SITE-SPECIFIC INSTABILITY IN *DROSOPHILA MELANOGASTER*: EVIDENCE FOR TRANSPOSITION OF DESTABILIZING ELEMENT

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ABSTRACT

In this study, we show that at least one lethal mutation at the 3F–4A region of the *X* chromosome can generate an array of chromosome rearrangements, all with one chromosome break in the 3F–4A region. The mutation at 3F–4A (secondary mutation) was detected in an *X* chromosome carrying a reverse mutation of an unstable lethal mutation, which was mapped in the 6F1–2 doublet (primary mutation). The primary lethal mutation at 6F1–2 had occurred in an unstable chromosome (*Uc*) described previously (LIM 1979). Prior to reversion, the 6F1–2 mutation had generated an array of chromosome rearrangements, all having one break in the 6F1–2 doublet (LIM 1979, 1980). In the *X* chromosomes carrying the 3F–4A secondary lethal mutation the 6F1–2 doublet was normal and stable, as was the 3F–4A region in the *X* chromosome carrying the primary lethal mutation. The disappearance of the instability having a set of genetic properties at one region (6F1–2) accompanied by its appearance elsewhere in the chromosome (3F–4A) implies that a transposition of the destabilizing element took place. The mutant at 3F–4A and other secondary mutants exhibited all but one (reinversion of an inversion to the normal sequence) of the eight properties of the primary lethal mutations. These observations support the view that a transposable destabilizing element is responsible for the hypermutability observed in the unstable chromosome and its derivaties.

HYPERMUTABILITY in an unstable *X* chromosome (*Uc*) and its derivatives is associated with a set of eight genetic properties (LIM 1979, 1980): (1) the occurrence of recessive lethal mutations at one site, the 6F1-2 doublet, on the *X* chromosome, (2) the frequent reversion of these mutations, (3) the generation of different rearrangements with a common breakpoint in the 6F1-2 doublet, (4) the appearance of identical but independent chromosome aberrations in the progeny of different flies, (5) the apparent confinement of chromosome breakage events to the *X* chromosome, (6) the occasional reinversion of an inversion to the normal sequence, (7) the presence of a unique abnormal cytological structure or "amorphous mass," and (8) the leapfrogging of a breakpoint, *i.e.*, the involvement of a pre-existing breakpoint in the generation of additional aberrations. A transposable destabilizing element inserted in the 6F1-2 doublet of the *X* chromosome was thought to be responsible for the hypermutability.

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In this paper, we report the isolation and characterization of secondary lethal mutations, *i.e.*, lethal mutations detected in *X* chromosomes carrying reversions of the primary lethal mutations at 6F1-2. The majority of the secondary lethals were localized at the 3F-4A region, the 6F region, or at 25.8 of the *X* chromosome. The secondary lethal mutations exhibited all but one (reinversion) of the eight properties of the primary lethal mutations mentioned above. One of the secondary mutations mapped at the 3F-4A region having the amorphous mass generated an array of chromosome rearrangements each having one break in the 3F-4A region. The 6F1-2 region of *X* chromosomes which carried 3F-4A mutations was apparently normal and stable. Elimination of the instability at one locus (6F1-2), followed by its appearance elsewhere in the *X* chromosome (3F-4A), implies that a transposition of the destabilizing element took place.

MATERIALS AND METHODS

Flies were reared on standard cornmeal–molasses–brewers yeast–agar medium at 25°. A description of mutant symbols used in this study is summarized in Table 1. Table 2 gives a description of the chromosomes used. Additional information on mutant symbols and chromosomes may be found in LINDSLEY and GRELL (1968). A description of males carrying *X*-chromosome deficiencies and compensating duplications for mapping purpose is presented in Table 3.

Hereafter, a line (mutant line, revertant line, etc.) refers to the individuals descended from one fly. The single fly cultures established from individuals of a given line will be defined as sublines. A mutant line in a specific generation (16th or 20th generation, etc.) refers to the number of generations starting from the time the mutant line was established from a single carrier female.

Revertant males used in this study were isolated from the *59b-z Uc-l* stocks of LIM (1979). Females heterozygous for *Df(1)Basc* and *59b-z Uc-l X* chromosomes were mated individually to *Df(1)Basc/sc*^{8}*.Y* males, and revertant males detected among the progeny were mated individually to *C(1)DX,yf/Y* females to establish stocks. These were subsequently maintained by matings between revertant males and *C(1)DX,yf/Y* females.

Isolation of the secondary lethal mutations

Revertant males, *59b-z Uc-l*^{r}*/Y*, were individually mated to females heterozygous for the *Df(1)Basc* and *FM6 l*^{69a} *X* chromosomes. Twenty *FM6 l*^{69a}*/59b-z Uc-l*^{r} and 20 *Df(1)Basc/ 59b-z Uc-l*^{r} F_1 daughters from each revertant male were mass-mated to *FM6/Y* males for four days, and then the inseminated females were placed individually into vials for screening the lethal mutations. The second experiment was conducted in a similar manner except ten *Df(1)Basc/59b-z Uc-l*^{r} F_1 daughters were sampled, instead of 40 F_1 daughters, from each revertant male.

Localization of the secondary lethal mutations

Females of the constitution *FM6/59b-z Uc-l'*, where *Uc-l'* means a secondary lethal mutation, were mated individually to various males carrying deficiencies and compensating duplications (see Table 3) for mapping purpose. The mutations detected in five of the 11 revertant males could not be mapped using the deficiency/duplication stocks. The recombination data were used to determine the position of these lethals. The recombination data for each of these lethal lines were based on at least 2,000 sons from *59b-z Uc-l'/m* females. The markers in the females were *y*^{59b} *z w*^{i} *ct*^{6} *f Uc-l'/m*. The analysis of recombination events to the left of *w* (1.5) was not attempted, however, since the leftmost lethal was mapped in the region between *w*^{i} and *ct* (7.6 ± 1.3).

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TABLE 1

Synopsis of gene symbols used in text

Symbol	Phenotype	Chromosome and location
B	Bar eye, eye restricted to vertical narrow bar	X–57.0
ct^6	cut-6, wings cut to points and edges scalloped	X–20.0
ct^{J6}	cut-J6, cut wings associated with $Df(1)ct^{J6}$	
dm	diminutive, bristles and body small and slender, female sterile	X– 4.6
f	forked, bristles bent and shortened	X–56.7
Ki	Kinked, bristles and hairs shortened and twisted	3–47.6
m	miniature, size of wing reduced	X–36.1
mal	maroonlike, brownish purple eye color	X–64.8
sc^8	scute-8, allele of sc, scute bristle	X– 0.0
sn	singed, bristles and hairs twisted and shortened	X–21.0
sn^{13a}	singed-13a, sn associated with $Dp(1;3)\ sn^{13a}$	
sn^{+72d}	wild allele of sn associated with $T(1;2)\ sn^{+72d}$	
w^{+64b13}	wild allele of w, white eye, associated with $T(1;2)w^{+64b13}$	X– 1.5
w^a	white-apricot, yellowish-orange eye color	
w^i	white-ivory, eyes very light yellow	
y^2	yellow-2, yellow body color with black bristles	X– 0.0
y^{31d}	yellow-31d, yellow body color with black bristles	
y^{59b}	yellow-59b, yellow body color with brownish-yellow bristles, y^{59b}/y^2 is wild-type	
z	zeste, eyes of females lemon yellow, wild-type in males. Requires two copies of w to express itself	X– 1.0

TABLE 2

Chromosomes used in the experiment

Abbreviation	Description
59b-z	A normal X chromosome with the standard sequence carrying five recessive markers y^{59b}, z, w^i, ct^6, and f.
59b-z Uc	An unstable X chromosome derived from *59b-z* (*Unstable chromosome*).
59b-z Uc-l	An X-linked lethal mutation of "spontaneous" origin generated in the *unstable chromosome* (*Primary lethal mutation*).
59b-z Uc-l^r	A revertant chromosome of *Uc-l* (*revertant*).
59b-z Uc-l'	An X-linked lethal mutation of "spontaneous" origin in the *Uc-l*^{r} (Secondary *lethal mutation*).
C(1)DX,yf	An attached-X chromosome homozygous for y and f.
FM6	First multiple 6; a full description is included in LINDSLEY and GRELL (1968).
FM6-l^{69a}	An *FM6* chromosome carrying a recessive lethal mutation, l^{69a}.
Df(1)Basc	A *Basc* chromosome carrying $Df(1)sc^8$, which represents a deletion of at least four recessive-lethal loci to the left of sc, including y, and sc.
FM6K	An *FM6* chromosome with w^i and dm^+.
$sc^8 \cdot Y$	A Y chromosome with a tip of $In(1)sc^8$ including l^{J1}, y^+, and ac^+ but not sc transferred to tip of Y^L distal to KL. The sc^8 segment covers the deficiency in *Df(1)Basc*.

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TABLE 3

Males carrying deficiency and compensating duplication chromosomes used for the complementation analysis

Genotype of males	Cytology	
	Deficiency	Duplication
$Df(1)\ y^{75e}/y^{2}\ Y^{67g}$	1A1–2;1B6–9	tip to 2B17–2C3
$Df(1)\ ct^{J6}/Y;Dp(1;3)\ sn^{13a}/Ki$	6E1;7C1	6C11;7C9
$Df(1)\ sn/Y;T(1;2)\ sn^{+72d}$	7A8;8A5*	7A8;8A5
$Df(1)\ m^{259-4}/y^{+}\ Y\ v^{+}B^{-}$	10C2–3;10E2–3	9F3;10E3–4;20B
$Df(1)\ mal^{3}/y^{+}\ Y\ mal^{106}$	19A1–2;20A	1A2;1B2;18F
$Df(1)\ w/Y;T(1;2)\ w^{+64b13}$	3C2;5A1;26D	3C2;5A1;26D**

* Haploinviable in females.
** Males carrying a normal chromosome and the duplication are inviable.

Study of revertability

To assess the revertability of the secondary lethal mutations, ten *FM6/59b-z Uc-l'* females from each lethal line were mated individually to *FM6/Y* males. The presence of revertant males (*59b-z Uc-l'/Y* males) was checked until at least 30 FM6 male progeny from each female were examined.

Cytological analysis

Progressive changes in the chromosome structure: The larvae with light brown mouth parts (*59b-z/59b-z Uc-l'*) from *FM6/59b-z Uc-l'* females who had been mated to *59b-z/Y* males were used for the examination of polytene chromosomes. Only slides with at least 30 analyzable nuclei were used. The technique for polytene chromosome preparation has been described by LIM and SNYDER (1968).

For the lethal mutations isolated in the first experiment, the chromosomes were examined when the mutants were in the third generation and again in the 15th generation. The chromosomes of lethal mutations from the second experiment were also analyzed at two different times, in the first and in the sixth generations. The rationale for examining the chromosomes at different times was to detect progressive changes in the chromosome structure, as was done by LIM (1980).

Amorphous mass: The cytological analysis of several *FM6K/59b-z Uc-l'* females in the 20th generation revealed the presence of an abnormal structure called the amorphous mass at the 3F–4A region in the *Uc-l'* chromosome. Some examples of the amorphous mass are shown in Figure 1. Henceforth, *Am* will be used as the abbreviation for the amorphous mass. One of the females carrying *Am* in her *59b-z Uc-l'* chromosome, designated as *27e3*, was mated to *FM6/Y* males to obtain *FM6/59b-z Uc-l'* daughters. Twenty *FM6/59b-z Uc-l'* daughters were mated individually to *59b-z/Y* males, and the polytene chromosomes of five larvae with light brown mouth parts (*59b-z/59b-z Uc-l'*) from each cross were examined.

As a control, a single female from *27d1*, a lethal line related to *27e3* but lacking the amorphous mass, was mated to *FM6/Y* males. A sample of 20 daughters was taken from the cross, and 100 slides were prepared in the same manner.

RESULTS

Isolation of secondary lethal mutations

The results from the experiments to detect secondary lethal mutations are summarized in Table 4. From the first experiment, 18 lethal mutations were detected in 1,651 sperm sampled for a frequency of 1.09 ± 0.64%. In the second experiment, 17 lethal mutations were detected in 1,575 sperm sampled, for

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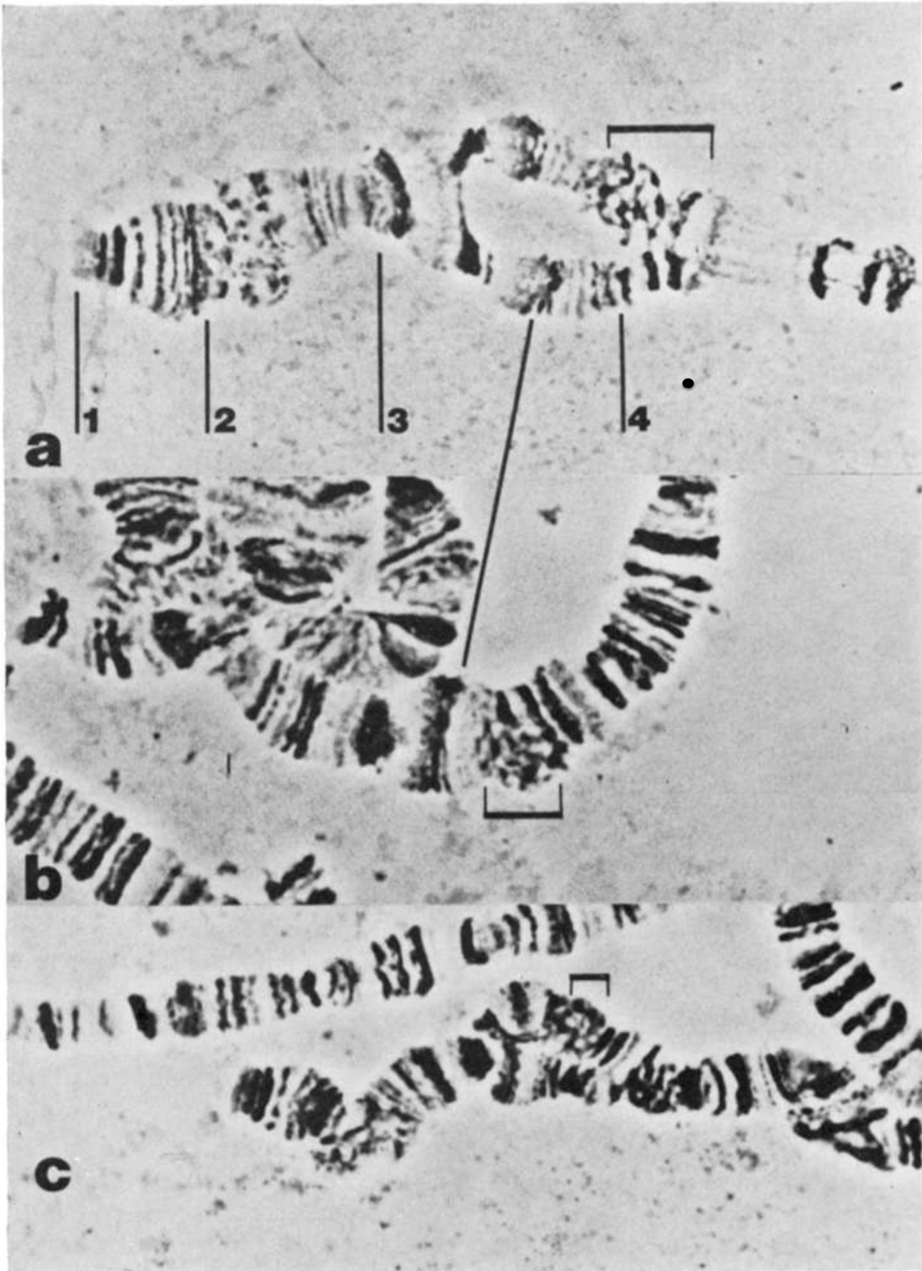

FIGURE 1.—Amorphous mass observed in the progeny from *27e3*. The brackets indicate the location of the amorphous mass. (a) An amorphous mass at 3F to 4C region in the upper chromosome. (b) An amorphous mass in the lower chromosome at 3F to 4A region. (c) The upper chromosome represents *Dp(1;1)3D;4A* with an amorphous mass at 4A–B. The new sequence is: tip to 3F2–5/3D2–4 to 4A/amorphous mass/4B1–2 to base. Note a sharp, dark, doublet representing 4B1–2 immediately next to the amorphous mass, and two distinct strands connecting the amorphous mass and 3D.

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TABLE 4

Summary of the experiments for lethal mutation detection

Source		Revertant males tested	F_1 females tested	F_1 females with lethals
Experiment 1				
IJA3–10		18	688	0
IJA3–10		1 .... *13**	40	1
IJA3–15		16	629	0
IJA3–15		1 .... *23*	40	1
IJA3–15		1 .... *27*	35	7
IJA3–15		1 .... *29*	20	1
IJA3–15		1 .... *33*	40	8
IJA3–16		4	159	0
	Total	43	1,651	18
Experiment 2				
IJA3–10		32	317	0
IJA3–15		34	314	0
IJA3–15		1 .... *22j*	10	8
IJA3–16		28	266	0
IJA3–16		1 .... *41d*	10	1
IJA3–16		1 .... *43a*	7	1
IJA3–16		1 .... *43b*	10	5
IJA3–16		1 .... *43g*	10	1
IJA3–16		1 .... *44h*	10	1
IJA3–17		34	328	0
IJA3–19		33	283	0
	Total	167	1,575	17

* The italicized numbers represent the designation of the revertant males that produced lethal-bearing sperm.

a frequency of 1.08 ± 0.65%. The weighted formula of ENGELS (1979) was used to calculate the standard deviation. These figures are more than fivefold higher than the widely accepted "background" frequency for *X*-linked recessive lethal mutations.

The fertility of the F_1 females was exceptionally good for both experiments. Less than 2% of the F_1 females were sterile in each of the experiments.

Localization of the secondary lethal mutations

The results of experiments for localizing the lethal mutations are summarized in Table 5. Twenty-three of the 35 lethal mutations detected from both experiments were mapped by using the set of deficiency and compensating duplication stocks listed in Table 3. All members within a cluster of lethal mutations were mapped to the same region on the *X* chromosome. Like the primary mutants (LIM 1979) a number of secondary mutations of independent origin were mapped at the same region of the *X* chromosome. Thus, lethal mutations from two males in the first experiment (*27* and *33*) were mapped in the 3C2-5A1 region, and lethal mutations from three males in the second experiment (*43a*, *43b*, and *44h*) were mapped in the 6F1-7C1 region.

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TABLE 5

Map position of the lethal mutations

Lethal lines	Lethal loci
Lethal mutations detected in the first experiment:	
23	19A1–2 to 20A as defined by *Df(1) mal*^{3}
27 (cluster of seven lethals)	3C2 to 5A1 as defined by *T(1;2) w*$^{+64b13}$
33 (cluster of eight lethals)	3C2 to 5A1 as defined by *T(1;2) w*$^{+64b13}$
*13**	25.7 ± 1.0
*29**	7.6 ± 1.3
Lethal mutations detected in the second experiment:	
43a	6E1 to 7C1 as defined by *Df(1) ct*^{J6}
43b (cluster of five lethals)	6E1 to 7C1 as defined by *Df(1) ct*^{J6}
44h	6E1 to 7C1 as defined by *Df(1) ct*^{J6}
*22j** (cluster of eight lethals)	25.9 ± 1.0 (only one mapped)
*41d**	56.1 ± 0.3
*43g**	50.6 ± 0.9

* Based on recombination data.

The lethal loci for the remaining 12 lines were determined from recombination data. In mapping the lethal mutations from *22j*, only one lethal line was used to determine its position (25.9 ± 1.0). The remaining seven lethal lines from *22j* were assumed to have a lethal lesion at the same position.

Revertability of the secondary lethal mutations

The instability of the secondary lethal mutations was indicated by frequent reversions in the cluster of seven lethal lines descended from male *27* (48 of the 67 *FM6/59b-z Uc-l'* females produced at least one revertant male). The cluster of five lines from male *43b* reverted with a low frequency (three of the 49 carrier females produced at least one revertant male), but the remaining 23 lines were quite stable. These 23 lines included a cluster of eight from male *33* in which the lethals mapped in the 3F-4A region, and another cluster of eight from male *22j* where one of the lethals mapped at 25.9.

Cytological analysis

Progressive changes in the chromosome structure: The chromosome aberrations observed in the secondary lethal mutations are summarized in Table 6. In the earlier analysis, three kinds of chromosome aberrations were detected in 12 of the 34 lethal lines; and four kinds of chromosome aberrations were detected in eight of the 35 lines in the later generation. Difference in the chromosome structure was noted for lethal lines from males *27* and *23* in experiment 1. Due to extremely low viability, one of the 35 lines, *43a*, was not analyzed in the earlier analysis. One each of a duplication and a deficiency, two different double deficiencies, and one translocation were observed in the analysis.

During the earlier analysis, *Df(1)6F2-5;7A4-5* was seen in the cluster of five lethal mutations from *43b*. When studied again in the later generation, one of

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TABLE 6

Summary of chromosome aberrations observed in the secondary lethal mutations

Lethal lines	Earlier analysis	Later analysis
Experiment 1		
23a	Appears normal	*T(1;3) 3F2–4;98B* in two larvae
27a	*Dp(1;1) 3E;4A*	Appears normal
27b	Appears normal	Appears normal
27c	*Dp(1;1) 3E;4A*	Appears normal
27d	*Dp(1;1) 3E;4A*	Appears normal
27e	*Dp(1;1) 3E;4A*	Appears normal
27f	*Dp(1;1) 3E;4A*	Appears normal
27g	*Dp(1;1) 3E;4A*	Appears normal
Experiment 2		
43a	Not analyzed	*Df(1) 6F2–5;7A4–5*
43b-1	*Df(1) 6F2–5;7A4–5*	*Df(1) 6F2–5;7A4–5*
43b-2	*Df(1) 6F2–5;7A4–5*	*Df(1) 6F2–5;7A4–5*
43b-3	*Df(1) 6F2–5;7A4–5*	*Df(1) 6F2–5;7A4–5*
43b-4	*Df(1) 6F2–5;7A4–5*	*Df(1) 6F2–5;7A4–5* plus *Df(1) 7A5–7;7B7–C1*
43b-5	*Df(1) 6F2–5;7A4–5*	*Df(1) 6F2–5;7A4–5*
44h	*Df(1) 6E2–4;6E5–F1* plus *Df(1) 6F2–5;6F6–7A1*	*Df(1) 6E2–4;6E5–F2* plus *Df(1) 6F2–5;6F6–7A1*

the five lethal lines (*43b-4*) had acquired another deficiency, *Df(1)7A5-7;7B7-C1*, in addition to the original deficiency. This was the only instance of progressive change noted in this material; however, it does support the idea that ongoing mutations and chromosome breakage events take place in the secondary lethals.

To date, LIM (1979, 1980 and unpublished) has observed 58 independently originating chromosome aberrations in the material derived from the *59b-z Uc-l* lines. All of the simple aberrations, except one, were two-break rearrangements. The exception was an intrachromosomal transposition involving three breaks. All of the 117 breaks were confined to the *X* chromosome. Moreover, some of the simple aberrations can produce additional aberrations to yield complex rearrangements; these additional breaks in the complex rearrangements were also confined to the *X* chromosome (LIM 1980).

In the lethal line from *23a*, a translocation *T(1;3)3F;98B* was observed in two of the five larvae from one female carrying the lethal mutation. This was the first case of interchromosomal rearrangement observed in the unstable chromosomes. This translocation indicates that such an aberration can be detected under the experimental conditions employed, but that the frequency of occurrence must be very low.

Amorphous mass: The analysis of polytene chromosomes from 100 larvae derived from *27e3* is summarized in Table 7. A total of 13 different chromosome aberrations shown in Figure 2 were observed. The founder female, *27e3*, had

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TABLE 7

Number of larvae with each kind of chromosome rearrangement

Chromosomes	Sublines A through T from *27e3*																			
	–A	–B	–C	–D	–E	–F	–G	–H	–I	–J	–K	–L	–M	–N	–O	–P	–Q	–R	–S	–T
Normal	5		1	1	4					2	2		3		3	5				
Dp(1;1)3E;4A							2													
Dp(1;1)3F;4C								5												
Dp(1;1)3F;4A													2							
Dp(1;1)4A;4C																			5	4
Dp(1;1)3D;4A & Am(1)4B																	1			
Df(1)3F;4B						4								5	2					1
Df(1)3D;4A							1													
Df(1)4A;4B												5								
Df(1)3D;4B																	3			
Am(1)3F;4A		5	2	4	1		2			3	2						1			
Am(1)3F;4C											1									
In(1)3F;4D						1														
Is(1)3F;4A			2						5									5		

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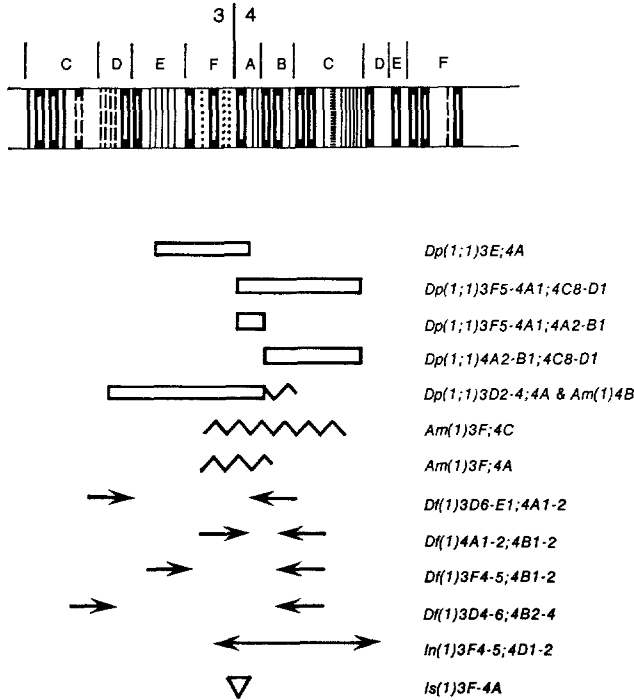

FIGURE 2.—Chromosome rearrangements and abnormalities observed in 100 larvae derived from a single *FM6K/59b-z Uc-l'* grandparent, *27e3*. At the top is a diagrammatic representation of the 3C to 4F region of the *X* chromosome. Below the cytological map are five classes of aberrations. A duplication is indicated by a narrow rectangle, an amorphous mass is represented by a wavy line, the extent of a deletion is marked by two arrows facing each other, an inversion is shown by the parting arrows connected by a horizontal line, and the position of an insertion is given by a triangle.

an amorphous mass at the 3F-4A region of the *X* chromosome in the 20th generation. Therefore, the chromosome structures other than *Am(1)3F-4A* shown in the table and the figure represent changes at the region. Only 20 of the 100 larvae in the 22nd generation had amorphous masses. Some of the aberrations observed in these larvae are shown in Figure 3.

In contrast to the above results, none of the 100 larvae sampled in the same manner from *27d1* carried a chromosome aberration. These data clearly indicate a high degree of site-specific instability in the derivatives of a chromosome with the amorphous mass.

Four of the 20 females from *27e3* (-C, -G, -K, and -Q) had three different chromosome structures among their five daughters. In addition, seven of the 20 females had two different chromosome structures among their five daughters. This reflects a high degree of genetic instability.

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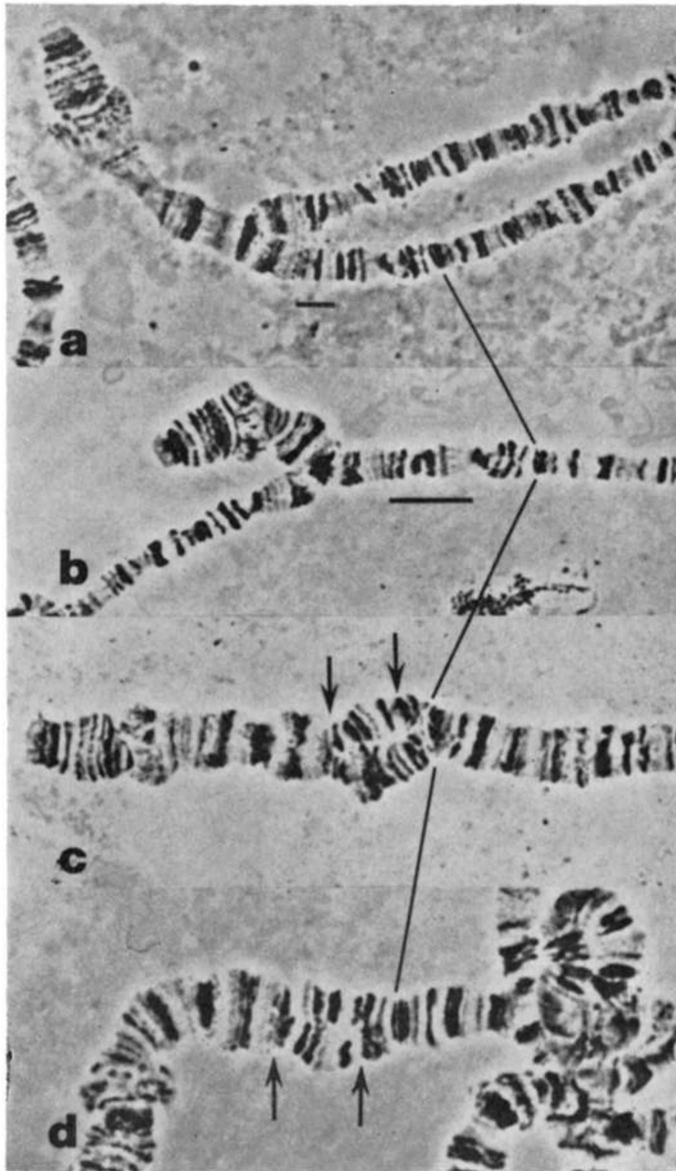

FIGURE 3.—Chromosome aberrations observed in the progeny from *27e3*. (a) *Df(1)3F3–4;4B1-2*, the deficiency in the upper chromosome and its normal homolog at the bottom with a horizontal bar indicating the extent of deleted section. (b) *Df(1)3F3–4;4C16–D1*, the deficiency chromosome running to the left and its normal homolog running to the right with a horizontal bar indicating the deleted section. (c) *Dp(1;1)3F3–4;4C8–D1*, the inverted repeats in the lower chromosome and its normal homolog at the top with two arrows indicating the approximate position of the breaks. (d) *In(1)3F4–5;4D1–2*, the upper chromosome with the inverted section and its normal homolog at the bottom. The approximate positions of the breaks are indicated by arrows. In the inversion chromosome, 4F1–4 are displaced and look as if they are deleted.

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Somatic mutations in the *59b-z/59b-z Uc-l'* larvae will result in mosaic salivary glands. At least 13 of the 100 larvae sampled clearly showed the mosaic glands. Many of the chromosome aberrations observed in the progeny from *27e3* were small aberrations, which are difficult to study. In many cases, mosaicism cannot be estalished with certainty. Since such glands were not tallied as mosaics, the above figure (13%) is an underestimate.

DISCUSSION

Evidence for transposition of destabilizing element

Our results show that the revertant chromosomes are highly mutable, although they may be less so than the unstable chromosome. Two aspects of the revertant chromosomes demonstrate the hypermutability. First, the revertant chromosomes generate "spontaneous" *X*-linked recessive lethal mutations with a frequency of about 1.1% (see Table 4). Second, the cluster of five secondary mutations from male *27* revert to the nonlethal state with a high frequency, and another cluster of five secondary mutations from male *43b* revert with a low frequency.

We also show that the mutational events in the revertant chromosomes, like those in the primary mutations, are site-specific. This was demonstrated by occurrence of two independent mutations each in the 3F-4A region, and at 25.8, and three independent occurrences of mutations in the 6F region (see Table 5). It is also indicated by the appearance of identical but independent chromosome aberrations in the progeny of different flies (see Table 7).

Finally, we show that secondary lethal mutations at the 3F-4A region of the *X* chromosome have genetic properties similar to the primary lethal mutations at the 6F1-2 doublet. These include: (1) aberrations with a break in the 3F-4A region, as shown in Figure 2, (2) almost exclusive occurrence of chromosome breakage events within the *X* chromosome (see Tables 6 and 7), and (3) appearance of the abnormal structure called the amorphous mass at the 3F-4A region (see Figure 1).

Except for the reinversion of an inversion to the normal sequence, all of the eight properties exhibited by the primary lethal mutation were observed in the secondary lethal mutations. It should be noted, however, that not as large a sample of chromosomes in the secondary lethal mutations were examined. All of these indicate that the secondary lethal mutations generated in the revertant chromosomes have genetic properties very similar to the primary lethal mutation in the unstable chromosomes. However, there is one significant difference, the map position of the two classes of mutations. The primary mutation is mapped in the 6F1-2 doublet while the secondary mutations of interest are mapped in the 3F-4A region. The 6F region of the *X* chromosome carrying a secondary mutation in the 3F-4A region is stable and *vice versa.*

These observations indicate that reversion of the primary mutation is accompanied by elimination of the instability at the 6F1-2 doublet, and that the instability can appear in a new position (3F-4A). Elimination of the instability at 6F1-2 followed by its appearance elsewhere in the *X* chromosome (3F-4A) implies that a transposition of the destabilizing element took place.

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The transposition of the element, suggested above, was deduced from the resemblance in the genetic properties between the primary lethal mutations at 6F and the secondary lethal mutations at 3F-4A. The sequence of events between the loss of lethality at 6F and appearance of a secondary lethal mutation cannot be traced with the data presented here. It is not likely, however, that the destabilizing element moved from the 6F region (primary lethal site) to the 3F-4A region (secondary lethal site) by way of the autosomes, since almost all of the chromosome breakage events in the *Uc* and its derivatives are confined to the *X* chromosome (LIM 1979, 1980). Confinement of the breakage events to the *X* chromosome and confounding of the mutations not generated by the destabilizing element make it very difficult, if not impractical, to trace the movement of the element between the *X* chromosome and autosomes.

Can every chromosome with an amorphous mass produce an array of chromosome aberrations like *27e3*? Our data are too limited to answer this question. The samples taken from a chromosome without the *Am*, *27d1*, are comparable to those taken from *27e3*. However, none of the chromosomes derived from *27d1* was associated with an aberration. This does not necessarily mean that the chromosomes without *Am* are stable; we have observed many aberrations produced by chromosomes without such an abnormal structure (LIM 1979, 1980, and this study).

Transposable elements and chromosome instability

Dissociation, *Ds*, in the now classic *Ds-Ac* system was discovered through a chromosome break generated by *Ds* in the short arm of chromosome *9* in maize (MCCLINTOCK 1951). Intensive study of *Ds* led to the formulation of the concept of the controlling element; the controlling element can be characterized by its ability to: (1) regulate, modulate, suppress, inhibit or control the activity of known "structural" genes; (2) break a chromosome at its site of insertion; and (3) transpose to a new site, which is often accompanied by the generation of gross rearrangements having a common break. Among the rearrangements observed are: translocations, inversions, ring chromosomes, duplications, and deficiencies. As pointed out by MCCLINTOCK (1951, 1956), the initial "burst" of newly arisen mutable loci appeared in the self-pollinated progeny of plants that had undergone the breakage–fusion–bridge cycle. The site specificity of the actions of *Ds* and its dependence on *Ac* has also been clearly indicated (MCCLINTOCK 1951, 1956).

Although there were earlier studies on mutable genes in Drosophila (for a summary, see DEMEREC 1941), we must credit GREEN (1967, 1969a,b, 1973) for the first evidence linking the concept of controlling elements with mutable genes in Drosophila. From the very first paper on *white-crimson* mutants, GREEN (1967) pointed out the parallelisms between his mutants and the controlling elements of maize and with lambda phage.

Our results, summarized in this and previous papers (LIM 1979, 1980) indicate that the putative destabilizing element in the *Uc* and its derivatives show all of the genetic properties of the controlling elements in maize. First, the control of gene activity by our destabilizing element was reflected in the repeated

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generation of lethal mutations at a given site. Second, the generation of chromosome breakage events at a given locus by our destabilizing element was clearly shown by the production of different rearrangements having a common breakpoint, *and* by the appearance of identical but independent chromosome aberrations in the progeny of different flies. Finally, the ability of our destabilizing element to transpose was demonstrated by the leapfrogging of a breakpoint (LIM 1980), *and* by the elimination of instability at one site (6F1-2) followed by its appearance at a new site (3F-4A) in the *X* chromosome.

Another feature in our system resembles the *Ds-Ac* system of controlling elements. The breakage events of the *Ds* element frequently involves the centromere and chromosome knobs (MCCLINTOCK 1951). The chromosome breakage events in the *Uc* and its derivatives frequently involve the centromere of the *X* chromosome. This was shown by the production of attached-*X* chromosomes in males carrying the *Uc* and its derivatives (LIM 1981). The structure of our amorphous mass resembles that of heterochromatin at the chromocenter. Whether or not the amorphous mass corresponds to the knobs in maize is not possible to establish at present.

Chromosome breakage events and enhanced mutability in dysgenic hybrids are similar to many aspects of site specific instability in our unstable chromosomes (ENGELS 1980, SIMMONS and LIM 1980). These include site-specific instability, involvement of a mobile element known as the *P* factor, confinement of chromosome breakage events within the *X* chromosome, and the appearance of identical but independent chromosome aberrations in the progeny of different flies. The relationship between hybrid dysgenesis and the instability of the *Uc* is not clear at this time.

In Drosophila, many families of DNA sequences with moderate degrees of repetition have been shown to be mobile (ILYIN *et al.* 1977; STROBEL, DUNSMUIR and RUBIN 1979; POTTER *et al.* 1979; YOUNG 1979). Recent studies (DUNSMUIR *et al.* 1980; LEVIS, DUNSMUIR and RUBIN 1980) have revealed that these DNA sequences (hereafter referred to as *copia*-like elements) have structural features strikingly similar to transposable elements in prokaryotes (for review, see KLECKNER 1977; CALOS and MILLER 1980). In addition, the distribution of *copia*-like elements in different individuals of a strain as determined by *in situ* hybridization indicate their movement within the genome. Movements of *copia*-like elements, however, are apparently not associated with the generation of cytologically detectable chromosome rearrangements.

Transposing elements (TE) of ISING and RAMEL (1976) are another category of mobile elements in Drosophila. Unlike the *copia*-like elements, the movements of the TE can be traced cytologically. A recent summary by ISING and BLOCK (1980) shows transposition of the elements to some 150 sites throughout the genome. Yet, none of these transposition activities apparently is associated with gross rearrangements. In a letter dated May 29, 1982, ISING informed us that at least three (TE20, TE81, and TE90) and possibly five of these TE-generated movements are associated with gross aberrations. This frequency is at least ten times lower than that found in the *Uc*-generated lethal mutations (LIM 1979, 1980).

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This brief discussion of transposable elements and chromosome instability in Drosophila is not intended to be complete or exhaustive. Rather, our intention is to point out that some transpositions apparently are not associated with chromosomal gross rearrangements. In this respect, the activity of the destabilizing element studied here differs from that of the *copia*-like element.

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