SITE-SPECIFIC INSTABILITY IN DROSOPHILA MELANOGASTER: 
THE ORIGIN OF THE MUTATION AND CYTOGENETIC 
EVIDENCE FOR SITE SPECIFICITY*

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ABSTRACT

During a study of delayed mutations, an unstable X chromosome (Uc) 
was detected. Spontaneous X-linked recessive lethal mutations were detected 
in 34 of 993 sperm sampled from 50 males carrying this chromosome. All 
but three of the 34 lethals originated as clusters in three of the 50 males 
Cytogenetic and complementation analyses revealed 14 intrachromosomal 
rearrangements: ten inversions, two reverse repeats, one deficiency and 
one transposition. Eight of the 14 rearrangements have one break in the 6F1–2 
doublet and two rearrangements have a break in 6F1–5 of the X chromo-
some. The remaining four rearrangements have in addition to the aberrations 
a lethal point mutation between 6F1 and 6F5. Though each of the lethal 
lines was established from a single lethal-bearing female, chromosome poly-
morphism is evident in 17 of the 18 lines having rearrangements, with certain 
aberrations recurring in several lines. The lethal mutations revert frequently 
to the nonlethal state, and cytological evidence indicates that more than one 
mutational event may occur at the unstable locus of the chromosome during 
one generation. Two lethal lines had more than one type of chromosome 
rearrangement sharing a common breakpoint. These observations are consis-
tent with the view that the instability in the Uc lines is caused by a transpos-
able element capable of site-specific chromosome breaks and perpetual genera-
tion of mutations. The mutagenic and genetic properties of transposable 
elements can be related to the two-mutation theory of Knudson (1971) for 
cancer initiation.

ONE of the most exciting recent developments in molecular genetics is the find-
ing that genetic instabilities in microorganisms can be caused by transposable 
elements (IS elements, Tn elements and episomes). Higher organisms also exhibit 
genetic instabilities, but the explanation for these has not been elucidated.

To be sure, the controlling elements of maize elegantly investigated and docu-
mented by McClintock (1950, 1951, 1956) are formally quite similar to the 
transposable elements of bacteria [for literature and discussion, see the reviews 
by Fincham and Sastry (1974) and by Nevers and Saedler (1977)], but their 
molecular nature is not known. In Drosophila, controlling elements have been

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linked with genetic instability by Green (1967, 1969a, 1969b), who studied white-crimson (w^c) mutants. Additional reports on several distinct classes of unstable mutants involving the white locus have been published (Kalisch 1970; Kalisch and Becker 1970; Judd 1967, 1969; Gethmann 1971; Rasmuson, Green and Karlsson 1974). More recently, Green (1977b) has found that MR (male recombination) chromosomes are capable of inducing mutations at the sn, ras and y loci of the X chromosome. Judging by the high reversion rate, Green concluded that the MR-generated mutations are due to insertion elements. Cytologically, however, these MR-generated mutations are free of detectable chromosome rearrangements.

The present paper describes the origin of X chromosomes that exhibit a highly site-specific instability, and reports some genetic properties of the mutations resulting from this instability. The unstable chromosomes (Uc) generate an unusually high frequency of spontaneous recessive lethal mutations originating in the 6F1–6F5 region of the X chromosome. These mutations arise in clusters, frequently revert to the nonlethal state and are often associated with chromosome rearrangements. Cytogenetic evidence supports the view that the instability is caused by an agent capable of transposition to other locations on the chromosome.

MATERIALS AND METHODS

Flies were reared on standard corn meal-molasses-brewers yeast-agar medium at 25°. The abbreviations and sources of mutants and chromosomes used in this study are summarized in Table 1, and Table 2 lists the mutant symbols used in text. Additional information can be found in Lindsley and Grell (1968). Of the duplication and deficiency stocks listed in Table 1, Df(1)yg^s6 and Df(1)TEM501 were induced with triethylenemelamine at Eau Claire; the rest were obtained from the Caltech Stock Center. Df(1)ctJ4, Df(1)ctJG and Dp(1;3)sn13a are described more fully in LeFevre and Johnson (1973). The procedure used in preparing polytene chromosomes for examination has been described by Lim and Snyder (1968).

The origin of the unstable chromosomes: These experiments concern five related X-chromosome lines designated IJA, IJB, IJC, IJD and IJE. Each line carries an unstable X chromosome (Uc), marked with the mutants y^s9b, z, wi, ct6 and f. This combination of mutants is abbreviated 59b-z. The unstable chromosomes were ultimately derived from a male fly, designated 117-13, whose grandfather was fed ethyl methanesulfonate (EMS), and were identified by noting a propensity to mutate to the lethal condition.

A summary of mutagenesis experiments related to the male fly, 117-13, follows. Young males carrying the 59b-z chromosome were fed 25 mM EMS in 1% sucrose solution for 15 hrs according to the method of Lewis and Bacher (1968). These were then mated to FM6K/FM6K females, and their F_1 daughters were tested individually for X-linked recessive lethal mutations. Complete absence of 59b-z males in the F_2, in the presence of more than 30 FM6K males, was the criterion for defining a lethal mutation. Among 473 F_2 females, in the treated group, there were 216 lethal carriers, while only six of the 1,510 control F_1 females carried lethal mutations.

One 59b-z male from each of the nonlethal F_2 lines was mated to Df Basc/Df w^o1 females and ten Df Basc/59b-z progeny females from each cross were mated individually to FM6K males to permit a second test for X-linked recessive lethal mutations. Through the test, F_2 gonadal mosaics could be detected in the F_4. Among the 217 F_2 males descended from treated grandfathers, four males had one lethal-bearing daughter and nine daughters without lethal mutations. The male fly, 117-13, is one of these four F_2 males with a gonadal mosaic.

The immediate pedigree of the five chromosome lines from 117-13 is shown in Figure 1. Among the nine nonlethal F_4 lines, two (G and I) produced lethal-carrying daughters. GJ,
one of the ten FM6K/59b-z females sampled from G, was a carrier of a lethal mutation, and II and IJ, two of the ten FM6K/59b-z females from I, were also lethal carriers. GC, one of the nine nonlethal siblings of GI, apparently had a mosaic ovary since one of her ten FM6K/59b-z daughters (GCF) was a lethal carrier.

The F1 lethal lines (GI, II and IJ) were then tested more thoroughly. From each of these, ten FM6/59b-z females were taken for testing, and a few days later, ten more females were taken from the same cultures and tested. All of the F1 females from GI and II behaved as if they were carriers of a stable lethal mutation. The first ten F1 females from IJ behaved as if they were carrying a stable lethal mutation, but the sample of ten F1 females taken a few days later included five lethal lines (IJF, IJG, IJH, IJJ, and IJL) and five nonlethal lines (IJA, IJB, IJC, IJD, and IJE). Retesting each of these ten lines for three additional generations revealed that all lethal lines were stable as lethals, while the nonlethal lines were extremely unstable.

The lethal lines (underlined in Figure 1) were maintained in the usual manner; a single FM6K/59b-z I female was taken as a founder fly and mated to FM6 males, and ten of her FM6K/59b-z I daughters were mated to their FM6K brothers. Ten sib-mated females carrying the 59b-z chromosome were used to produce each generation thereafter. The same procedure was employed for establishing and maintaining the five nonlethal lines (IJA through IJE).

Each of the nonlethal lines produced FM6K males and 59b-z males, as well as FM6K/FM6K females and FM6K/59b-z females. However, the 59b-z males were sedentary, very slow to mate and often sterile. When the sib-FM6K males were present in the same vial, 59b-z/59b-z females were very seldom produced. Initially, attempts were made to maintain the nonlethal lines in a homozygous condition (i.e., 59b-z/59b-z), or to maintain them as males mated to attached-X chromosome females. Since the chromosomes were so unstable, however, neither of these schemes was satisfactory.

**TABLE 1**

The chromosomes used in the experiment

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Genotype</th>
<th>Source, description</th>
</tr>
</thead>
<tbody>
<tr>
<td>59b-z</td>
<td>γ⁵₉bz⁵₉ct⁵⁶</td>
<td>A recombinant chromosome from females heterozygous for γ⁵₉bz⁵₉ct⁵⁶.</td>
</tr>
<tr>
<td>FM6K</td>
<td>In(1)FM6γ⁴⁺⁴sc⁴w⁺dm⁺⁺B</td>
<td>From the City of Hope Medical Center.</td>
</tr>
<tr>
<td>Df Basc</td>
<td>In(1)Bascm(1)sc⁵w⁺</td>
<td>ISOLATED FROM FM6K BY K.K. KIDD.</td>
</tr>
<tr>
<td>Df w⁺3I</td>
<td>Df(1)w⁺3Iγ⁵w⁺sep⁵sn²</td>
<td>BURKE H. JUDD</td>
</tr>
</tbody>
</table>

Duplication and deficiency chromosomes used for mapping:

<table>
<thead>
<tr>
<th>Genotype of males</th>
<th>Deficiency</th>
<th>Cytology</th>
<th>Duplication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Df(1)γ⁷⁵es/γ⁵₉Y70</td>
<td>1A1-2 ; 1B6-9</td>
<td>tip to 2B17-2C3</td>
<td></td>
</tr>
<tr>
<td>Df(1)TEM501/w⁺Y</td>
<td>2D4-E1 ; 3C1-3</td>
<td>2D ; 3D</td>
<td></td>
</tr>
<tr>
<td>Df(1)ct⁵sl/γ⁴⁺⁺δ</td>
<td>7A2 ; 7C1</td>
<td>6C11 ; 7C9</td>
<td></td>
</tr>
<tr>
<td>Df(1)ct⁵sl/γ⁴⁺⁺δ</td>
<td>6E1 ; 7C1</td>
<td>6C11 ; 7C9</td>
<td></td>
</tr>
<tr>
<td>Df(1)ct⁵sl/γ⁴⁺⁺δ</td>
<td>7A8 ; 8A5</td>
<td>7A8 ; 8A5</td>
<td></td>
</tr>
<tr>
<td>Df(1)ct⁵sl/γ⁴⁺⁺δ</td>
<td>10C2-3 ; 10E2-3</td>
<td>9F3 ; 10E3-4 ; 20B</td>
<td></td>
</tr>
<tr>
<td>Df(1)ct⁵sl/γ⁴⁺⁺δ</td>
<td>14B13 ; 15A9</td>
<td>14B13 ; 15A9</td>
<td></td>
</tr>
<tr>
<td>Df(1)ct⁵sl/γ⁴⁺⁺δ</td>
<td>19A1-2 ; 20A</td>
<td>1A2 ; 1B2 ; 18F</td>
<td></td>
</tr>
</tbody>
</table>

* Haploinviable in females.
RESULTS

Enhanced mutability associated with the unstable chromosomes

The mating scheme used for detecting, testing and maintaining the lethal mutations is shown in Figure 2. Ten 59b-z males were tested from each of the Uc lines, and each male was represented by 20 daughters of the constitution Df Basc/59b-z Uc. The complete absence of 59b-z Uc sons from a carrier female was the criterion for lethality throughout the experiments.

TABLE 2
Synopsis of gene symbols used in text

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Phenotype</th>
<th>Chromosome and location</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>Bar eye, eye restricted to vertical narrow bar</td>
<td>X-57.0</td>
</tr>
<tr>
<td>ct6</td>
<td>cut-6, wings cut to points and edges scalloped</td>
<td>X-20.0</td>
</tr>
<tr>
<td>ct14</td>
<td>cut-14, cut wings associated with Df(1)ct14</td>
<td></td>
</tr>
<tr>
<td>ct16</td>
<td>cut-16, cut wings associated with Df(1)ct16</td>
<td></td>
</tr>
<tr>
<td>da</td>
<td>daughterless, homozygous da produce no daughters</td>
<td>2-39.3</td>
</tr>
<tr>
<td>dm</td>
<td>diminutive, bristles and body small and slender, female sterile</td>
<td>X-4.6</td>
</tr>
<tr>
<td>ec</td>
<td>echinus, eyes large and bulging</td>
<td>X-5.5</td>
</tr>
<tr>
<td>f</td>
<td>forked, bristles bent and shortened</td>
<td>X-56.7</td>
</tr>
<tr>
<td>Fl</td>
<td>Female lethal, renamed Sx1F1</td>
<td>X-19.1</td>
</tr>
<tr>
<td>Ki</td>
<td>Kinked, bristles and hairs shortened and twisted</td>
<td>3-47.6</td>
</tr>
<tr>
<td>m</td>
<td>miniature, size of wings reduced</td>
<td>X-36.1</td>
</tr>
<tr>
<td>mal</td>
<td>maroonlike, brownish purple eye color</td>
<td>X-64.8</td>
</tr>
<tr>
<td>r</td>
<td>rudimentary, wings truncated to about tip of abdomen</td>
<td>X-54.5</td>
</tr>
<tr>
<td>ras</td>
<td>raspberry, eye color dark ruby</td>
<td>X-32.8</td>
</tr>
<tr>
<td>sc6</td>
<td>scute-8, allele of sc, scute bristles</td>
<td>X-0.0</td>
</tr>
<tr>
<td>sn</td>
<td>singed, bristles and hairs twisted and shortened</td>
<td>X-21.0</td>
</tr>
<tr>
<td>sn8</td>
<td>singed-3, allele of sn</td>
<td></td>
</tr>
<tr>
<td>sn13a</td>
<td>singed-13a, sn associated with Dp(1;3)sn13a</td>
<td></td>
</tr>
<tr>
<td>sn+7ad</td>
<td>wild allele of sn associated with T(1;2)sn+7ad</td>
<td></td>
</tr>
<tr>
<td>spl</td>
<td>split, split bristles and smaller eyes</td>
<td>X-3.0</td>
</tr>
<tr>
<td>Sx1F1</td>
<td>Sex lethal, Females-specific #1, lethal to da females</td>
<td>X-19.2</td>
</tr>
<tr>
<td>Sx1M1</td>
<td>Sex lethal, Male-specific #1, lethal to males</td>
<td></td>
</tr>
<tr>
<td>w5</td>
<td>white-apricot, yellowish-orange eye color</td>
<td>X-1.5</td>
</tr>
<tr>
<td>w7</td>
<td>white-crimson, orangish eye color, allele of w</td>
<td></td>
</tr>
<tr>
<td>w1</td>
<td>white-ivory, eyes very light yellow, allele of w</td>
<td>X-3.0</td>
</tr>
<tr>
<td>w−</td>
<td>white-deleted, associated with Df(1)w−</td>
<td></td>
</tr>
<tr>
<td>w+J1</td>
<td>white-recombinant of Judd #1, white eye color</td>
<td></td>
</tr>
<tr>
<td>γ2</td>
<td>yellow-2, yellow body color with black bristles</td>
<td>X-0.0</td>
</tr>
<tr>
<td>γ21d</td>
<td>yellow-31d, yellow body color with black bristles</td>
<td></td>
</tr>
<tr>
<td>γ59b</td>
<td>yellow-59b, yellow body color with brownish-yellow bristles, γ59b/γ2 is wild type</td>
<td></td>
</tr>
<tr>
<td>z</td>
<td>zeste, eyes of females lemon yellow. Wild-type eye color in males</td>
<td>X-1.0</td>
</tr>
</tbody>
</table>

The 59b-z males in the five nonlethal lines from IJA, IJB, IJC, IJD, and IJE were chosen for further study of the genetic instability. These five lines will hereafter be referred to as the five Uc lines. 59b-z chromosomes subjected to the same manipulations as the unstable chromosomes, but derived from untreated ancestors, were used as controls in these experiments.
Data on the enhanced mutability in the males of the Uc lines are summarized in Table 3. Among the 50 males tested, six produced at least one lethal-bearing sperm. None of the 50 males in the control lines produced lethal-bearing sperm.

A more detailed account of the incidence of mutations among the progeny of these six males follows. One male, IJA3, derived from IJA, produced a cluster of 19 lethal lines and one nonlethal line. The lethal lines were designated IJA3-1 through IJA3-16, -18, -19 and -20, and the only nonlethal line was designated IJA3-17. A cluster of seven lethals (lines IJD4-1 through IJD4-7) and 13 nonlethals was detected in the progeny from IJD4. A brother of IJD4, IJD3, produced five daughters carrying a lethal mutation (lines IJD3-1 through IJD3-5) and 15 daughters without the lethal mutation. Each of the remaining three males produced one lethal and 19 nonlethal lines. These three lethal lines (IJD15, IJC9 and IJE10) are henceforth referred to as singletons. The over-all frequency of the spontaneous lethal mutations in the experimental lines, adjusting for the clusters, is 3.4 ± 2.1%. The standard deviation was calculated using the formula of Engels (1979).

Each of the 34 lethal stocks identified above, plus the only nonlethal line from
**Figure 2.**—The mating scheme used for isolating, retesting and maintaining the spontaneous recessive lethal mutations detected in the unstable chromosomes. The 59b-z/Y males in the P generation represent the males carrying the 59b-z chromosome in control lines or in the five Uc lines with the 59b-z Uc chromosome.

IJA3, IJA3–17, was retested by examining the progeny of ten FM6K/59b-z Uc females from each F2 culture. From each of the 35 stocks, a single lethal progeny line in the F3, or in F6, was taken at random and ten FM6K/59b-z Uc females in each generation were tested individually.

The three lethal lines detected as singletons (IJD15, IJC9 and IJE10) were stable lethals—all of the ten females tested for each of the F2, F3 and F6 generation (total of 30) were carriers of a stable lethal mutation. In contrast, many lines in the clusters of lethals from IJA3, IJD3 and IJD4 were unstable. The retest results from IJA3 lethal lines are shown in Table 4 to illustrate the degree of instability. The ratios in columns 3 to 6 indicate the number of lethal-carrying females to the number of females tested. In the F2, for instance seven of the 20 lines studied were
totally lethal; in these cases, all the $F_2$ females tested proved to carry lethals. In two cases, however, none of the $F_2$ females sampled carried lethal mutations. The $F_2$ females from the remaining eleven lines, including IJA3–17, which was not a lethal carrier in the $F_1$, were composed of both lethal carriers and females producing viable $59b-z Uc$ sons (nonlethals.)

The instability displayed by these lines can be expressed by calculating the frequency with which lethal mutations revert to the nonlethal state. The apparent reversions observed in lethal-carrying females in the lines from IJA3, IJD3 and IJD4 are summarized in Table 5; these data are based on data similar to those in Table 4.

The sign test (see SNEDECOR and COCHRAN 1967) was used to test for differences in the apparent reversions. In IJA3, the greatest difference was observed between the $F_2$ and the $F_0$ females, but the difference was not significant ($p = 0.057$). None of the paired comparisons of the differences in the apparent reversions observed in IJD3 lines or IJD4 lines was found to be significant.

A noteworthy characteristic of the lethal-carrying females in the IJA3 lines is sterility. As shown in Table 5, approximately 25 to 30% of the females carrying lethal mutations were sterile. These females never produce eggs, owing to underdevelopment of ovaries. Female sterility of similar nature and comparable frequency has not been observed in the IJD3 and IJD4 lines.

### TABLE 3

*Spontaneous X-linked recessive lethal mutations detected in the males carrying the unstable X chromosome*

<table>
<thead>
<tr>
<th>Materials</th>
<th>Number of males tested</th>
<th>Number of mosaic testes*</th>
<th>Designation†</th>
<th>Number of $F_1$ females tested</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Uc</em> lines:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IJA</td>
<td>10</td>
<td>1</td>
<td>IJA3</td>
<td>198</td>
</tr>
<tr>
<td>IJB</td>
<td>10</td>
<td>0</td>
<td>——</td>
<td>199</td>
</tr>
<tr>
<td>IJC</td>
<td>10</td>
<td>1</td>
<td>IJC9</td>
<td>198</td>
</tr>
<tr>
<td>IJD</td>
<td>10</td>
<td>3</td>
<td>IJD3</td>
<td>200</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>IJD4</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>IJD15</td>
<td></td>
</tr>
<tr>
<td>IJE</td>
<td>10</td>
<td>1</td>
<td>IJE10</td>
<td>198</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>6</td>
<td></td>
<td>993</td>
</tr>
<tr>
<td>Control lines:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control-1</td>
<td>10</td>
<td>0</td>
<td>——</td>
<td>196</td>
</tr>
<tr>
<td>Control-2</td>
<td>10</td>
<td>0</td>
<td>——</td>
<td>198</td>
</tr>
<tr>
<td>Control-3</td>
<td>15</td>
<td>0</td>
<td>——</td>
<td>298</td>
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<tr>
<td>Control-4</td>
<td>15</td>
<td>0</td>
<td>——</td>
<td>297</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>0</td>
<td></td>
<td>989</td>
</tr>
</tbody>
</table>

* Number of males yielding at least one lethal-bearing daughter.
† The designation of males with lethal-bearing daughters.
Mapping and complementation analyses

Male flies carrying the deficiency and duplication chromosomes listed in Table 1 were used to map the lethal loci of the 35 lines (34 lines plus an F3 lethal line isolated for IJA3–17). Surprisingly, all of the 35 lines, except IJC9 and IJE10, can be complemented by the $Dp(1;3)SN^{18}$ (6C11 to 7C9). The lethal line from IJE10 was covered by the duplication segregant of $T(1;2)SN^{+72d}$ (7A8–8A5). None of the deficiency and duplication chromosomes was useful for locating the lethal in IJC9. Based on recombination data, it is located at 40.6 ± 1.0.

The 33 lines with lethals localized by coverage with $Dp(1;3)SN^{18}$ were tested and confirmed by individual matings. Five carrier females ($FM6K/59b-z\ UC-1$) per lethal line were mated individually to $Df(Z)cP/Y; Dp(2;3)SN^{18}/JKi$ males. The results of the test are summarized in Table 6. The noncomplementing females belonged to either class 1, class 2 or class 3. The control females carrying the lethal mutation complementing with the deficiency were placed in class 4. The class 1 females cannot produce $59b-z\ UC-1/Df(1)cP; Ki/+\ daughters$, although they can produce more than 20 $59b-z\ UC-1/Df(2)cP; Ki/f\ daughters$ and many $59b-z\ UC-1/Y; Dp(1;3)SN^{18}/+\ sons$. The class 2 females can be recognized by their inability to produce either $59b-z\ UC-1/Df(1)cP; Ki/+\ daughters$.

### Table 4

<table>
<thead>
<tr>
<th>Lines</th>
<th>Category in F2</th>
<th>Number of lethal-carrying females</th>
<th>Number of females tested</th>
<th>Fraction</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>F2 females</td>
<td>F3 females</td>
<td>F4 females</td>
</tr>
<tr>
<td>IJA3–1</td>
<td>lethal</td>
<td>8/8</td>
<td>5/6</td>
<td>7/7</td>
</tr>
<tr>
<td>IJA3–2</td>
<td>lethal</td>
<td>4/10</td>
<td>9/10</td>
<td>6/6</td>
</tr>
<tr>
<td>IJA3–3</td>
<td>lethal</td>
<td>3/7</td>
<td>7/7</td>
<td>9/10</td>
</tr>
<tr>
<td>IJA3–4</td>
<td>lethal</td>
<td>5/7</td>
<td>4/6</td>
<td>8/10</td>
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<tr>
<td>IJA3–5</td>
<td>lethal</td>
<td>4/9</td>
<td>6/10</td>
<td>10/10</td>
</tr>
<tr>
<td>IJA3–6</td>
<td>lethal</td>
<td>8/8</td>
<td>9/9</td>
<td>9/9</td>
</tr>
<tr>
<td>IJA3–7</td>
<td>lethal</td>
<td>6/6</td>
<td>6/6</td>
<td>6/6</td>
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<tr>
<td>IJA3–8</td>
<td>lethal</td>
<td>6/7</td>
<td>5/7</td>
<td>6/7</td>
</tr>
<tr>
<td>IJA3–9</td>
<td>lethal</td>
<td>4/6</td>
<td>4/4</td>
<td>6/6</td>
</tr>
<tr>
<td>IJA3–10</td>
<td>lethal</td>
<td>7/7</td>
<td>8/8</td>
<td>6/6</td>
</tr>
<tr>
<td>IJA3–11</td>
<td>lethal</td>
<td>9/9</td>
<td>7/7</td>
<td>9/9</td>
</tr>
<tr>
<td>IJA3–12</td>
<td>lethal</td>
<td>0/9</td>
<td>3/7</td>
<td>2/3</td>
</tr>
<tr>
<td>IJA3–13</td>
<td>lethal</td>
<td>5/5</td>
<td>7/8</td>
<td>8/9</td>
</tr>
<tr>
<td>IJA3–14</td>
<td>lethal</td>
<td>7/7</td>
<td>6/6</td>
<td>9/9</td>
</tr>
<tr>
<td>IJA3–15</td>
<td>lethal</td>
<td>2/6</td>
<td>7/8</td>
<td>10/10</td>
</tr>
<tr>
<td>IJA3–16</td>
<td>lethal</td>
<td>2/3</td>
<td>6/6</td>
<td>8/8</td>
</tr>
<tr>
<td>IJA3–17</td>
<td>Nonlethal</td>
<td>6/10</td>
<td>6/7</td>
<td>1/1</td>
</tr>
<tr>
<td>IJA3–18</td>
<td>lethal</td>
<td>0/9</td>
<td>4/5</td>
<td>8/10</td>
</tr>
<tr>
<td>IJA3–19</td>
<td>lethal</td>
<td>1/8</td>
<td>4/5</td>
<td>9/9</td>
</tr>
<tr>
<td>IJA3–20</td>
<td>lethal</td>
<td>7/8</td>
<td>9/10</td>
<td>7/9</td>
</tr>
</tbody>
</table>
### Table 5

**Results from progeny testing of lethal-carrying females in the lethal mutations detected as clusters**

<table>
<thead>
<tr>
<th>Source of lethal mutation</th>
<th>IJA3</th>
<th>IJD3</th>
<th>IJD4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>F₃ females:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of females mated</td>
<td>183*</td>
<td>50</td>
<td>70</td>
</tr>
<tr>
<td>Number of sterile females</td>
<td>44</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Number of females tested</td>
<td>139</td>
<td>47</td>
<td>69</td>
</tr>
<tr>
<td>Number of apparent reversions</td>
<td>51</td>
<td>12</td>
<td>7</td>
</tr>
<tr>
<td>% apparent reversions ± s.d.</td>
<td>36.7 ± 11.4</td>
<td>25.5 ± 14.8</td>
<td>10.1 ± 5.9</td>
</tr>
<tr>
<td><strong>F₄ females:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of females mated</td>
<td>180†</td>
<td>50</td>
<td>70</td>
</tr>
<tr>
<td>Number of sterile females</td>
<td>53</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Number of females tested</td>
<td>127</td>
<td>48</td>
<td>68</td>
</tr>
<tr>
<td>Number of apparent reversions</td>
<td>12</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>% apparent reversions ± s.d.</td>
<td>9.5 ± 2.6</td>
<td>4.0 ± 2.7</td>
<td>4.4 ± 2.2</td>
</tr>
</tbody>
</table>

* Only three females were available for retest in one of the 19 lines (IJA3-16).
† Two lethal lines detected in F₃ (IJA3-12 and IJA3-18) produced nonlethal lines only in the F₄. Data and F₃ females in these lines are not included in the table.
‡ IJA3-12 and IJA3-18 produced lethal lines in the F₄. Ten F₄ females from a lethal culture in each of these lines are included for testing.

### Table 6

**A summary of results from complementation tests involving Df(1)ct16/Y; Dp(1;3)sn13a/Ki**

<table>
<thead>
<tr>
<th>Class 1†</th>
<th>Number of carrier females in:</th>
<th>Class 4</th>
<th>Number of lethal lines</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Class 2</td>
<td>Class 3</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>-</td>
<td>-</td>
<td>9</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>1</td>
<td>4</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>-</td>
<td>4</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>-</td>
<td>5</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>5</td>
<td>2*</td>
</tr>
</tbody>
</table>

* Complementing lines (IJC9 and IJE10) used as controls.
† See text for significance of class differences.
or 59b-z Uc-l/Y; Dp(1;3)sn^{18s}/+ sons, although they can produce more than 25 daughters of the constitution 59b-z Uc-l/Df(1)ct^{18s}; Dp(1;3)sn^{18s}/+. The class 3 females distinguish themselves by producing one or a few 59b-z Uc-l/Df(1)ct^{18s}; Ki/+ daughters as well as occasional 59b-z Uc-l/Y; Ki/+ sons, in addition to more than 20 59b-z Uc-l/Y; Dp(1;3)sn^{18s}/+ sons. The class 1 females represent those females carrying the lethal mutation in the deleted region of Df(1)ct^{18s}. The class 2 females are those with at least two lethal mutations in which one of the lethals is in the deleted region, or carriers of a deficiency or an inversion. The class 3 females are most easily interpreted as representing reversions.

Five carrier females (FM6K/59b-z Uc-l) from each of the 33 lines were progeny tested with Df(1)ct^{18s}/Y; Dp(1;3)sn^{18s}/Ki males. All of the 165 females produced five or more 59b-z Uc-l/Df(1)ct^{18s}; Ki/+ daughters. The results of the complementation tests, using the two overlapping deficiencies, indicate that all of the 33 lethal lines have at least one of the lethal lesions in the region between 6E1 to 7A2. Using the data shown in Table 6, a reversion frequency of 9.7 ± 2.1% was obtained for the 165 females in the 33 lethal lines.

Allelic complementation tests for those lethal mutations localized in 6E1 to 7A2 region were conducted by mating each of five females carrying a given lethal mutation (FM6K/59b-z Uc-l) to two males carrying the lethal mutation to be tested [59b-z Uc-l/Y; Dp(1;3)sn^{18s}/+]. Complementation was indicated by the presence of 59b-z Uc-l/59b-z Uc-l daughters, but absence of 59b-z Uc-l/Y sons. The lethal lines GJ and GCF from the earlier work (see Figure 1) were included in the test since these lines mapped in the region between 6E1 to 7A2. Two lethal lines having a lesion in the X chromosome other than 6E1 to 7A2 region (IJC9 and IJE10) were used as controls.

The instability and female sterility associated with the lethal clusters from IJA3 posed some difficulty. Convincing evidence was obtained, however, for the complementation pattern shown in Table 7. It is interesting to note that all members of the clusters of lethal mutations are in one and the same complementation group.

**TABLE 7**

*Complementation pattern of 35 lethal lines localized in the region between 6E1 to 7A2*

<table>
<thead>
<tr>
<th>Complementation group #1</th>
<th>Number of lines</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lethal cluster from IJA3</td>
<td>20</td>
</tr>
<tr>
<td>Lethal cluster from IJD3</td>
<td>5</td>
</tr>
<tr>
<td>Lethal cluster from IJD4</td>
<td>7</td>
</tr>
<tr>
<td>Total</td>
<td>32</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Complementation group #2</th>
</tr>
</thead>
<tbody>
<tr>
<td>IGJ</td>
</tr>
<tr>
<td>IJD15</td>
</tr>
<tr>
<td>Total</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Complementation group #3</th>
</tr>
</thead>
<tbody>
<tr>
<td>IGJ</td>
</tr>
</tbody>
</table>
**Cytogenetic analyses**

The polytene chromosomes of female larvae having light-brown mouth parts (59\(b\)-z \(Uc\)-l/59\(b\)-z) from the cross between the \(FM6K/59\(b\)-z \(Uc\)-l females and 59\(b\)-z/Y males were examined. At least ten individual cultures were established for each of the 35 lines, and the first satisfactory slide containing one pair of glands for each culture was analyzed to represent the culture. A satisfactory slide contained spreads of chromosomes, relatively free of distortion, from at least 30 nuclei. The 59\(b\)-z chromosomes from \(FM6/59\(b\)-z females in the control lines were examined in the same manner to compare with the lethal lines. The chromosomes from the lethal lines IJA3-1 through IJA3-10 were analyzed in the \(F_3\) generation. Those of the remaining 25 lines were analyzed in the \(F_9\) generation.

Fourteen chromosome rearrangements, shown in Figure 3, were detected among 18 of the 35 lethal lines. These rearrangements include ten inversions, two reverse repeats, one transposition and one deficiency. The 18 lethal lines with chromosome rearrangements are listed in Table 8. All of the chromosomes examined in the remaining 17 lethal lines appear to be free of cytologically detectable rearrangements. Detailed cytological examination of 150 larvae from the control lines revealed no evidence of aberrations.

---

**Figure 3.** A list of chromosome rearrangements detected in the 35 lethal lines from the five \(Uc\) lines. The \(X\) chromosome is diagrammatically represented by the uppermost thick line with its 20 cytological regions. The symbols |-|, -|-, and --> represent deficiency, duplication, inversion and transposition, respectively. The extents of the rearranged segments are shown to scale. The designations are shown to the left and the breakpoints are shown to the right of the rearrangement symbols. A point mutation is indicated by a dot (\(\cdot\)).
<table>
<thead>
<tr>
<th>Lethal lines</th>
<th>Normal</th>
<th>c4*</th>
<th>c4e-8G</th>
<th>Dp*</th>
<th>Dp*</th>
<th>Inversion</th>
<th>transposition</th>
</tr>
</thead>
<tbody>
<tr>
<td>IIA3-1</td>
<td>6</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>11</td>
</tr>
<tr>
<td>IIA3-5</td>
<td>12</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>11</td>
</tr>
<tr>
<td>IIA3-6</td>
<td>6</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>11</td>
</tr>
<tr>
<td>IIA3-7</td>
<td>7</td>
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<td>5</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>11</td>
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<tr>
<td>IIA3-9</td>
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<td>11</td>
</tr>
<tr>
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<td>2</td>
<td>11</td>
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<td>11</td>
</tr>
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<td>11</td>
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<td>12</td>
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<tr>
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<td>4</td>
<td>4</td>
<td>4</td>
<td>12</td>
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<tr>
<td>IIA3-18</td>
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<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>12</td>
</tr>
<tr>
<td>IIA3-19</td>
<td>7</td>
<td>6</td>
<td>6</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>12</td>
</tr>
<tr>
<td>IIA3-20</td>
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<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>11</td>
</tr>
<tr>
<td>IID3-4</td>
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<td>1</td>
<td>1</td>
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<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>11</td>
</tr>
</tbody>
</table>

*Df = Deficiency; Dp = Duplication; Tp = Transposition*
The rearrangements detected among the lethal mutations are remarkable in two respects: (1) distribution of their breakpoints is not random, and (2) they are extremely unstable. The similarity of breakpoints is indicated by the fact that ten of the fourteen rearrangements have one of the breaks in the 6F region of the X chromosome; eight of these ten are within the 6F1–2 doublet. At least one of the breakpoints in each of the remaining four rearrangements was near the 6F region; \( \text{In}(1)7C–9A \) and \( \text{In}(1)7C–10F \) each have a break in 7C1–4, one of the breaks in \( \text{In}(1)6D–10F \) is in 6D–E1, and \( \text{Tp}(1)7B–7E \) has a break in 7B5–C1. Although a cytological break in 6F was not observed in these four rearrangements, these lines do have a lethal lesion in 6F1–4, as will be discussed below (see discussion on transposability).

The most unusual aspect of the aberrations, however, was the presence of three different chromosome rearrangements sharing identical breakpoints. Thus, as shown in Figure 4, \( \text{Df}(1)ct^{st} \), \( \text{Dp}(1;1)ct^{st} \) and \( \text{Zn}(1)6F–7C \) each have breakpoints in 6F1–2 and 7C1–2. This is clear indication of a remarkably high specificity in the breakage of chromosomes in the \( Uc \) stocks.

Chromosome rearrangements were detected in 106 of the 365 larvae from the 35 lethal lines examined. After adjusting for the clusters, this corresponds to a frequency of 29.0 ± 6.9%. As shown in Table 8, all of the lines with aberrations, except IJD15, were heterogeneous in that they included a mixture of individuals with different types of chromosomes. Three chromosome types were detected among the ten larvae examined for each of IJA3–9, IJA3–11 and IJA3–12. Since each of these lines was established from a single carrier female, this chromosome polymorphism must reflect chromosome instability.

Two classes of related chromosome rearrangements were found in IJA3–7. Three of the ten larvae examined cytologically had \( \text{Dp}(1;1)ct^{st} \) and seven larvae had an inversion, as well as a duplication [designated as \( \text{In}(1)\text{Dp}(1;1)ct^{st} \)]. To produce these two classes, there must have been at least two independent breakage and fusion events, with some of the events resulting in a transmissible rearrangement. The larvae examined were in the F3 generation from the time when the line was established from a single carrier female, indicating a remarkable instability of the chromosome.

Two separate lines of evidence suggest that the events leading to the production of at least some chromosome rearrangements take place in somatic cells during the development of larvae. First, some larvae exhibit mosaic salivary glands and, second, some larvae appear to have intrastrand rearrangements of the polytene chromosomes. Neither of these exceptional types of rearrangements was included for tally in Table 8.

A total of four unequivocal cases of mosaic salivary glands was observed. One of the larvae sampled from IJA3–15 had a gland composed primarily of cells with the normal X chromosome, but with a sector of at least seven cells with a reverse repeat. The breakpoints of the duplication were 5B–C and 6F1–2. A larva from IJA3–18 had a sector of cells with a normal X chromosome and a sector of cells with \( \text{In}(1)6F–7C \). Two of the larvae sampled from IJA3–17 had mosaic glands. The first had a sector of cells with \( \text{Df}(1)6F–7C \) and another sector of at
Figure 4.—Polytene chromosomes showing the regions 6 and 7 of the X chromosome: (a) A well-stretched normal X chromosome. (b) The X chromosomes from a female heterozygous for a normal X chromosome and Df(1)ct¹⁸. The arrow indicates the band formed by fusion of left part of 6F1-2 and right part of 7C1-2. (c) An X chromosome with Dp(1;1)ct²⁸. The separated normal homologue is not shown. (d) The X chromosome from a female heterozygous for In(1)6F-7C and a normal X chromosome.
least six cells with an inverted segment extending from 11A to 19E, as well as the deficiency. The second mosaic gland from IJA3–17 was composed of a sector carrying In(1)6F–11A and a sector of cells with Df(1)6F–7C. In each of these four cases, there was at least one nucleus in which the separation of homologous chromosomes made possible a clear cytological interpretation of the rearrangements involved.

Among nuclei in which homologous X chromosomes were separated in the central region, there were sporadic cases of what appeared to be intrastrand chromosomal aberrations. One of some 15 such cases observed is shown in Figure 5. Region 6 and 7 of the X chromosomes shown in the figure are from a single nucleus of a female larva of the constitution 59b-z/59b-z Uc-l from IJA3–3. One of the two X chromosomes, shown in Figure 5b, appears normal. The other X, shown in 5a, has an obvious structural abnormality in the region between 6F and 7B.
This structure appears to be an intrastrand inversion. If, during the early stages of polytenization, one of the sister strands within one of the pair of homologous chromosomes underwent an inversion, somatic pairing between the inversion strands and their normal sister strands should yield a structure like that in Figure 5a. A few cases of what appeared to be intrastrand deletions were also observed. In these, about one-half or one-fourth of the 6F1–2 doublet or 7C1–2 doublet seemed clearly deleted in one of the homologues.

Kalisch (1970) observed re-inversion of \( \text{In}(1)w^{\text{ae}} \) to the normal sequence, which was associated with reversion of his mutable \( zw^{ \text{ae}} \) to the pigmented phenotype. To date, conclusive cases of such re-inversion to the normal sequence have not been detected among the \( U_c \)-generated lethal mutations.

**Morphological abnormalities**

Mutational alterations in four recessive markers (\( y^{59b}, w^i, ct^i, \) and \( f \)) were checked by observing 59b-z/59b-z \( U_c \)-1 daughters from the cross between the carrier females and males carrying the 59b-z chromosome. The only mutational change detected for the recessive markers was a reversion of \( ct^e \) to \( ct^+ \) in IJA3–3. The significance of the reversion is not evident at present.

Many flies from the lines shown in Figure 1, descended from the F₂ male, 117–13, exhibited a peculiar abnormality of the abdomen. The lethal lines from 117–13 (\( l^u, l^v, l^w, l^6, \) and the five lethal lines from \( l^u \)), as well as the five \( U_c \) lines, all exhibit this trait, with the proportion of affected flies varying from line to line and from one generation to the next within a line. Although the proportion of affected flies in a given line ranged from between 5 and 40% of the \( B/B^+ \) females (\( FM6K/59b-z \ U_c \) or \( FM6K/59b-z \ U_c-1 \)), the transmission of the trait was continuous, never skipping a generation. The abnormalities appeared to be limited to the abdomen of the affected flies. The trait is characterized by incomplete formation of tergites and sternites, exposing a thin, crinkled cuticle and elimination of the hairs and bristles on the corresponding parts of the abdomen. In most of these, the cuticle is so thin that tracheae can be seen clearly. Lack of an entire tergite spanning the abdomen is not uncommon, and usually more than one area of the abdomen exhibits the abnormality. Frequently, abnormal patches involving two or more tergites are present on only one side of the abdomen, and the patches are often so small that only a few bristles or hairs are missing. The phenotype resembles abnormal abdomen (\( A \)) described by Morgan (1915) and Sobels (1952) and is very similar to the etched abdomen in extreme bobbed flies. The trait is expressed almost exclusively by the females carrying a \( U_c \) or \( U_c-l \) chromosome. Although it is, on rare occasions, observed in the sibling females homozygous for \( FM6K \), and sibling males carrying \( FM6K \) or the 59b-z \( U_c \) chromosome, in these flies, the expression of the trait is so mild that it usually escapes detection.

The relationship, if any, between the instability and the abdominal cuticle morphology is not evident. Preliminary observations, however, suggest a possible relationship between female sterility in some of the lethal lines and the abnormal abdomen. A study is underway to investigate the relationships between instability, sterility and abdomen morphology.
SITE-SPECIFIC INSTABILITY

DISCUSSION

Genetic properties of the unstable chromosomes

Genetic instability: A number of features in \( Uc \) lines, such as revertability, site-specific instability, generation of somatic mutations and occurrence of clusters of mutations, make it difficult to estimate the mutation frequency. Nevertheless, quantitative as well as qualitative (cytological) differences in the mutability of the \( Uc \) lines and the control lines are real.

Many lines of \( Uc \)-generated lethal mutations revert to a nonlethal state with a disturbingly high frequency. Most of the lethal lines that originated in clusters are so unstable that they would have been quickly discarded if these lines had to conform to classical lethal standards. Yet, these are authentic lethal mutations, many of which are associated with chromosome rearrangements.

Mosaic salivary glands, apparent intrastrand chromosome aberrations and chromosome polymorphism imply that a given chromosome may go through a number of mutational events during the period between zygote formation and gametogenesis.

Unfortunately, very little can be said about the molecular basis of the origin of the \( Uc \) chromosome. It did originate from EMS-treated sperm; however, whether the treatment had anything to do with generation of the element and subsequent association with the 6F region of the \( X \) chromosome is difficult to establish at this time. Green (1977a) presented a charming argument and evidence that \( w^i \) (also \( y^s \) and \( f^{rn} \)) is an insertion mutant. Both \( w^c \) of Green and the \( Uc \) chromosome described in this paper are chromosomes that carry \( w^i \). Whether this is just a coincidence or \( w^i \) is the source of the transposable element for the \( Uc \) chromosome is not at all clear.

Transposability: In IJA3–9, five of ten larvae exhibited an inversion having breakpoints at 6F2–5 and 7B5–C1. A transposition, \( Tp(1)7B–7E \), detected in three of the remaining five sibling larvae from the same line, had a segment between 7B5–C1 and 7E1–2 inserted at 9C2–D1. One of the two inversions detected in IJA3–14 had breakpoints at 6F1–2 and 7C1–2; while the second had breakpoints at 7C1–4 and 10F–11A1. These two lines have "leapfrogging rearrangements," which are defined as a set of two or more aberrations, present among siblings, sharing a common breakpoint. The leapfrogging rearrangements suggest that the aberrations in each of these lines were generated by a transposable agent. Presumably, this agent was initially at 6F1–4.

\( In(1)7C–9A, In(1)7C–10F, In(1)6D–10F \) and \( Tp(1)7B–7E \) do not have a chromosome break in 6F1–5. These rearrangements, however, do not complement \( In(1)3C–6F, \) in which the lethal break is at 6F1–5, and the second break, not associated with lethality, is at 3C10–D1. Complementation tests with \( In(1)3C–6F \) indicate that the four stocks each have a lethal point mutation at 6F1–5. The situation implies a rearrangement-free transposition with a lethal lesion generated at the initial site (6F1–5), followed by an aberration-generating transposition. The characteristics of these four stocks, as well as those showing leapfrogging rearrangements, are most easily explained by the action of a transposable element.
Site specificity: All of the rearranged chromosomes from Uc lines had either a chromosome break or a lethal point mutation in 6F1-5 of the X chromosome. In addition, all but two of the 35 lines had a lethal lesion in the 6E1 to 7A2 region of the X chromosome. This remarkable degree of site specificity, along with the transposability just mentioned, suggested a model for the nature of Uc-generated lethal mutations. The X chromosome of the Uc lines has a transposable element inserted at 6F1-5, which may or may not impair genetic function in its vicinity. This element may transpose itself to a new location within the X chromosome, frequently to 7C1-2, less frequently to 10F-11A, and still less frequently, elsewhere. The transposition may or may not induce a lethal lesion at 6F1-5, and may or may not generate a chromosome rearrangement.

Viewed in this way, two of the 35 lethal lines not having a lethal lesion in 6F1-5 (IJC9 having a lethal point mutation at 40.5 ± 1.0 and IJE10 having a lethal point mutation in the region between 7C9 and 8A5) are presumably cases of transpositions without rearrangement, without lethality at 6F1-5, but with the insertions causing a lethal effect at new loci. Alternately, these two lines represent spontaneous mutations. Four of the 14 rearrangements not having a chromosome break in 6F1-5 [In(l)6D-10F, In(l)7C-9A, In(l)7C-10F and Tp(l)7B-7E] are cases where the transpositions resulted in no rearrangement involving the 6F1-5 region, but in lethality at 6F1-5. Subsequent transpositions produced rearrangements, however, in areas other than 6F1-5. The remaining ten rearrangements with a chromosome break in 6F1-5 represent transpositions causing rearrangements and lethality at the break in 6F1-5. Finally, the lines having a lethal point mutation in the 6E1 to 7A2 region are transpositions accompanied by the induction of lethal mutations at 6F1-5, with or without lethality at the new sites.

Chromosome rearrangements: Approximately one-third of the larvae carrying the Uc-generated lethal mutations are associated with chromosome aberrations. All of the aberrations had either a chromosome break or a lethal point mutation in 6F1-5 of the X chromosome. These site-specific aberrations associated with many of the mutations are the most crucial evidence indicating that the instability is associated with mutational events.

At least one of the Uc-generated breaks, a break at 3C10-D1 in In(l)3C-6F, is not associated with lethality. Many breaks are in the bands (see Figure 3 and 4) rather than in the interband regions, and interchromosomal aberrations, such as translocations, are absent.

A fixed endpoint for insertion-element-induced rearrangements is clearly documented by LEFEVRE and GREEN (1972), and GREEN (1973) has pointed out that deletion is not accompanied by the loss of the insertion. The only type of aberration detected in GREEN's wc mutants and their derivatives was deficiency, and the MR-generated sn, y and ras mutants investigated by GREEN (1977b) were free of cytologically detectable rearrangements. In contrast, the most frequent type of aberration generated in the Uc lines was inversion and the least frequent types were deficiency and transposition. This situation may reflect a difference in the nature of the element involved in each case.

LEFEVRE and JOHNSON (1973) found that deficiency for bands 7C5-9 is associ-
ated with dominant lethality. According to LEFEVRE (personal communication) the dominant lethality is actually a manifestation of a strong Minute locus in the region. The relative scarcity of deficiency-associated lethals among the Uc-generated mutations may be attributable to the haploinviable locus at 7C5–9. The Sxl locus in 6E–F region can be another cause for relatively infrequent occurrence of deficiency. A dominant female-lethal gene (Fl) of MULLER and ZIMMERING (1960) has been renamed SxlF' by CLINE (1978). The SxlF' is at 19.1, cytologically placing it in 6E–F, and its relationship to SxlF and the da locus in the second chromosome has been documented by CLINE (1978). The relationship between these loci and Uc, if there is any, is not known at present.

The four genetic properties of the Uc-generated lethal mutations discussed above, taken together, constitute persuasive evidence for involvement of a transposable element in the Uc lines. The conclusion is based on the remarkable similarity between the observed genetic properties of the Uc-generated lethal mutations and the well-documented characteristics and properties of transposable elements (IS elements, Tn elements and episomes) in Escherichia coli and its plasmids (see reviews by STARLINGER and SAEDLER 1976; STARLINGER 1977; NEVERS and SAEDLER 1977; and KLECKNER 1977; and the monograph edited by BUKHARI, SHAPIRO and ADHYA 1977).

The significance of the unstable chromosome

As an hereditary mutagen, a transposable element differs from any physical or chemical mutagen in at least three characteristic ways. First, the action of a transposable element is persistent. Once in a cell, the cell will experience recurring damage and repair of a number of different genes until the host organisms dies or until the element is eliminated from the cells. In this regard, its mutagenic effect resembles the effects of a chronic exposure to a mutagen. An important difference is that the targets for the activities of a transposable element are not random, especially for the primary locus (the locus where the element was initially located).

Second, it is likely to produce mutations with incomplete penetrance and variable expressivity for a given locus. The variation in the manifestation and expression of a gene at the primary locus reflects the net results of the transpositional activities of the element. These activities reflect where in the genome it was transposed to, when it was transposed in regard to development, how many transpositions occurred during a given time, and what happened at molecular level in the primary and secondary locus (the new location of the element). Since the transposition activities can differ from one event to the other, the manifestation and expression of the gene at the primary locus are expected to be modified accordingly.

Third, a mutation generated by a transposable element involves at least two mutational events at a time: one in the primary locus and the other at the secondary locus. This is an extremely important feature of the element, which can be related to the two-mutation theory of KNUDSON (1971) for cancer initiation. According to this theory, at least two successive mutations, and probably more, are necessary for initiation of both hereditary and nonhereditary forms of cancer. The nature of a mutagen that might favor such activities leading to initiation of
cancer, especially by a transposable element, has not been elaborated by Knudson (1971) or in his subsequent studies (Knudson, Strong and Anderson 1973; Hethcote and Knudson 1978).

The three feature expected of a transposable element as a hereditary mutagen make it a logical type of mutagen in initiating cancer. In this regard, it did not escape my attention that some retinoblastomas, perhaps the most extensively investigated of all cancers in relation to the genetic basis of cancer, are associated with site-specific chromosome breaks and rearrangements (Sparkes et al. 1979; Yunis and Ramsey 1978; Knudson et al. 1976). The intriguing situation in retinoblastoma associated with chromosome rearrangements in mother and daughter reported by Sparkes, et al. (1979) can easily be explained by involvement of a transposable element.

The important role such elements may play in evolution is obvious, and their effects on development, aging, fertility and chromosome segregation deserve careful scrutiny.

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SITE-SPECIFIC INSTABILITY


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