta-retention, symbolized as *Da*, is found to be located at 24.9 of chromosome 2 (Minamori and Sugimoto 1973). These findings suggest that delta may be a gene-product. If this is true, the information for delta may be transmitted in a dual way: chromosomally and extrachromosomally. 2) Two variants of delta, [b] and [r], are known (Minamori 1971), and they are assumed to be a product of multiple-alleles of the gene *Da*; [delta b] is produced by *Da^b* and [delta r] by *Da^r*. *Da^b* is carried by a sensitive chromosome symbolized by *S^b* and an insensitive chromosome symbolized by *ID^b*. *Da^r* is carried by a sensitive chromosome symbolized by *S^r*. [Delta b] kills *S^b/S^b* and *S^b/S^r* zygotes, and [delta r] kills *S^r/S^r* but not *S^b/S^r* zygotes (*loc. cit.*). 3) [Delta b] is observed to be a necessary element for the gametogenesis of a certain *ID^b* chromosome line (Minamori and Ito 1972). In a natural population *Da* alleles are maintained at a high frequency (average 30%; Minamori *et al.* 1970, 1973); hence, a polymorphism is established as to *Da* locus in the population. Delta appears to have been maintained for a longer period in the evolutionary process and to become a normal component of the genetic system of the fly. 4) A most characteristic nature of delta may be its mutagenic action to chromosomes. [Delta b] induces frequent recessive lethal mutations on 2nd chromosomes (Minamori and Ito 1971). Both [delta b] and [r] are considered to cause elimination of a specific homologue of chromosome 2, and gametes receiving the nullo-2 chromosome set are assumed to become dysfunctional (unpublished). When the elimination occurs at an early embryonic stage, the gametes recovered carry only one kind of homologue and not the counterpart (one-sided gamete recovery; Minamori 1970). [Delta r] correlates with "Segregation-Distortion" phenomenon caused by *SD* gene (Sandler *et al.* 1959); this delta appears to damage *SD⁺*-bearing chromosomes in the presence

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of *SD* at a stage after sex differentiation. [Δ r] induces translocations and crossing over in males, and increases recombination frequency in females (unpublished).

As reported in a previous paper (Minamori and Ito 1971), [Δ b] retained by a 2nd chromosome line (*ID^b-45*) induces recessive lethal mutations. The frequency estimated with three 2nd chromosome lines was 1.47%, being 3.6 times the spontaneous mutation rate (0.41%). The distribution of lethals along the chromosome was not at random; most lethals (82%) were located on the right arm and, moreover, concentrated near Lobe region (50%; *L*, 2—72.0). A similar distribution pattern was observed by Gershenson (1965) in lethals induced by feeding calf-thymus DNA to *Drosophila* larvae. He found that the same agent did not increase markedly the frequency of sex-linked lethals. The same result was obtained by Mathew (1965) in experiments employing the same procedure, and by Gershenson *et al.* (1971) in experiments inoculating viruses or injecting DNA's extracted from the viruses which are non-infectious to the fly. In the present study an attempt was made to clarify whether or not [Δ b]; is similar to the biopolymer in its mutagenicity for X chromosomes. The results disclosed that [Δ b] did not increase significantly the frequency of lethals.

MATERIALS AND METHODS

In the present study [Δ b]'s retained by four sublines of *ID^b-45* chromosome which had been employed for estimation of lethal mutation rates induced on 2nd chromosome were also used. The *ID^b-45* chromosome steadily retains [Δ b], but is insensitive to this Δ . Twenty-four X chromosome lines (Table 2) were examined as to their lethal mutation rates in the presence of [Δ b]; 19 were extracted from wild flies collected in Hiroshima City, and the other 5 were carried by laboratory stocks (*O-1*, *Or-R*, *cn bw*, *Iso-Amherst*, *Cy/Pm*). All these X chromosome lines retained no Δ .

The frequencies of recessive lethals were estimated under various combinations of X chromosomes and [Δ b]'s retained by the above sublines of *ID^b-45* chromosome. X chromosomes carried by *M-5/X; Cy/ID^b-45* (*M-5*; Muller-5 chromosome) females which retained an appreciable amount of [Δ b] were individually tested for their viability in the non-*M-5* grandsons by the ordinary procedure for detecting lethals. The above females were obtained by mating females of *M-5/M-5; Cy/ID^b-45* which retained an appreciable amount of [Δ b] with males of *X/Y; Cy/Pm*. Lethal cluster obtained from a single female was counted as one mutation. When all X chromosomes recovered from a single female were lethal, these mutants were omitted from the data. For the estimation of spontaneous mutation rate, X chromosomes carried by *M-5/X; Cy/Pm* females which retained no Δ were tested for their viability. Flies were cultured in an incubator kept at $25 \pm 1^\circ\text{C}$.

RESULTS AND DISCUSSION

The frequency of spontaneous recessive lethal mutations was estimated with five

Table 1. Frequencies of sex-linked recessive lethals induced by [delta b] which were retained by different sublines of *ID^b-45* chromosome

[Delta b] retaining sublines	No. of chromosomes tested	No. of lethals	Percent of lethals
<i>ID^b-45 o-9</i>	3,440	4	0.12
<i>T-2</i>	2,189	2	0.09
<i>y-8</i>	6,037	4	0.07
<i>A-5</i>	2,121	1	0.05
Pooled	13,787	11	0.08

X chromosome lines extracted from the Hiroshima population. The frequency was, on an average, 0.02% (1/6,314). This is very low, being only one-twentieth of the frequency of lethals arising spontaneously on 2nd chromosomes (0.41%; Minamori and Ito 1971).

The frequencies of recessive lethals induced on X chromosomes by [delta b]'s retained by four sublines of *ID^b-45* chromosome are given in Table 1. The frequencies induced by [delta b]'s varied appreciably; however, this variation was not significant ($\chi^2=1.38$, $0.75>P>0.50$). It may be stated that [delta b]'s retained by different sublines do not vary as to their mutagenicity for X chromosomes. The average frequency was 0.08%, being approximately four times the spontaneous mutation rate. This difference, however, was not significant ($\chi^2=1.99$, $0.25>P>0.10$). Accordingly, the conclusion may be that the mutagenic action of [delta b] to X chromosomes is not so effective as to 2nd chromosomes (1.47%; *loc. cit.*). This conclusion well coincides with those obtained in the experiments employing calf-thymus DNA (Gershenson 1965; Mathew 1965) and virus DNA (Gershenson 1969; Gershenson *et al.* 1971). An appreciable variation was observed among frequencies of lethals induced on 24 X chromosome lines, ranging from 0 to 0.30% (Table 2). However, it was not admitted to be heterogeneous ($\chi^2=18.65$, d.f.=23, $0.75>P>0.50$).

In the course of experiment visible mutants were also obtained: six yellows, three whites, and one vermilion. Moreover, one lethal cluster and two visible clusters (one yellow, and one white) were obtained. These mutations appear to have been induced in premeiotic stages.

As mentioned above, all the 24 X chromosome lines tested were shown to be not mutable in the presence of [delta b]. This result sharply contrasts with that obtained with 2nd chromosomes (Minamori and Ito 1971) in which only three lines were tested and all these lines, respectively, showed significantly higher mutation rates in the presence of [delta b]. It is now clear that X chromosomes differ from 2nd chromosomes as to their mutability to [delta b]. The origin of this difference may be explained from the aspect of natural selection.

The maintenance of variation is necessary for the survival of populations under changing environment. In fact, natural populations are observed to contain an enormous amount of variation. However, the maintenance leads to an inevitable production of ill-adapted individuals—the reduction of fitness of the population. Therefore, an extremely high and an extremely low mutation rate would be both unfavorable for

Table 2. Frequencies of recessive lethals induced by [δ b] on various X chromosomes

X chromosome lines	No. of chromosomes tested	No. of lethals	Percent of lethals
27	1,002	3	0.30
0-1	404	1	0.25
29	409	1	0.24
Or-R	444	1	0.23
5	456	1	0.22
cn bw	513	1	0.19
92	555	1	0.18
15	2,379	2	0.08
23	23	0	0
14	121	0	0
30	326	0	0
31	335	0	0
2	401	0	0
28	408	0	0
8	421	0	0
12	432	0	0
521	441	0	0
39	461	0	0
13	476	0	0
19	502	0	0
414	561	0	0
Iso-Amherst	573	0	0
Cy/Pm	1,014	0	0
26	1,130	0	0
Pooled	13,787	11	0.08

the survival of the population. The mutation rate may be a plastic trait which can undergo rapid adaptive changes in the process of evolution. Natural selection will strike a compromise between the variability and the reduction of fitness, consequently an optimum mutation rate may be established in individual populations (Kimura 1960). The spontaneous mutation rate may reflect such an optimum rate.

The comparison of mutation rates between X and 2nd chromosomes has been made by many authors. The data reported hitherto with *D. melanogaster* are summarized in Table 3, and classified into mutations arising spontaneously, and those induced by irradiation, chemicals, viruses, DNA's and [δ b]. To make a comparison, the ratio of the frequency of lethals on X chromosomes to that on 2nd chromosomes was obtained (symbolized as X/II). The ratio is expected to be about 50% under the assumption that mutation rates are proportional to chromosome length. However, as shown in the table, it is generally lower than expected, especially in [δ b]-induced lethals. With respect to natural selection, the important difference of X from autosomal chromosomes is that one-third of the former would be exposed, due to hemizyosity in males, to

Table 3. Comparison of the frequency of lethals induced on X chromosomes with that induced on 2nd chromosomes

Mutagen	Percent of lethals		Percent X/II	Literature
	X	II		
A. Lethals arising spontaneously				
	0.12	0.34	35.3	Gershenson <i>et al.</i> 1971
	0.09	0.56	16.1	Mathew 1965
	0.02	0.41	4.9	Minamori and Ito 1971; present study
	0	1.84	0	Alexander 1967
B. Lethals induced by irradiation				
X-ray (1,000R)	2.13	3.81	55.9	Thompson 1962
X-ray (1,000R)	2.86	7.02	40.7	Chandley and Bateman 1960
X-ray	3.84	20.48	18.8	Berg 1937
C. Lethals induced by chemicals				
Formaldehyde (0.18-0.25%)	5.49	11.50	47.7	Auerbach and Moser 1953
Ethylenimine	2.66	6.67	39.9	Paik 1968
S-2-chloroethylcysteine ($0.27 \times 10^{-2}M$)	4.52	11.74	38.5	Farmy and Farmy 1960
S-2-chloroethylcysteine ($0.19 \times 10^{-2}M$)	6.99	19.89	35.1	Farmy and Farmy 1960
Ethylenimine	3.99	15.29	26.1	Alexander 1967
Ethylenimine	3.85	20.00	19.3	Alexander 1967
D. Lethals induced by viruses which are non-infectious to the fly				
P. V.*	1.24	2.43	51.0	Gershenson 1969
P. V.* (2,1000 p.f.v.)	0.64	1.96	32.7	Gershenson <i>et al.</i> 1971
T. I. V.** (0.001-0.005 mg)	0.50	2.02	24.8	Gershenson <i>et al.</i> 1971
E. Lethals induced by DNA				
Calf-thymus DNA (13%) in females	0.77	2.82	27.3	Mathew 1965
Calf-thymus DNA (13%) in males	0.31	1.39	22.3	Mathew 1965
Calf-thymus DNA (10%)	0.15	1.20	12.5	Mathew 1965
T. I. V.** (0.012-0.015 μg)	0.28	7.76	3.6	Gershenson <i>et al.</i> 1971
F. Lethals induced by [delta b]				
[Delta b]	0.08	1.47	5.4	Minamori and Ito 1971; present study

* poliomyelitis virus.

** tipula iridescent virus.

natural selection in each generation. In contrast, only square of the frequency of individual lethal allele on autosomes would become homozygous and be selected. Berg (1937) predicted that, since X chromosomes are more unfavorably selected than 2nd chromosomes, the mutable X chromosomes would be eliminated from the population, and then the mutation rate on X chromosomes may become lower. Her prediction was ascertained to be true in X-ray irradiation experiments (Table 3, B).

As shown in the table, there is a variation among ratios X/II obtained by different mutagens. On an average, the ratio is 14.1% in spontaneous, 38.5% in irradiation-induced, 34.4% in chemicals-induced, 36.2% in virus-induced, 16.4% in DNA-induced and 5.4% in [delta b]-induced. These mutagens can be grouped into two: those show-

ing ratios higher than 34% (irradiation, chemicals, virus) and those showing ratios less than 17% (spontaneous, DNA, [delta b]). This variation may also be interpreted from the aspect of natural selection. In natural populations, flies may seldom be exposed to X-ray and mutagenic chemicals in high dose, or not infected with viruses shown in Table 3, D. Accordingly, X chromosomes appear not to have been selected by these mutagenic agents in the evolutionary process. However, delta appears to have operated as a selecting force for the chromosomes. The reason is that delta appears to be an indispensable element for fertility of a certain chromosome line, and *Da* gene which is assumed to produce deltas is polymorphic in a natural population. In the evolutionary process, X chromosomes carrying mutable loci for delta would be eliminated, and ultimately unmutable chromosomes have spread in the population. As noted earlier, delta is a striking mutagen for 2nd chromosome inducing lethal and visible mutations, chromosome elimination, translocations and recombination. If the hypothesis that delta is a gene-product is valid, delta is a gene-produced mutagen: a mutagen produced by the fly itself. It is predictable that various kinds of such a mutagenic agent may exist in *Drosophila* flies and other organisms. The mutagenic nature of DNA's found by Gershenson (1965), Mathew (1965) and Gershenson *et al.* (1971), though these DNA's are foreign to the fly, suggests that some of the above agents may be associated with some extrachromosomal DNA's. Such an agent might select against mutable X chromosomes. Thus, the chromosomes which survived may show a lower mutation rate in the natural condition (spontaneous) or when they happen to fall in with foreign DNA's and/or their derivatives. An extreme reduction in the ratio X/II of tipula iridescent virus by employing its DNA (Table 3, E) appears to favor the above view. The similarity of delta to calf-thymus DNA or virus DNA in mutagenicity for X and 2nd chromosomes permits us to infer that delta may be an agent associated with some extrachromosomal DNA or with its derivatives.

SUMMARY

In a previous study (Minamori and Ito 1971) the extrachromosomal element [delta b] was shown to induce recessive lethal, semilethal and visible mutations on 2nd chromosomes. In this study the mutagenic action of this delta to X chromosomes was examined. The frequency of recessive lethals induced was 0.08%, being four times the spontaneous mutation rate (0.02%). The difference, however, was statistically insignificant. Accordingly, the conclusion may be that [delta b] has no effective mutagenicity for X chromosomes. The differential behaviors of X and 2nd chromosome to mutagenicity of [delta b] were discussed from the aspect of natural selection.

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