VITAL GENES THAT FLANK SEX-LETHAL, AN X-LINKED SEX-DETERMINING GENE OF DROSOPHILA MELANOGASTER

JANICE A. NICKLAS¹ AND THOMAS W. CLINE²

Department of Biology, Princeton University, Princeton, New Jersey 08544

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ABSTRACT

The X-chromosome:autosome balance in D. melanogaster appears to control both sex determination and dosage compensation through effects on a maternally influenced sex-linked gene called Sex-lethal (Sxl; 1-19.2). To facilitate molecular and genetic analysis of Sxl, we attempted to determine the locations of all ethyl methanesulfonate (EMS)-mutable genes vital to both sexes in the region between 6E1 and 7B1. This area includes approximately 1 cM of the genetic map on each side of Sxl and was reported by C. B. BRIDGES to contain 26 salivary gland polytene chromosome bands. The region appears rather sparsely populated with genes vital to both sexes, since the 122 recessive lethal mutations we recovered fell into only nine complementation groups. From one to 38 alleles of each gene were recovered. There was a preponderance of embryonic lethals in this area, although the lethal periods of loss-of-function mutations included larval, pupal and adult stages as well. Since the screen required that mutations be recessive and lethal to males, our failure to recover new Sxl alleles was the result expected for a gene with a female-specific function. An attempt was made to identify recessive male-specific lethals in this region, but none were found. Precise map positions were determined for eight of the nine vital genes. An interesting feature of the map is the location of Sxl in the middle of a 0.6- to 0.7-cM interval that appears to be devoid of genes vital to both sexes. The genetic location was determined of breakpoints near Sxl for all available chromosome rearrangements. Sxl is most likely located just to the left of band 7A1. We determined the relationship of our EMS-induced mutations in these nine genes to alleles induced by others. From this we conclude that the various genes appear to differ significantly from each other in their relative sensitivity to mutation by EMS vs. X rays.

An important step in the study of specific genes in Drosophila melanogaster that merit intensive analysis is the characterization of the vital genes that flank them. As a useful by-product, such efforts generate a considerable amount of detailed information regarding gene organization and its relationship to chromosome structure, as well as information on gene mutability. We report here our efforts to isolate and characterize all vital loci in the 6E1-7B1 region of the X chromosome. This study is part of a project designed to explore, eventually at the molecular level, the functioning of a master regulatory gene called Sex-lethal (Sxl; 1-19.2) which is located in the middle of this region.

In Drosophila melanogaster, the ratio of the number of X chromosomes to

¹ Present address: Immunobiology Research Center, University of Minnesota, Minneapolis, Minnesota 55455.
² To whom correspondence should be sent.

the number of sets of autosomes controls both sex determination and X-chromosome dosage compensation. At least in somatic tissues, the effects of this X:A balance developmental signal on both processes appears to be mediated by regulation of Sxl or its product (see Lucchesi and Skripsky 1981; Cline 1983). Sxl+ activity imposes the female morphogenetic sequence and a lower overall rate of sex-linked gene transcription (a rate appropriate for an X:A balance of 1) on cells that would follow the male morphogenetic sequence and exhibit a higher overall rate of sex-linked gene transcription (appropriate only for an X:A balance of 0.5) in its absence. As a consequence, loss-of-function Sxl mutations behave as female-specific lethals, whereas gain-of-function (constitutive) alleles behave as male-specific lethals. Zygotic functioning of Sxl+ is dependent on maternal functioning of an autosomal gene called daughterless (da; 2-41.5). For this reason, wild-type embryos derived from eggs produced in the absence of maternal da+ activity develop as if they lacked Sxl+ activity (Cline 1978, 1980, 1983).

Because of its sex-specific functions and its interaction with da, both loss-of-function and gain-of-function (regulatory) variants of Sxl can be generated and characterized with unusual ease (Cline 1981); consequently, study of Sxl should be useful not only for understanding developmental regulation, but also for understanding the nature of mutations in higher eukaryotes. Particularly relevant to the present paper is the question of how closely the organization of regions surrounding regulatory genes such as BX-C, ANT-C and N, in addition to Sxl, resembles that surrounding genes such as ry, Adh and Ddc whose products have no obvious regulatory function.

MATERIALS AND METHODS

Culture conditions and stocks: Flies were raised at 22° on a standard cornmeal-yeast-sugar-agar medium (Cline 1978). For descriptions of most mutations and chromosomes see Lindsley and Grell 1968; Cline 1978; and Leefevre 1981. The exceptions are listed, followed by the appropriate reference.

Df(1)ct6
Df(1)ct4
Df(1)HA32
Df(1)Sxl+  
CLINE, unpublished  
Generated as revertants of the dominant male lethality of
Df(1)Sxl+  
CLINE, unpublished  
Sxl+ (see Cline 1981).
Dp(1;3)sn
Genotype updated in Leefevre 1981 (= Dp(1;3)ct+ in figures)
Dp(1;2)sn ct+y+Y
Cytology updated in Leefevre 1981

The TM3 chromosome used here was In(3LR)TM3, ri p+ sep bx+ e+ Sb Ser. The attached-X was C(1)DX, y f.

Isolation of lethal mutations in the 6D1-7D1 region: Ethyl methanesulfonate (EMS) was administered according to the method of Lewis and Bacher (1966) with a range of concentrations between 0.012 and 0.050 M. The highest percentage of lethals consistent with adequate male fertility was generated at 0.044 M, the concentration used most frequently. Figure 1 presents the scheme used to isolate lethals in the 6D1-7D1 region, the first step in the isolation of Sxl-region lethals. The mutagenized X chromosome indicated in this figure is marked with ct+y; however, in 7% and 25% of the crosses, respectively, a cm or a cm v chromosome was used instead so that we might recover differently marked alleles of most genes. Note that both cm and ct are closely linked to Sxl, and both are covered by Dp(1;3)ct+. Each bottle for cross A carried 50 mating pairs from which progeny were collected for 6 days. The fertility of males in cross B proved to be extremely low at 29°, so we
abandoned our plan to screen for temperature-sensitive alleles and conducted all crosses at 22°C. Sixty-five percent of the single-male matings in cross B were fertile at this temperature.

From 55,923 successful step B matings, we recovered only 463 lethals (0.84%) in a region (6D1-7D1) that represents, based on polytene chromosome bands, approximately 5% of the X chromosome. This figure suggests an X-linked lethal frequency of only 0.18/chromosome. The low yield almost certainly reflects a low overall mutation rate in the experiments, rather than a particularly low mutability of genes in the 6D1-7D1 region. During the mutagenesis, we estimated the mutation rate by comparing the cross A progeny sex ratio to that from a parallel unmutagenized male control. Although this estimate suggested an average rate of X-linked lethals of 0.87/chromosome, consistent with our initial expectations, subsequent analysis of the rate of induction of visible mutations in these same experiments indicated that the progeny sex-ratio effect must have led to a gross overestimate of the true mutation rate. Our results for y and w (66 nonmosaic w males, 25 nonmosaic y males) compared with those of Lee, Sega and Bishop (1970), and our results for e (92 e males and females) compared with those of Keppy and Denell (1979), suggest that our mutation rate was approximately fivefold lower than that generally found with EMS mutagenesis under these conditions, in agreement with our observed value of 0.18 based on 6D1-7D1 region lethals. Physiological complications caused by Dp(1;3)sn131 may have been responsible for the disappointing mutation rate with EMS. By brute force we managed to isolate an adequate number of alleles for this study, but we cannot recommend schemes in which males with this duplication are mutagenized.

Since the scoring of cross B lines was accomplished rapidly by observation of active flies through the sides of vials, subtle visible mutations would have been missed; however, mutations with a substantial effect on wing shape or head features should have been detected in the course of inspection for the ct wing phenotype or the cm v eye color. In fact, a variety of visibles covered by Dp(1;3)sn131 were observed, affecting wings, bristles, eye shape or eye color, but only five to ten new eye color mutants phenotypically indistinguishable from cm proved to be within the 6E1-7B1 interval studied here (unfortunately, these were not tested for complementation with cm).
Assignment of mutations to complementation groups: The pattern of complementation of new mutations with the three deficiencies shown in Figure 2 allowed us to determine which were in the 6E1-7B1 subregion of interest, and to which side of Sxl these mapped. Complementation tests of 6E1-7B1 lethals were generally done in groups of four to eight new mutants on the same side of Sxl, each initially being tested against all others in the group. When a complementation group acquired several members, a particular allele was chosen as the tester for categorizing lethals subsequently. For each complementation test, we examined at least 65 daughters from the cross: Lethal/Binsinscy, y w sn B & X & lethalal/Y; Dp(1;3)sn130/TM3, Sb Ser males. For mutants which complemented, we expected 16 lethalal/lethalal; TM3/+ daughters in a brood of 65 females. When fewer than five such daughters were recovered in a brood of this size, or if they exhibited obvious defects, a larger sample of flies was checked and/or the test was repeated. In this way, most lethals were tested with several alleles in their complementation group and with several lethals from each of the other groups on the same side of the map from Sxl. For example, of the 38 jnR1 alleles, three were crossed to five or more jnR1 alleles, 18 were crossed to four, six were crossed to three, six were crossed to two and five were crossed to only one allele. Because of the difficulty assigning jnR4 alleles to a group, all putative alleles of this gene were crossed inter se.

Mapping crosses: One member from each complementation group was mapped with respect to SxlM' in the cross: lethalal, (cm or ct6) v/y Hw SxlM'; sn. B; TM3/+ & X +/+; SxlM' is a dominant male-specific lethal (CLINE 1978). Map distance was calculated as 2 x (n sons)/(n daughters), with gene order indicated by the orientation of outside markers in the surviving sons. Many lethals were mapped pairwise from the cross: lethalal, ct6 v/lethalal, cm v; TM3/+ & X +/+; y/Y. A control mating to ct6 v/Y males served to indicate whether the TM3 balancer present in these crosses significantly affected the recombination frequency. Ninety-five percent confidence limits were calculated based on a Poisson distribution when more than 50 recombinant males were recovered; when the number was lower, the tables of STEVENS (1942) were used.

Lethal period determination: Lethal periods were determined for two alleles of each complementation group, usually those that had been used in mappings and as tester stocks in the complementation tests. From lethalal, (cm or ct6) v/+ females who had mated to wild-type males, 150-200 eggs (embryos) were counted and placed into vials. Several days later the percentage of egg hatch was determined, and the presence of any brown eggs (dead embryos) was noted. The sex and phenotype of eclosed adults, as well as the presence of any dead pupae, were recorded after 2 weeks. When lethal periods for two alleles differed, the earlier period was taken for Figure 3 as more indicative of the effects of a more complete loss of that gene’s function.

RESULTS

EMS-induced lethals near Sxl

A total of 463 hemizygous lethal mutations covered by the 6D1-7D1 duplication in males were recovered. Of these, 375 were tested for complementation with deficiencies SxlM', ct6 and ct55 which define the 6E1-7B1 interval as illustrated in Figure 2. The balance of 88 lethals (included in Figure 3 but not Figure 2) was tested only for their complementation with Df(1)SxlM', the 6F5-7B1 deficiency. From the collection of 375 lethals, 104 (28%) were determined to be within the 6E1-7B1 interval which contains 51% of the salivary chromosome bands (BRIDGES 1938). Seventy-nine lethals (21%) were located between 7B2 and 7B6-8, a region with a maximum of 14% of the bands surveyed. This region contains the highly mutable ct locus. Four lethals were uncovered by Df(1)ct45 but not Df(1)ct55; the right breakpoints of these two deficiencies, therefore, must be different (see Figure 3 in LEFEVRE and JOHNSON 1973). No lethals uncovered by the 6F5-7B1 deficiency were not also uncovered by the loss of 7A1-7B8; thus, the 6F5-6F11 region appears to be devoid of genes vital to both sexes.

The 122 new mutants generated in the 6E1-7B1 region fell into nine complementation groups indicated in Figure 3. jnR1 through jnR4 alleles were isolated
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survival of genotype:

lethal ct v. Dp(1;3)ct+ & Y ; TM3

lethal ct v. TM3 ; Df(1)Sxr &

lethal ct v. TM1 ; Df(l)ct+ &

lethal ct v. Df(l)ct+ &

bands in interval

doublets = 1)
muts recovered

FIGURE 2.—Assignment of 375 of the original 463 recessive lethals to subsections of the 6D1-7D1 region based on their pattern of complementation when heterozygous with each of three deficiencies ("+-" = failure to survive). The schematic of the salivary gland polytene chromosome and the band counts are based on Bridges' 1938 drawings (band width proportional to staining intensity). The number in parentheses indicates a lower limit to the band count, with Bridges' doublets counted as single bands.

FIGURE 3.—Complementation groups formed by 122 6E1-7B1 region recessive lethals, including 104 (33 + 71) mutations from Figure 2, plus an additional 18 mutations from 88 6D1-7D1 lines that were only screened against the 6F5-7B1 deficiency. Values in parentheses indicate the numbers of alleles we would have expected if all 463 6D1-7D1 lethals had been screened according to the scheme in Figure 2.

from a screen of all 463 6D1-7D1 lethals, whereas jnL1 through jnL4 alleles were from the subset of 375. To facilitate comparisons of the genes' mutability, the values in parentheses indicate the number of jnL1-4 alleles we would have expected to recover if all 463 lethals had been screened over the entire 6E1-7B1
region. Records were kept regarding the genealogy of all induced lethals. In only one case did we recover two alleles of the same gene (jnR4) from the same group of 50 females (step A, Figure 1); thus, we can conclude that essentially all alleles represent independent mutational events.

The relative number of alleles recovered for these nine genes clearly fail to fit a Poisson distribution. The low number for jnLX may reflect a failure of the screen to recover mutations with such leaky phenotypes (see later); however, such an explanation could not account for the recovery of only a single jnL3 allele, a larval-pupal lethal with no escapers at 22°C.

Figure 3 includes information on the lethal periods for mutations in these genes. The region appears to be unusually rich in embryonic lethals. For only 13 of the 70 vital genes (19%) studied by Shannon et al. (1972), Bryant and Zornetzer (1973) and Gausz et al. (1981) did loss of function cause embryonic lethality. Of the nine vital genes characterized here, loss of function at five (56%) caused embryonic lethality. This count does not include Sxl itself, which is also an embryonic lethal for females.

All jnR4 alleles allowed a fraction of the mutant individuals to survive to the adult stage; mutant males exhibited incompletely rotated genitalia, incomplete fusion of the mesothoracic disc derivatives and/or unexpanded wings. Even the rare morphologically normal escapers, however, could be distinguished from wild type, since all mutant males invariably died within a few days. Complementation analysis of the jnR4 group was complicated by the fact that homozygous and heteroallelic females had a less extreme phenotype; nevertheless, when all combinations of these mutant alleles were examined, a clear pattern of noncomplementation emerged. Only one allele remained ambiguous; it was placed in this group on the basis of its similar phenotype and map position.

The single EMS-induced allele of jnLX allowed most animals to eclose. They were normal with respect to external morphology but generally failed to expand their wings, quickly became stuck on the food surface and died. Hemizygous and homozygous mutant females displayed a phenotype similar to that of the males. In contrast to mutations at jnR4, several percent of the jnLX escapers remained healthy as adults, and some of these were fertile. This made precise mapping of jnLX difficult. The other gene for which we isolated only a single representative, jnL3, proved to be allelic to an EMS-induced adult-viable mutation called CB8 which was isolated in a behavioral screen for adults with impaired visual ability (Kankel and Lipshitz 1983).

Rare cases of intracistronic complementation were observed. JnR1 allele 9s1 partially complemented two (3n1 and 6j2) of the five jnR1 alleles against which it was tested. This seemed to be a rather special characteristic of mutant 9s1, since 3n1 and 6j2 failed to complement any of the other 15 or 22 alleles, respectively, against which they were tested. Viability of the heteroallelic combination was only about 25%, but the surviving adults were healthy and fertile. Less than one in a thousand of the sons survived from such heteroallelic females. One partially complementing combination of jnR2 alleles was also discovered.

Figure 4 shows map positions for eight of the nine new genes described here, relative to the standard position of the marker mutation, cm (band 6E6).
FIGURE 4.—Genetic map of the 6E1-7B1 region. Positions were determined from crosses between adjacent vital genes. These data are indicated on the top map, with the number of recombinant males recovered given in parentheses. The heavy line designates a 0.6- to 0.7-cM region surrounding Sxl that seems to contain no genes vital to both sexes. Additional mapping data between nonadjacent genes are indicated on the bottom map. These distances were generally consistent with the sums (values in parenthesis) of the smaller distances on which the map is based.

Positions were determined from the data presented on the map with the gene designations above it. The number of recombinant males recovered in the crosses used to construct this map is indicated in parentheses. The 95% confidence interval follows the "±." Recombination frequencies observed in crosses between nonadjacent genes are presented at the bottom of the figure. They are in good agreement with the sum of the shorter distances (values in parentheses) on which the map is based. Our single jnLX allele was not mapped precisely due to the problems with escapers. We did, however, determine an approximate map position for it relative to jnL2 by individually testing surviving sons of I(1) jnL2 ct v/HC217 females. HC217 is a somewhat less leaky X-ray-induced jnLX
allele (see later). We recovered five wild-type males among 2642 females. This places \textit{jnLX} at 18.5, a position consistent with the deficiency analysis that located it to the left of \textit{jnL1} and to the right of \textit{jnL4} (see Figure 3).

The calculated distance between marker mutations \textit{cm} and \textit{ct^6} on this map is 1.38 cM, somewhat longer than the standard value of 1.1 cM. JOHNSON and JUDD (1979) also reported a \textit{cm–ct^6} distance longer than the standard. Their distance of 1.3 cM (2600 recombinants) corresponds almost exactly to ours if we make a 5% correction for the interchromosomal effect of the TM3 balancer that was present in all of our mapping crosses. This correction (not included in the Figure 4 data) is based on an observed \textit{ct^6–v} distance in our control crosses of 13.6 cM (3047 recombinants) vs. the standard value of 13.0 cM. JOHNSON and JUDD also found, however, that the \textit{ct^6–sn^3} distance was shorter than the standard; consequently, the position of \textit{cm} at 18.9 seems the most reliable reference value for the assignment of standard map positions to the lethals we describe here.

The positions of chromosome rearrangement breakpoints on the genetic map are based on complementation data shown in Figure 3 and on our determination of allelism with LEFEVRE’s X-ray-induced mutations (see later). It should be noted that JOHNSON and JUDD (1979) cite a slightly different left breakpoint (7A1 vs. 7A3) for \textit{Df(1)ct^I4} than LEFEVRE and JOHNSON (1973). From the cytological and recombination data, it seems most likely that \textit{Sxl} is located just to the left of band 7A1.

The most striking aspect of the mapping data is the large distance (indicated by the heavier line) between the two vital genes, \textit{jnL1} and \textit{jnR1}, that flank \textit{Sxl}. \textit{Sxl} is in the middle of a 0.6-cM stretch of the genetic map that appears to contain no genes that are vital to both sexes.

Are there male-specific vital genes near \textit{Sxl}?

\textit{Sxl^+} function appears to be required only in females. Our failure to recover any \textit{Sxl} alleles in the present study is consistent with a female-specific function for this gene, since our screen demanded that mutations behave as recessive lethals in both sexes. In view of the absence of genes near \textit{Sxl} that are vital to both sexes, we wondered whether this region might contain genes with male-specific vital functions. Such genes do exist elsewhere with functions related to those of \textit{Sxl} (BELOTE and LUCCHESI 1980; SKRIPSKY and LUCCHESI 1980; CLINE 1982; UENOYAMA, FUKUNACA and OISHI 1982). Recessive male-specific lethals in the 6D1–7D1 region would have been included in the first selection step of the screen (Figure 1), but regardless of their true location, they would have been discarded in the subsequent tests (Figure 2) based on their complementation with the tester deficiencies in females.

To determine whether some of our 6D1–7D1 lethals might be male-specific, we tested 56 of the 186 recessive lethals which appeared to be outside the 6E1–7B1 region based on the tests in Figure 2, asking whether they might be homozygous viable in females; any male-specific recessive lethal in this group would be viable in females when homozygous. All 56 mutants tested were homozygous lethal and, thus, could not have been male-specific lethals. From this result we can conclude that there is not likely (\textit{P} < 0.035) to be a male-
specific vital gene in this region as mutable as the average vital gene that is not sex specific. Of course we realize that these results do not preclude a lethal of unusually low mutability; only analysis of Sxl-region deficiencies in males will be definitive on this point.

Relationship of our EMS lethals to lethal alleles generated by other mutagenic agents

Lim (1979) described three genes in the 6E1-7A1 region defined by recessive lethals that were generated by an extremely unstable transposable element at band 6F1.2. We attempted to determine the relationship of Lim's genes to those we describe here. Complementation tests revealed only one correspondence: Lim's group 2 (defined by l^{ScF}) and jnL1; however, this group 2 mutant appears to be a cytologically cryptic deficiency, since it also fails to complement the closely linked gene, cm, unlike all jnL1 alleles. The group 1 allele, l^{JA3-3}, was extremely leaky in our hands and, therefore, could not be assigned to a complementation group. The group 3 lethal allele, l^{Gd}, was no longer as originally characterized by Lim, since it mapped far (>10 cM) from jnR1 and jnR2 and complemented Df(l)ct*. The instability of these mutations limits their analysis.

Recently Lefevre (1981) isolated a number of X-ray-induced point mutations and rearrangements in the vicinity of Sxl. We examined all mutants still available that were listed on Lefevre's Figure 2 as between 6D8 and ct (7B3-4). This included alleles listed at 6D8 (RA52), 6E4 (HC217), 6E6 (EA22), 7A3 (JA9), 7A6 (EF465), 7B1 (RC21) and a deficiency from 7A5 to 7B1 (RFZ9). As Lefevre points out, the gene sequence and locations he indicated were only approximate, particularly for those genes with no euchromatically rearranged lethal alleles. Consistent with its assignment to 6D8, mutant RA52 was beyond Df(1)ct* and, thus, outside our region of interest. Mutant HC217 (6E4) corresponded to jnLX, EA22 (6E6) to jnL1, JA9 (7A3) to jnR1, and EF465 (7A6) to jnR3. Lefevre's deficiency RF19 uncovered jnR2, jnR3 and jnR4. Mutant RC21 (breakpoint at 7B1) is not included in our Df(1)Sxl"", but it is uncovered by RF19. Thus, there appears to be at least one additional vital gene between jnR4 and ct. Unfortunately, since RC21 is a rearrangement, we cannot use it to define more precisely the right breakpoint of Df(1)Sxl"" on the genetic map.

DISCUSSION

The present study adds the 6E-7A region containing a homeotic gene, Sxl, to the list of segments of the D. melanogaster genome that have been examined extensively with respect to the content and organization of vital genes. It represents one of only a small group of such studies that include extensive information on the specific map positions of adjacent genes, determined by recombination frequency. As is generally the case (Barrett 1980), large differences in the mutability of vital genes in the region confound attempts to determine by statistical criteria how close we are to having saturated the Sxl region. Our attempt to discover all vital genes near Sxl is at least as extensive as any except the combined studies of the z-w interval; however, definitive tests of whether we have identified all vital genes in the vicinity of Sxl will
involve the analysis of overlapping deficiencies, rather than additional searches for point mutations.

The possibility of a one-to-one correspondence between larval salivary gland polytene chromosome bands and complementation groups has received considerable attention. Two fundamental problems encountered in efforts to resolve this point are the difficulty in unambiguously identifying all bands and rearrangement breakpoints in a particular region, and the near impossibility of knowing how many complementation groups remain to be identified. Recent results of Young and Judd (1978) and Wright et al. (1981) seem to argue against such a correspondence. Less controversial but more relevant to the present paper is the finding that in every region for which efforts have been made to identify all vital genes (Hochman 1976; Schalet and Lefevere 1976; Woodruff and Ashburner 1979; Hilliker et al. 1980; Gausz et al. 1981; Zhimulev et al. 1981), the number of genes vital to both sexes has proven to be only a bit less than the number of bands. The region of the X chromosome from 3C1 to 3D4 does seem likely to a major exception to this rule: in this 16-band interval, only one vital gene, Notch, has been identified (Lefevere 1981), although it should be noted that a concerted effort to saturate this region for lethal mutations has not yet been made. Lefevere (1981) also noted that the region from 10B to 10F may be sparsely populated with vital genes in proportion to its band count; curiously, this region contains a newly discovered female-specific lethal, called sisterless, with functions intimately related to Sxl (T. W. Cline, unpublished data).

The work we report here identifies the interval between 6E1 and 7B1 as an exceptionally "lethal-free" region based on this general relationship between vital genes and band counts. Bridges (1938) assigned 26 bands to this area; yet, we have found it to contain only nine generally lethal complementation groups. A number of workers have concluded that many of Bridges' doublet bands are more properly classified as singlets (Berendes 1970; Lefevere 1976; Sorsa and Saura 1980); however, even if all of Bridges' doublets in the 6E1-7B1 region are scored as singlets (see Figure 2), the band count, 15-16, is still considerably in excess of the number of vital genes.

Several other observations are consistent with a relative scarcity of vital genes in this region. In the mutant screening, we found that only one-quarter of the lethals covered by a duplication from 6D1 to 7D1 were included in deficiencies from 6E1 and 7B1, despite the fact that this subregion is half the physical length of the duplication in polytene chromosomes (Lefevere and Johnson 1973) and contains half of the bands by Bridges' count. Furthermore, Lefevere (1973) reported that region 7A had a higher than average frequency of nonlethal rearrangement breakpoints, the result expected for a region with a frequency of vital genes that is below average.

On his Figure 2, Lefevere (1981) placed three complementation groups between jnl1 and jnr1, based on his isolation of cytologically rearranged lethals which, unfortunately, are no longer available for analysis. For one of these, the lethal effect was discovered not to be associated with the breakpoint (G. Lefevere, Jr., personal communication); however, if the cytological assignments of the other two are correct, there may be two vital genes near Sxl that we failed to identify.
in our screen of EMS-induced mutants. On the other hand, it was not the purpose of Lefevre's screen to define the cytological location of these breakpoints with extreme precision, a particularly difficult task in any event with heterochromatically rearranged lethals such as one of those in question. With only minor reassignments of their location, the two genes represented by these rearrangements could very well include \textit{jnL2}, \textit{jnL3}, \textit{jnR2} or \textit{jnR4}; nevertheless, a possibility that must be considered is that this region may contain genes like \textit{Tpl} (Keppy and Denell 1979; Roehrdanz and Lucchesi 1980) and \textit{dpp} (Spencer, Hoffmann and Gelbart 1982) whose functions are disrupted much more strongly by chromosomal rearrangements than by point mutations. Another possibility is that there could be vital genetic units in the region that we could not identify by EMS mutagenesis on account of the unusual functional relationships between them, a situation similar to that for genes between \textit{w} and \textit{N} (Lefevre and Green 1972).

The rate of recovery of mutant alleles of the nine vital genes between bands 6E1 and 7B1 varied over a 30-fold range; we isolated only a single mutant allele for \textit{jnLX} and \textit{jnL3} but recovered 38 alleles of the most mutable genes, \textit{jnR1}. A similar range in gene mutability was found in other studies. For example, the number of alleles recovered for genes between \textit{gt} and \textit{w} (combined results of Judd, Shen and Kaufman 1972; Lim and Snyder 1974; Liu and Lim 1975) ranged from a minimum of three to a maximum of 57. Genes \textit{zw1} and \textit{zw2} were hotspots which, like \textit{jnR1} characterized here, each had about twice as many alleles as the next most mutable gene.

Map distances between adjacent vital genes in the 6EZ-7B1 region varied over a wide range, from 0.045 cM for \textit{jnL1} and \textit{jnL2} to the rather large distance, 0.57–0.74 cM, between \textit{jnL1} and \textit{jnR1}. The maximum distance measured between adjacent lethals in the \textit{gt-}w region was 0.30 cM. Zhimulev et al. (1981) observed a maximum distance of 0.48 cM between adjacent lethals \textit{BP4} and \textit{BP5} in their saturation study of a region with a recombination frequency comparable to that near \textit{Sxl}. Although the \textit{jnL1-jnR1} distance exceeds this, it is less than the remarkable 1.5 cM that separates \textit{N} from the nearest known point lethals on either side (Lefevre and Green 1972; Lefevre 1981).

The size of the \textit{Sxl} locus itself does not appear to be responsible for the large distance between the flanking lethals, \textit{jnR1} and \textit{jnL1}. Fine-structure analysis of seven \textit{Sxl} heteroalleles showed the gene to be only 0.014 cM long and not unusually mutable (Cline 1978, 1981); however, these conclusions could be biased by the type of mutations that were analyzed: mostly intragenic suppressors of the dominant male lethality of \textit{Sxl}^\text{Mer}, mapped with respect to the male-specific lethal.

In studies of the \textit{gt-w} region, the relative frequencies of EMS-induced mutant alleles of each gene appeared to be similar to those for X-ray-induced mutations, although of course the absolute frequencies for the two mutagenic treatments were quite difficult. We found that our EMS hotspot, \textit{jnR1}, corresponded to Lefevre's X-ray hotspot at 7A3; however, for other genes in this region, there do appear to be differences with respect to their relative sensitivity to EMS vs. X rays. Lefevre found seven X-ray-induced lethal alleles of \textit{jnR3}, but only one
allele of jnL1. His collection could have included no more than two alleles of jnR2. In contrast, we found jnL1, jnR2 and jnR3 to be equally mutable by EMS. Both studies produced a single allele of jnLX, despite the fact that ours included more than five times the number of alleles of the hotspot, jnR1.

The aim of this study was to generate information that would be useful in the future analysis of Sxl and its interactions with other genes. Of particular interest is the question of whether Sxl function is indeed required only by females. Our failure to isolate recessive lethals or recessive male-specific lethals closely linked to Sxl is consistent with such a sex-specific function, but a definitive test of this hypothesis requires the demonstration that males who lack this region of the X chromosome can survive. Work we report here suggests that it should not be difficult to construct synthetic deficiencies for such a test.

The discovery of intracistronic complementation between alleles of jnR1, the vital gene closest to Sxl on the centromere proximal side, also promises to be useful. It has allowed us to synthesize easily a small tandem duplication of Sxl+ (see Gelbart and Chovnick 1979). With this we should be able to analyze partially complementing heteroallelic Sxl mutant combinations in males and increase the multiplicity of Sxl+ through unequal recombination for use in dose-effect studies.

For genes such as Adh, ry and Ddc, which do not have obvious regulatory functions in development, flanking vital loci appear to be very closely linked. Although the organization of genes surrounding homeotic elements of the Antennapedia complex has been determined (Lewis et al. 1980a, b), the precise linkage relationships of these flanking vital genes have not yet been reported. Nor is this kind of information yet available for the classic homeotic gene complex, bithorax (Lewis 1978). N is the most conspicuous example of a gene like Sxl that appears to be relatively isolated from functionally unrelated vital loci on either side. This is curious, since N, like Sxl, seems to establish and perhaps maintain specific developmental pathway commitments (see Lehman et al. 1981, and discussion in Wright 1970). A determination of the significance of this observation awaits analysis of the genes surrounding the other homeotic loci mentioned.

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