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The control of mutational instability by a new mutator gene of *Drosophila melanogaster**

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SUMMARY

The isolation and genetic characterization of a new mutator gene, *Mutator-forked*^{3N} (*Mu-f*^{3N}), of *Drosophila melanogaster* are described. This mutator gene is unique in that it seems to increase specifically the reversion frequency of the unstable mutant *forked*^{3N} (*f*^{3N}, 1–56.7), since the frequency of spontaneous sex-linked recessive lethals in males and females and the frequency of reverse mutations at eight additional X-linked alleles were unaffected by *Mu-f*^{3N}. The mutator is a dominant gene that has been mapped to the region between *f*^{3N} (1–56.7) and *Beadex-2* (*Bx*^{2}, 1–59.4) in the *X* chromosome, and it seems to function only in the 'cis' configuration. The mode of action of *Mu-f*^{3N} is compared with that of other mutator genes.

1. INTRODUCTION

Genes which increase spontaneous mutation frequencies have been observed in a variety of organisms (see Drake, 1973). In prokaryotes, the mode of action of these mutator genes has been analysed in detail, but there is no comparable analysis in eukaryotes.

In Drosophila, mutator genes have been identified mainly by their ability to increase the frequency of spontaneous recessive lethal mutations or of spontaneous visible mutations (Demerec, 1937; Neel, 1942; Mampell, 1943; Ives, 1945, 1950; Slatko & Hiraizumi, 1973). In one case a mutator gene was identified by its influence on the frequency of reversion of a specific mutant (Green, 1970). In addition, mutator genes in Drosophila have been postulated to affect the mutation frequency of unstable mutants, i.e. mutants which are characterized by frequent changes to other mutant states and/or high reversion frequencies (Demerec, 1929; Green, 1970; Woodruff, Bowman & Simmons, 1970, 1972). For example, recent studies have suggested that a mutator gene may be partially responsible for the mutational instability of the mutant *forked-3N* (*f*^{3N}, 1–56.7) of *Drosophila melanogaster*. Green (1970) has reported the isolation of a third chromosome mutator gene which increases the reversion frequency of *f*^{3N} and has suggested that the reported high spontaneous reversion frequency of *f*^{3N} (Green, 1957; Lefevre & Green, 1959; Altenburg & Browning, 1962) may be a result of an unknown mutator gene present in these stocks. Woodruff *et al.* (1970, 1972) have suggested that the reduc-

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tion in the spontaneous f^{3N} reversion frequency observed in some outcrossed strains may be the result of removal of a mutator gene.

This paper describes the isolation and genetic characterization of a new dominant sex-linked mutator gene, *Mu*-f^{3N}, of *Drosophila melanogaster*.

2. MATERIALS AND METHODS

Stocks. The mutant genes, chromosomal aberrations, wild-type stock, and special chromosomes used in this study are listed in Table 1. All stocks were maintained at an ambient temperature of about 24 °C on a standard cornmeal-agar-brewer's yeast-sugar medium with propionic acid added as a mold inhibitor.

Table 1. *Mutants, chromosome aberrations, wild-type stock, and special chromosomes* (*Lindsley & Grell, 1968*)

Symbol	Name (structure affected)	Location and/or remarks
B	Bar (eye)	1–57.0
Bx^2	Beadex-2 (wing)	1–59.4
car	carnation (eye)	1–62.5
Cy	Curly (wing)	2–6.1 (recessive lethal, 2nd inversions)
CxD	Dichaete (wing)	3–40.7 (recessive lethal, 3rd inversion)
Df(1) B^{263-20}	Deficiency (1) Bar^{263-20}	Deficient for *Bar*
f^{3N}	forked-3N (bristle)	1–56.7
f^{36a}	forked-36a (bristle)	1–56.7
m	miniature (wing)	1–36.1
M(1)n	Minute(1)n (bristle)	1–62.7 (recessive lethal)
$Pm(bw^{v1})$	Plum (eye)	2nd inversion, recessive lethal
Sb	Stubble (bristle)	3–58.2 (recessive lethal)
un	uneven (eye)	1–54.4
v	vermilion (eye)	1–33.0
w	white (eye)	1–1.5
w^a	white-apricot (eye)	1–1.5
y	yellow (body)	1–0.0
y^2	yellow-2 (body)	1–0.0
y^{31d}	yellow-31d (body)	1–0.0
FM6	First Multiple 6	1 inversions (balancer)
FM7	First Multiple 7	1 inversions (balancer)
Canton-S	Canton-Special	Wild-type stock
C(1)RM, *v* f^{3N} *car*	Compound (1) Reversed Metacentric	Attached *X*, homozygous *v* f^{3N} *car*
C(1)DX, *y w f*	Compound (1) Double *X*	Homozygous *y w f*

Reversion experiments. The identification and characterization of *Mu*-f^{3N} was based on the ability of this mutator gene to increase the reversion frequency of the unstable mutant f^{3N} in females. In reversion experiments, the frequencies of f^{3N} reversion events were determined from crosses using females with free-*X* or attached-*X* chromosomes. In free-*X* crosses, f^{3N} females, with *X*-chromosome markers flanking f^{3N}, were mass mated to f^{36a} Bx^2 or f^{36a} *B* males. All F_1 progeny of these crosses were scored for the presence of presumptive f^{3N+} revertants by observing flies with wild-type macrochaetae and the appropriate X-chromosome marker phenotypes. Heterozygous f^{3N}/f^{36a} females have a forked phenotype. Since f^{36a} is apparently a non-reverting *forked* allele (Lefevre & Green, 1959), all F_1 offspring which had wild-type forked phenotypes were considered to be f^{3N+} revertants. All male presumptive f^{3N+} revertants were subsequently mated to $C(1)DX$, $y\ w\ f$ females to determine if they bred true for the revertant phenotype. In addition, these males were mated to homozygous f^{36a}, free-X, females to insure that they were not new forked mutants with mild forked phenotypes. A f^{3N+}/f^{36a} heterozygote would have a wild-type (not forked) phenotype, whereas a heterozygote of a mild forked mutation and f^{36a} would have a forked phenotype. All F_1 presumptive f^{3N+}/f^{36a} females were mated to f^{3N} male sibs, and their offspring were scored for the presence of phenotypically f^+ males with the appropriate X-chromosome marker phenotypes. This procedure eliminated the possibility of mistakenly scoring f^{36a+} revertants as f^{3N} reversion events. No f^{36a+} revertants were observed in this study. The reversion frequency of f^{3N} was determined in attached-X females by mating $C(1)RM$ females homozygous for v, f^{3N} and *car* to wild-type males. The F_1 attached-X females were then scored for f^{3N+} revertants. All presumptive f^{3N+} revertants were mated to wild-type males to determine if they bred true for the wild-type phenotype, and since $C(1)RM$, $v\ f^{3N}\ car$ females contain two X chromosomes, a recombination experiment was performed on each f^{3N+} revertant to determine if one or two f^{3N} alleles had reverted. All f^{3N} reversion events in attached-X females occurred in only one X chromosome.

In the above experiments, approximately 20 pairs of parents were mass mated in half-pint milk bottles. Every 4–5 days these flies were transferred to fresh medium for a total of four or five broods. Each bottle was coded to allow the identification of clusters of f^{3N} reversion events. No clusters of f^{3N+} revertants were observed during this study. The F_1 progeny of these crosses were scored until the eighteenth day from the time any one culture was initiated. By discarding the bottles after the eighteenth day, no F_2 progeny were mistakenly included in F_1 results. Precautions against contamination by extraneous flies were made by maintaining f^{3N+} revertants separately from other stocks, and by carefully checking the phenotypes of all flies before using them in any experiment.

The genetic frequencies in Table 2 are given as wild types recovered per f^{3N} locus scored. Scored flies were counted by an automatic counter similar to that designed by Keighley & Lewis (1950) or by hand. Fiducial limits on reversion frequencies were computed according to Stevens (1942).

Test for f^{3N} suppressor mutations. Although most presumptive f^{3N+} revertants have been observed to be true revertants (Green, 1957; Lefevre & Green, 1959; Woodruff *et al.* 1972), suppressors of f^{3N} have been observed (Lefevre & Green, 1959). It was necessary, therefore, to determine that the presumptive f^{3N+} revertants recovered in this study were true revertants of f^{3N} and not due to the induction of a suppressor of f^{3N}. None of the presumptive f^{3N+} revertants recovered during this study segregated from a dominant autosomal f^{3N} suppressor, i.e. no

Table 2. *Spontaneous reversion frequency of* f^{3N} *in females*

	f^{3N} chromosomes scored	Total revertants recovered	Reversion frequency
(A) Control†	183783	19	$10 \cdot 3 \times 10^{-5}$‡
(B) Effect of second and third chromosome replacement:			
C(1)RM; *v* f^{3N} *car*; $2^u/2^u$; $3^u/3^u$§	50614	6	$11 \cdot 9 \times 10^{-5}$
v f^{3N} *car*; $2^c/2^c$; $3^c/3^c$‖	109681	8	$7 \cdot 3 \times 10^{-5}$
(C) Effect of replacement of *X*-chromosome segments:			
y w v^+ *m* f^{3N} *car*‖ with replaced *X*-chromosome from distal end to *m*	110928	8	$7 \cdot 2 \times 10^{-5}$
v f^{3N} *B* car^+‖ with replaced *X*-chromosome from f^{3N} to proximal end	100499	2	$2 \cdot 0 \times 10^{-5}$**
(D) Effect of *Bar*:¶			
v f^{3N} B^+ car^+‖	100361	2	$2 \cdot 0 \times 10^{-5}$**
(E) Location of *Mu*-f^{3N} on the *X*-chromosome:			
v f^{3N} B^+ *Mu*-f^{3N} Bx^2 car^+‖ with replaced *X*-chromosome from *B* to the proximal end	75990	5	$6 \cdot 6 \times 10^{-5}$
(F) A test for the ability of *Mu*-f^{3N} to increase f^{3N} reversion events in a 'cis' and 'trans' configuration in y^2 *v* f^{3N} *B*/*v* f^{3N} *Mu*-f^{3N} *car* females:‖			
y^2 *v* f^{3N} *B* ('trans' chromosome)	58236	1	$1 \cdot 7 \times 10^{-5}$*
v f^{3N} *Mu*-f^{3N} *car* ('cis' chromosome)	58236	5	$8 \cdot 6 \times 10^{-5}$

† Control frequency represents combined data from *C(1)RM*, *v* f^{3N} *car* females, *v* f^{3N} *car* females (Woodruff *et al.* 1972) and *v* f^{3N} females. This frequency is similar to the previously reported spontaneous f^{3N} reversion frequency in females (52/698698 = $7 \cdot 4 \times 10^{-5}$) (pooled data from Green, 1957, and from Altenburg & Browning, 1962).

‡ This frequency has 95 % fiducial limits of $6 \cdot 2 \times 10^{-5}$ and $16 \cdot 2 \times 10^{-5}$ by the method of Stevens (1942).

§ Mated to wild-type males, and attached-*X* females progeny scored for f^{3N} reversion events (2^u and 3^u represent autosomes from an *un* Bx^2 containing stock).

‖ Mated to f^{36a} Bx^2 males and all progeny scored for f^{3N} reversion events (2^c and 3^c represent autosomes from a *Canton-S* stock).

¶ The *v* f^{3N} B^+ car^+ stock was synthesized by recombination from a *v* f^{3N} *car*/*Canton-S* female (see text).

** Significantly different from the control frequency at the 1 % level.

* Significantly different from the control frequency at the 5 % level.

homozygous f^{3N+} revertants produced any forked offspring, and a recessive autosomal f^{3N} suppressor would not be functional as a heterozygote. To test for the presence of sex-linked suppressors of f^{3N}, 15 of the 57 free-*X* presumptive f^{3N+} revertants reported in this study were selected and examined. Revertant chromosomes were made homozygous and females were mated to *Canton-S* males in mass. Their F_1 presumptive-f^{3N+}/*Canton-S* female offspring were then mated in mass to *Canton-S* males, and F_2 progeny were scored for the presence of f^{3N+} and f^{3N} males, the assumption being that any X-linked f^{3N} suppressor could be separated from the f^{3N} mutation by recombination, thereby producing f^{3N} products. A total of 24801 f^{3N+} and 0 f^{3N} males were recovered from crosses with the 15 presumptive f^{3N+} revertants, an average of 1653 f^{3N+} F_2 males scored per stock. Therefore, none of the 15 presumptive f^{3N+} revertants contained a sex-linked f^{3N} suppressor, unless the suppressor was very tightly linked to the forked locus.

Spontaneous sex-linked recessive lethal mutations. The frequencies of spontaneous sex-linked recessive lethal mutations were determined by standard Drosophila techniques (Abrahamson & Lewis, 1971) using the *X*-chromosome balancer *FM7* (Merriam, 1968), which contained the markers y^{31d} w^a *v B*. Mutation frequencies were determined in both males and females in a stock which contained the mutator gene, *v* f^{3N} *Mu*-f^{3N} *car*, and in a stock that did not contain the mutator gene, *v* f^{3N} *Mu*-f^{3N+} *B*. The mutation frequency in males was determined by crossing males which contained or did not contain the mutator gene to virgin *FM7*/*FM7* females in mass. Individual F_1 females from these crosses were mated to one or two F_1 *FM7* male sibs and F_1 parents were removed before scoring F_2 progeny. The F_2 progeny were examined in the vials for the presence of non-*FM7* males. Any F_1 mating which produced at least one F_2 *FM7* male and no F_2 non-*FM7* males was subsequently retested for the presence of an X-linked lethal by mating individual F_2 heterozygous *FM7* females to individual F_2 male sibs. The frequency of spontaneous sex-linked recessive lethal mutations in females was determined by the following crosses:

P_0 *FM7*/*FM7* ♀♀ × *v* f^{3N} *Mu*-f^{3N} *car*/Y ♂♂ or *v* f^{3N} *Mu*-f^{3N+} *B*/Y ♂♂;
P_1 *FM7*/*v* f^{3N} *Mu*-f^{3N} *car* ♀♀ or *FM7*/*v* f^{3N} *Mu*-f^{3N+} *B* ♀♀ × *FM7*/Y ♂♂;
F_1 individual *FM7*/*v* f^{3N} *Mu*-f^{3N} *car* ♀ or individual *FM7*/*v* f^{3N} *Mu*-f^{3N+} *B* ♀ × *FM7*/Y ♂♂.

The F_2 offspring were then scored for the presence of *FM7* and non-*FM7* males. The initial P_0 cross was performed to eliminate any pre-existing *X*-chromosome recessive lethal mutations in the chromosomes to be tested.

3. RESULTS

Green (1970) and Woodruff *et al.* (1970, 1972) have suggested that a mutator gene may be partially responsible for the high reversion frequency of f^{3N}. The validity of this hypothesis was determined by an attempt to identify such a gene in f^{3N} containing stocks.

The identification of a mutator gene was based on the assumption that replacement of the mutator by its wild-type allele would lead to a reduction in the frequency of f^{3N} reversion events. To test this assumption, crosses were performed in which part of the genome of f^{3N} containing stocks was replaced by the genome from non-f^{3N} containing stocks. The replacement of a f^{3N} segment which contains a mutator would be expected to lead to a reduction in the f^{3N} reversion frequency. This rationale led to experiments which were performed to determine if the *X*, second, or third chromosomes of f^{3N} stocks contain a mutator gene. The fourth chromosome was not considered in these studies because of its small size in relation to the other chromosomes.

The following crosses involving the balanced lethal stock *Cy/Pm; CxD/Sb* were used to replace the second and third chromosomes of a *C(1)RM, v* f^{3N} *car* stock with the second and third chromosomes of a stock containing the sex-linked mutations *un* Bx^2. The *un* Bx^2 containing stock was used in this experiment because of the reported reduced f^{3N} reversion frequency observed in progeny of crosses between *un* Bx^2 and f^{3N} stocks (Woodruff *et al.* 1972). In this experiment, the autosomes of the f^{3N} containing stock are designated 2^f and 3^f, and those of the *un* Bx^2 containing stock are designated 2^u and 3^u. Crosses:

G_{1A} *C(1)RM, v* f^{3N} *car*; $2^f/2^f$; $3^f/3^f$ ♀♀ × ±/Y; *Cy/Pm*; *CxD/Sb* ♂♂;
G_{1B} *Cy/Pm*; *CxD/Sb* ♀♀ × *un* Bx^2/Y; $2^u/2^u$; $3^u/3^u$ ♂♂;
G_2 *C(1)RM, v* f^{3N} *car*; *Cy*/2^f; *CxD*/3^f ♀♀ (from G_{1A}) × ±/Y; *Pm*/2^u; *Sb*/3^u ♂♂ (from G_{1B});
G_3 *C(1)RM, v* f^{3N} *car*; *Cy*/2^u; *CxD*/3^u ♀♀ × ±/Y; *Cy*/2^u; *CxD*/3^u ♂♂;
G_4 *C(1)RM, v* f^{3N} *car*; $2^u/2^u$; $3^u/3^u$ ♀♀ × ±/Y; $2^u/2^u$; $3^u/3^u$ ♂♂.

The resultant G_4 *C(1)RM, v* f^{3N} *car*; $2^u/2^u$; $3^u/3^u$ female is homozygous for f^{3N} and the second and third chromosomes of the *un* Bx^2 containing stock. If a mutator is linked to either of the replaced f^{3N} autosomes, the frequency of f^{3N} reversion events in the female will be significantly reduced. The data in Table 2, part B, show that replacement of the second and third chromosomes does not affect the f^{3N} reversion frequency. Although these data indicate that a mutator gene is not present on the second or third chromosomes, another explanation is that a mutator gene is common to both the *C(1)RM, v* f^{3N} *car* and the *un* Bx^2 stocks. Therefore, the following crosses were performed in which the second and third chromosomes of the free-*X v* f^{3N} *car* stock were replaced by the second and third chromosomes of a *Canton-S* wild-type stock (designated 2^c and 3^c). Crosses:

G_1 *v* f^{3N} *car/v* f^{3N} *car*; *Cy/Pm*; *CxD/Sb* ♀♀ × ±/Y; $2^c/2^c$; $3^c/3^c$ ♂♂;
G_2 *v* f^{3N} *car/v* f^{3N} *car*; *Cy/Pm*; *CxD/Sb* ♀♀ × *v* f^{3N} *car*/Y; *Cy*/2^c; *CxD*/3^c ♂♂;
G_3 *v* f^{3N} *car/v* f^{3N} *car*; *Cy*/2^c; *CxD*/3^c ♀♀ × *v* f^{3N} *car*/Y; *Cy*/2^c; *CxD*/3^c ♂♂;
G_4 *v* f^{3N} *car/v* f^{3N} *car*; $2^c/2^c$; $3^c/3^c$ ♀♀ × *v* f^{3N} *car*/Y; $2^c/2^c$; $3^c/3^c$ ♂♂.

The data in Table 2, part B, show that replacement of the f^{3N} second and third chromosomes with the second and third chromosomes of the *Canton-S* stock does not cause a significant reduction in the f^{3N} reversion frequency. Therefore, unless

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the mutator gene is common to a number of stocks, the second and third chromosomes of the *C(1)RM*, *v* f^{3N} *car* and free-*X* *v* f^{3N} *car* stocks do not contain a mutator gene which affects f^{3N} reversion events.

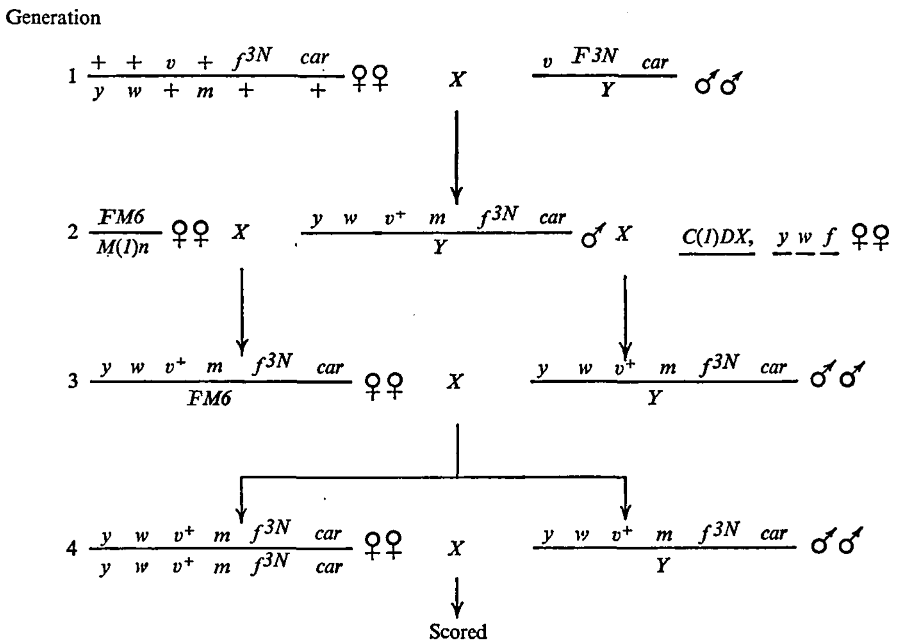

Fig. 1. The mating scheme employed to replace a section of the X chromosome to the left of f^{3N} in a v f^{3N} car/v f^{3N} *car* stock. The individual generation-2 male was recovered as a cross-over product between m and f^{3N} in the generation-1 females. The generation-2 male was double mated.

There is of course no *a priori* reason for limiting a mutator gene to the autosomes. The reduction in the f^{3N} reversion frequency which was reported in outcrossed f^{3N} stocks, could have resulted from removal of an X-chromosome mutator gene by recombination (Woodruff *et al.* 1972). Thus, the X chromosome of a f^{3N} stock was surveyed for a gene affecting f^{3N} reversion events by replacing sections of the X chromosome to the left and to the right of f^{3N} with a non-f^{3N} X chromosome. If a mutator is present in either of these X-chromosome sections, its replacement with its wild-type allele would lead to a reduction in the frequency of f^{3N} reversion events. The crosses given in Fig. 1 were performed to replace a section of the X chromosome to the left of f^{3N}. The X chromosome from at least the locus of miniature (m, 1–36.1) to the distal end has been replaced in the generation-4 (y w v^+ m f^{3N} *car*) offspring. The inclusion of the *yellow* (y, 1–0.0), *white* (w, 1–1.5), and *vermilion* (v, 1–33.0) markers in these crosses aided in the exclusion of multiple recombination events to the left of m in the above generation-1 cross. The f^{3N} reversion frequency in the y w v^+ m f^{3N} *car* generation-4 females is shown in part C of Table 2. Since the data show that the f^{3N} reversion frequency was not signifi-

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cantly reduced, a mutator does not appear to be in the X-chromosome region from the distal end to m.

A similar experiment was performed in which a section of the X chromosome to the right of f^{3N} was replaced by the crosses in Fig. 2. In these crosses, the f^{3N} containing X chromosome was replaced from at most f^{3N} to the proximal end. The results show that the f^{3N} reversion frequency in the $v\ f^{3N}\ B\ car^{+}$ generation-4 females was significantly reduced (Table 2, part C). It is therefore likely that the X chromosome from f^{3N} to the proximal end, approximately 13 map units, contains a gene which affects f^{3N} reversion events. The f^{3N} reversion frequency was

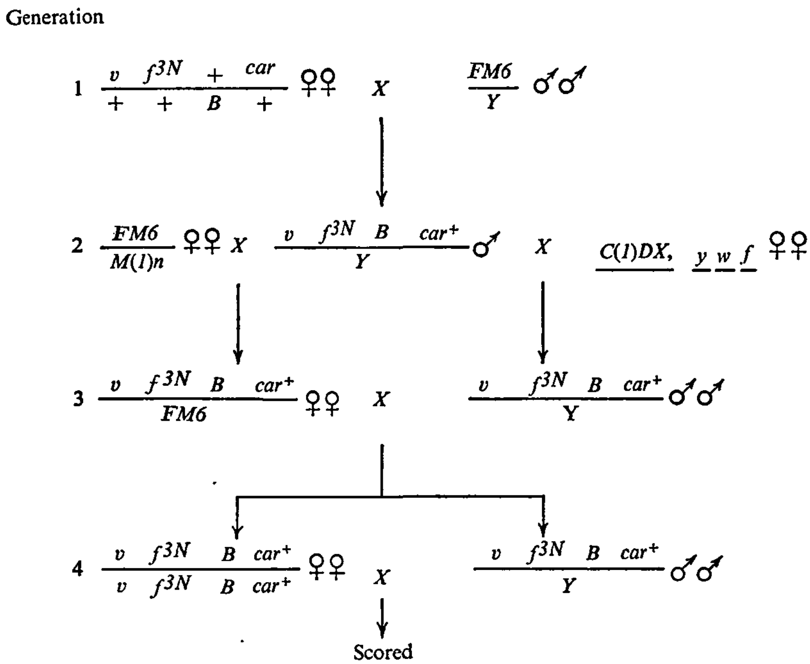

Fig. 2. The mating scheme employed to replace a section of the X chromosome to the right of f^{3N} in a $v\ f^{3N}\ car/v\ f^{3N}\ car$ stock. The individual generation-2 male was recovered as a cross-over product between f^{3N} and B in the generation-1 female. The generation-2 male was double mated.

also determined in the above $v\ f^{3N}\ B\ car^{+}$ males by crossing the males to $C(1)DX$, $y\ w\ f$ females and scoring patroclinous male offspring for f^{3N} reversions. No f^{3N+} revertants were observed among 51813 patroclinous male offspring.

Although the above data do suggest the presence of a mutator on the X chromosome, the reduction in the frequency of f^{3N} reversion events could be a consequence of the presence of *Bar* (B, 1–57.0) on the tested X chromosome. Since B is a tandem duplication located only 0·3 map units from the *forked* locus, it may interfere with the reversion of f^{3N}. To test this possibility, B in the above low f^{3N}-reverting females ($v\ f^{3N}\ B\ car^{+}$) was replaced by its wild-type allele through use of the Fig. 3 crosses. If B, instead of a mutator gene, is the cause of the reduced f^{3N} reversion frequency, this frequency in the $v\ f^{3N}\ B^{+}\ car^{+}$ generation-5 females should

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return to its previous high value. The data in part D of Table 2 show, however, that the f^{3N} reversion frequency is still significantly reduced. Hence, *B* is not the cause of the reduced f^{3N} reversion frequency. Again the simplest explanation is that a section of the *X* chromosome to the right of f^{3N} contains a mutator gene which influences f^{3N} reversion events. It should be noted that this mutator gene is absent in both types of tested females in parts C and D of Table 2, and in both cases a significant reduction in f^{3N} reversion frequency is observed.

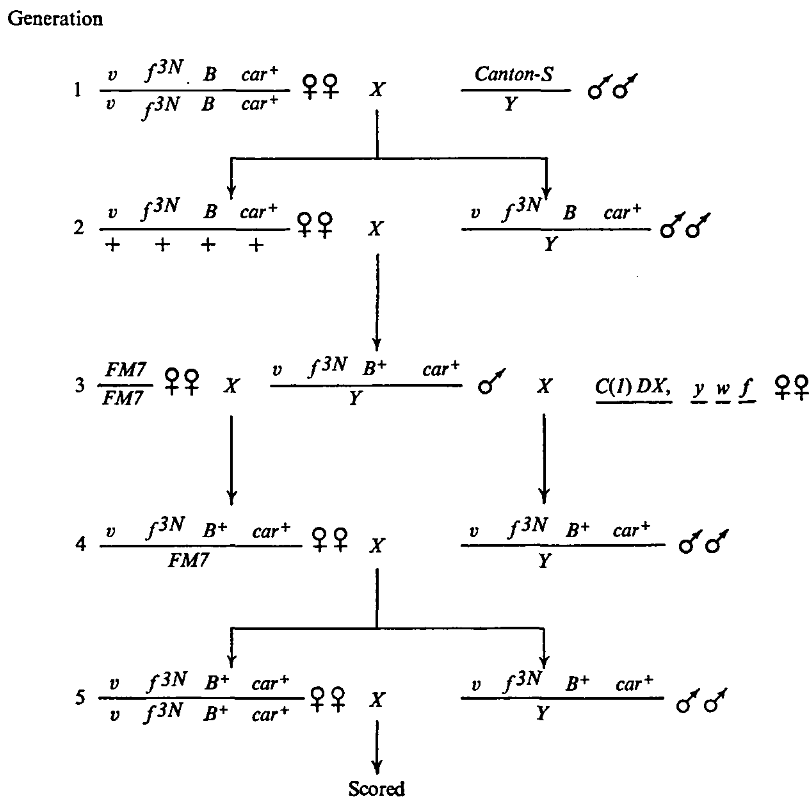

Fig. 3. The mating scheme employed to replace the *B* mutation by its wild-type allele in a low f^{3N}-reverting female ($v\ f^{3N}\ B\ car^+$). The individual generation-3 male was recovered as a cross-over product between f^{3N} and *B* in the generation-2 female. The generation-3 male was double mated.

The remainder of this study is an analysis of the genetic nature of the presumed mutator gene. To determine if the mutator gene is a dominant or a recessive mutation, f^{3N} reversion events were scored in females heterozygous for the mutator gene and its wild-type allele. If the mutator gene is recessive, its influence on f^{3N} reversion events would be masked in a heterozygote. On the other hand, a dominant mutator would increase the frequency of f^{3N} reversion events in a heterozygote. Hence, $v\ f^{3N}\ car$ females, which contained the mutator gene, were mated to $f^{36a}\ B$ males which did not contain the mutator gene. By means of this cross, the resultant $v\ f^{3N}\ car/f^{36a}\ B$ F_1 females would contain a mutator gene as a heterozygote with its wild-type allele. The reversion frequency of f^{3N} was determined by mating the $v\ f^{3N}\ car/f^{36a}\ B$ F_1 females to $f^{36a}\ B$ males and scoring $v\ f^{3N}\ car/f^{36a}\ B$ F_2 females and $v\ f^{3N}\ car$ F_2 males for f^{3N+} revertants. From 54682 scored f^{3N} F_2 chromosomes, five f^{3N+} revertants were recovered, frequency $= 9{\cdot}2\times10^{-5}$. In an additional experiment, a similar f^{3N} reversion frequency ($7/88865 = 7{\cdot}9\times10^{-5}$) was observed in females heterozygous for *Df(1)* B^{263-20} and $v\ f^{3N}\ car$. Since these frequencies are not significantly different from the control ($10{\cdot}3\times10^{-5}$ Table 2), the mutator gene appears to exert the same influence on f^{3N} reversion events in a homozygote and in a heterozygote. Hence, the mutator gene is dominant and has been given the name *Mutator*-f^{3N} (*Mu*-f^{3N}).

To map *Mu*-f^{3N} more precisely, one recombination event between *B* (1–57.0) and *Beadex-2* (Bx^2, 1–59.4) from $v\ f^{3N}\ car/B\ Bx^2$ females was analysed for the presence or absence of *Mu*-f^{3N}. The data in Table 2, part E, show no signficant reduction in the f^{3N} reversion frequency in the recovered $v\ f^{3N}\ B^+\ Bx^2\ car^+$ recombinant. This suggests that the recombinant contains *Mu*-f^{3N}.

With this in mind, an analysis of the possible origins of the recombinant provides an indication of the location of *Mu*-f^{3N}. Hence, the three possible locations of *Mu*-f^{3N} within the *X* chromosome are as follows.

(A) $\dfrac{v\ \ f^{3N}\ \ Mu\text{-}f^{3N}\ \ +\ \ +\ \ car}{+\ \ +\ \ \ \ +\ \ \ \ B\ \ Bx^2\ \ +}$

(B) $\dfrac{v\ f^{3N}\ +\ \ Mu\text{-}f^{3N}\ \ +\ \ car}{+\ \ +\ B\ \ \ +\ \ \ \ Bx^2\ \ +}$

(C) $\dfrac{v\ \ f^{3N}\ +\ \ +\ \ Mu\text{-}f^{3N}\ \ car}{+\ \ +\ \ B\ \ Bx^2\ \ \ +\ \ \ \ +}$

The $v\ f^{3N}\ B^+\ Bx^2\ car^+$ recombinant which contained *Mu*-f^{3N} would be possible in (A) and (B) by a single cross-over event between *B* and Bx^2, whereas in (C) the same recombinant would only be formed by an infrequent triple cross-over event between *B* and *car* (5·5 map units). The recovery of the $v\ f^{3N}\ B^+\ Bx^2\ car^+$ recombinant which contained *Mu*-f^{3N}, therefore, suggests that *Mu*-f^{3N} is located between f^{3N} (1–56.7) and Bx^2 (1–59.4), i.e. no more than 2·7 map units from the mutation it affects.

Some mutator genes in *Escherichia coli* (Cox, Degnen & Scheppe, 1972) and in yeast (von Borstel, Cain & Steinberg, 1971) have been shown to function in the 'trans' position to the genes they affect. These observations suggest that these mutator genes produce a diffusible product. It is of interest to determine if the eukaryotic mutator *Mu*-f^{3N} functions in the 'trans' position to f^{3N}, and, therefore, possibly produces a diffusible product.

To attain the above objective, f^{3N} reversion events were scored in $y^2\ v\ f^{3N}\ B/v\ f^{3N}$ *Mu*-f^{3N} *car* females mated to $f^{36a}\ Bx^2$ males. If *Mu*-f^{3N} functions only in the 'cis' configuration, then the reversion frequency of f^{3N} in the 'trans' position ($y^2\ v\ f^{3N}\ B$ chromosome) should be about $2{\cdot}0\times10^{-5}$, whereas the reversion frequency of f^{3N} in the 'cis' position ($v\ f^{3N}\ Mu\text{-}f^{3N}\ car$ chromosome) should be about $10{\cdot}3 \times 10^{-5}$. Yet, if $Mu\text{-}f^{3N}$ functions in the 'cis' and 'trans' configurations, then the f^{3N} reversion frequency in both of the X chromosomes should be about $10{\cdot}3 \times 10^{-5}$. The results in part F of Table 2 show that $Mu\text{-}f^{3N}$ seems to function only in the 'cis' configuration.

Table 3. *The frequency of spontaneous sex-linked recessive lethals in males and females in the presence and absence of* Mu-f^3N^ *(see text for details)*

	Chromosomes tested	Lethals	%
$v\ f^{3N}\ Mu\text{-}f^{3N}\ car$			
♀♀	1037	3	0·29
♂♂	1042	1	0·01
$v\ f^{3N}\ Mu\text{-}f^{3N+}\ B$			
♀♀	1098	2	0·18
♂♂	1024	0	0·00

From the above data it is apparent that some f^{3N} stocks do contain a mutator which influences the reversion of f^{3N}. These data do not, however, indicate if $Mu\text{-}f^{3N}$ is a specific mutator gene which affects only f^{3N} reversion events or if it is a general mutator gene which affects the mutation of many genes. Accordingly, the influence of $Mu\text{-}f^{3N}$ on the frequency of spontaneous sex-linked recessive lethal mutations was evaluated in males and in females. If $Mu\text{-}f^{3N}$ has a generalized effect on mutation, the frequency of lethals would be increased in its presence. The results of these experiments are shown in Table 3. It is apparent that $Mu\text{-}f^{3N}$ does not have a significant influence either in males or females, on the frequency of spontaneous sex-linked recessive lethals.

In addition, the data in this paper show that the reversion frequencies of eight sex-linked alleles other than f^{3N} are apparently not affected by $Mu\text{-}f^{3N}$. No reversions were observed in the presence of $Mu\text{-}f^{3N}$ for the following genes: *vermilion* (0/517838 chromosomes scored); *carnation* (0/509189); *yellow* (0/55464); *white* (0/55464); *miniature* (0/55464); *Beadex-2* (0/37995); *Bar* (0/26118) and *yellow-2* (0/26118). Additional experiments are in progress to test the influence of $Mu\text{-}f^{3N}$ on other unstable alleles.

4. DISCUSSION

The data in this paper support the hypothesis that a mutator gene, $Mu\text{-}f^{3N}$, is partially responsible for the instability of f^{3N}. It has been shown that $Mu\text{-}f^{3N}$ has the following genetic characteristics: (1) it is a dominant mutation, (2) it maps between positions 56.7 and 59.4 in the X chromosome, (3) it is apparently a specific mutator which affects only f^{3N} reversion events and (4) it functions only in the 'cis' configuration with f^{3N}.

Woodruff *et al.* (1972) proposed that a mutator gene is responsible for the sex-based dichotomy in the reversion frequency of f^{3N} (reversions of f^{3N} in females occur at a significantly higher frequency than in males). If this proposal is correct, the frequency of recessive sex-linked lethals in males (see Table 3) should not be influenced by $Mu\text{-}f^{3N}$. The data herein do support this proposal, but they do not prove it. For example, the spontaneous reversion frequency of f^{3N} in males which contain $Mu\text{-}f^{3N}$ (2/101 304 = $2{\cdot}0 \times 10^{-5}$) (Woodruff *et al.* 1972), is similar to the frequency in females which do not contain $Mu\text{-}f^{3N}$ (2/100 499 part C of Table 2 + 2/100 361 part D of Table 2 = $2{\cdot}0 \times 10^{-5}$). It is possible, however, that $Mu\text{-}f^{3N}$ causes a general increase in f^{3N} reversions in both sexes, and that the sex-based dichotomy of f^{3N} reversions is intrinsic to the f^{3N} allele. If this situation is true, the frequency of f^{3N} reversions in both females and males should be significantly different in the presence and absence of $Mu\text{-}f^{3N}$. In contrast, if $Mu\text{-}f^{3N}$ is female specific in increasing the reversion frequency of f^{3N}, then the frequency of f^{3N} reversions in males should be independent of $Mu\text{-}f^{3N}$. Therefore, to determine which of the above two possible modes of action of $Mu\text{-}f^{3N}$ is correct, the reversion frequencies of f^{3N} in both sexes in the presence and absence of $Mu\text{-}f^{3N}$ must be compared. Data from this paper and from previous experiments (Woodruff *et al.* 1972) show that the f^{3N} reversion frequency in females in the presence of $Mu\text{-}f^{3N}$ ($10{\cdot}3 \times 10^{-5}$) is significantly different from the frequency in females in the absence of $Mu\text{-}f^{3N}$ ($2{\cdot}0 \times 10^{-5}$). A comparable study with f^{3N} males is incomplete. It is known that f^{3N} reverts in $Mu\text{-}f^{3N}$ males at a frequency of $2{\cdot}0 \times 10^{-5}$ (2/101 304) (Woodruff *et al.* 1972), but the frequency of f^{3N} reversions in $Mu\text{-}f^{3N+}$ males has not been fully determined. A preliminary experiment showed no f^{3N} reversions in $Mu\text{-}f^{3N+}$ males among 51 813 scored chromosomes. Additional counts are presently in progress to determine more accurately the f^{3N} reversion frequency in $Mu\text{-}f^{3N}$ males, and, therefore, to delimit the mode of action of $Mu\text{-}f^{3N}$.

Green (1970) has reported the isolation of a third-chromosome mutator gene, *mt*, in *Drosophila melanogaster* which functions only in females. With this evidence, plus the observation that in *mt* females crossing over in the *X* chromosome is reduced, Green (1970) has suggested that *mt* is associated with the crossing-over event. Two observations indicate that this situation may also be true for $Mu\text{-}f^{3N}$. First, $Mu\text{-}f^{3N}$ may function only in females, the sex to which recombination is ordinarily restricted in *Drosophila melanogaster*; secondly, recombination in the proximal half of the *X* chromosome is reduced in the presence of $Mu\text{-}f^{3N}$ (Woodruff, 1973). On the other hand, f^{3N} reversion events in the presence of $Mu\text{-}f^{3N}$ are not dependent on exchange of outside markers (Woodruff *et al.* 1972). It might be informative to determine if $Mu\text{-}f^{3N}$ and *mt* function in females in the presence of recombination-deficient mutations.

From the above facts and from the observation that *mt* also increases the reversion frequency of f^{3N} in females (Green, 1970), one might surmise that *mt* and $Mu\text{-}f^{3N}$ have the same mode of action. Yet, these two genes differ in their ability to increase the spontaneous frequency of sex-linked recessive lethal mutations – *mt* increases this frequency (Green & Lefevre, 1972), whereas $Mu\text{-}f^{3N}$ apparently does not. It is possible that a small but significant influence of $Mu\text{-}f^{3N}$ on the spontaneous frequency of sex-linked recessive lethal mutations might not have been observed in this study because of the paucity of data reported in Table 3. At any rate, $Mu\text{-}f^{3N}$ does not increase this frequency to the same extent as *mt*.

Although not conclusive, this observation would suggest that the two mutators have different modes of action.

In prokaryotic organisms, several factors cause a general increase in the frequency of mutation events. These factors include a defective repair system (Bohme, 1967; Jyssum, 1968; Hill, 1970), a defective DNA polymerase (Speyer, 1965; Coukell & Yanofsky, 1970; Berg, 1971; Bernstein, 1971; Hall & Brammar, 1973), a defective DNA replication enzyme (Gross, Karamata & Hempstead, 1968; Bernstein *et al.* 1972), a defective ligase (Koch & Drake, 1973), and the production of an aberrant DNA precursor (Kirchner & Rudden, 1966). By analogy, the apparent inability of *Mu-*f^{3N} to increase the frequency of general mutation events, i.e. the frequency of spontaneous sex-linked recessive lethal mutations and the frequency of reverse mutations at alleles other than f^{3N}, suggests that *Mu-*f^{3N} does not function by any of these mechanisms. *Mu-*f^{3N}, because of its apparent allele-specific action, does resemble certain genes isolated in *Zea mays* (Rhoades, 1941; McClintock, 1965) and in *Antirrhinum majus* (Harrison & Fincham, 1968).

Mutator genes which increase the frequency of mutation of unstable genes have been observed in maize and in one other Drosophila species. Emerson (1929) hypothesized that a modifying gene or genes influenced the mutability of the variegated pericarp gene in maize. Demerec (1929) reported the isolation of five genetic factors which stimulated the mutation of the unstable *miniature* mutant of *Drosophila virilis*. Unlike *Mu-*f^{3N}, four of these genes only increased the frequency of somatic mutation events. Although somatic reversions of f^{3N} have been reported (Altenburg & Browning, 1962), no somatic f^{3N+} revertants were observed in this study either in the presence or absence of *Mu-*f^{3N}. This fact may indicate that a meiotic event is essential for the function of *Mu-*f^{3N}.

The inability of *Mu-*f^{3N} to increase the frequency of f^{3N} reversion events in the 'trans' configuration suggests that *Mu-*f^{3N} does not produce a diffusible product, implying that it has a localized effect. Due to this apparent localized effect, it might be conjectured that *Mu-*f^{3N} is associated with a chromosomal structural change. Yet, the salivary gland *X* chromosome of a *Mu-*f^{3N} stock seems to be structurally normal (unpublished data).

Finally, it should be noted that a large amount of data has been accumulated on the spontaneous reversion frequency of f^{3N}, making f^{3N} one of the most intensely studied mutants in eukaryotes. From the present study and from the previous study of Woodruff *et al.* (1972), the frequency of f^{3N} reversions in females which contain *Mu-*f^{3N} is $63/732799 = 8{\cdot}6 \times 10^{-5}$. In addition, Altenburg & Browning (1962), and Green (1957) have reported f^{3N} reversion frequencies in females of $38/400000 = 9{\cdot}5 \times 10^{-5}$ and $14/298698 = 4{\cdot}7 \times 10^{-5}$ respectively. It is not known whether *Mu-*f^{3N} was present in the f^{3N} stocks used by Altenburg & Browning (1962) and by Green (1957); however, the similarities in f^{3N} reversion frequencies between these two previous studies and the present study suggest that *Mu-*f^{3N} was present in all stocks. In any event, the combined f^{3N} reversion frequency in females is $115/1431477 = 8{\cdot}0 \times 10^{-5}$.

In contrast to the above frequency, the f^{3N} reversion frequency in females that do not contain $Mu\text{-}f^{3N}$ is $4/200\,860 = 2{\cdot}0 \times 10^{-5}$, whereas the f^{3N} reversion frequency in males with $Mu\text{-}f^{3N}$ is $2/101\,340 = 2{\cdot}0 \times 10^{-5}$ and in males without $Mu\text{-}f^{3N}$ is 0/51 813.

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