Genetic and Molecular Analysis of New Female-Specific Lethal Mutations at the Gene *Sxl* of *Drosophila melanogaster*

Begoña Granadino,* Miguel Torres,* Daniel Bachiller,*,[1] Eduardo Torroja,* José L. Barbero[†] and Lucas Sánchez*

**Centro de Investigaciones Biológicas, Velázquez 144, 28006 Madrid, Spain, and [†]Antibióticos Farma, S. A. Antonio López 109, 28026 Madrid, Spain*

Manuscript received October 16, 1990
Accepted for publication June 6, 1991

ABSTRACT

We have isolated three female-specific lethal mutations at the gene *Sex-lethal (Sxl)*: Sxl^{fb}, Sxl^{fc} and Sxl^{fd}. We have carried out the complementation analysis between these mutations and other previously reported Sxl^{f} mutations. It is possible to classify the alleles tested in this report into two complementation groups: the *bc* group defined by Sxl^{fb}, and Sxl^{fc}, and the *LS* group defined by Sxl^{fLS}. The other alleles tested affect both complementation groups albeit with different degrees. Contrary to what happens with mutations at the *LS* group, mutations at the *bc* group do not affect sex determination, nor late dosage compensation nor oogenesis. Both Sxl^{fb} and Sxl^{fc} present a DNA insertion of at least 5 kb between position −10 and −11 on the molecular map, within the fourth intron. On the contrary, Sxl^{fd}, a strong mutation affecting all *Sxl* functions, is not associated to any detectable DNA alteration in Southern blots, so that it seems to be a "point" mutation. In agreement with their phenotypes, both Sxl^{fc}/Sxl^{fLS} and Sxl^{fc} homozygous female larvae express only the late *Sxl* transcripts characteristic of females, while females homozygous for Sxl^{fLS} express only the late *Sxl* transcripts characteristic of males. Moreover, Sxl^{fc} presents a lethal synergistic interaction with mutations at either *da* or the X:A ratio, two signals that define the initial activity state of *Sxl*, while Sxl^{fLS} do not. These data suggest that the two complementation groups are related to the two sets of early and late *Sxl* transcripts, which are responsible for the early and late *Sxl* functions, respectively: Sxl^{fb} and Sxl^{fc} would affect the early functions and Sxl^{fLS} would affect the late *Sxl* functions.

IN *Drosophila melanogaster*, the gene *Sxl* controls the processes of somatic sex determination, dosage compensation and oogenesis. The functional state of *Sxl* is determined by the X:A ratio signal: in 2X;2A flies *Sxl* will be ON, while in X;2A flies *Sxl* will be OFF (CLINE 1978). Two X-elements of the X:A signal have been identified: *sisterless-a* (*sis-a*) (CLINE 1986) and a region of the *achaete-scute* complex that has been named *sisterless-b* (*sis-b*) (CLINE 1988) which corresponds to the gene *scute-T4* (*sc-T4*) (TORRES and SANCHEZ 1989; PARKURST, BOPP and ISH-HOROWIEZ 1990; ERICKSON and CLINE 1991). Activation of *Sxl* requires also the maternal *daughterless* (*da*) product (CLINE 1978). Once the state of *Sxl* is defined, an event that occurs at the blastoderm stage, the X:A signal is no longer used and the activity of *Sxl* remains fixed (SANCHEZ and NÖTHIGER 1983; BACHILLER and SANCHEZ 1991). The capacity of the gene *Sxl* to function as a stable "switch" is thought to be due to a positive autoregulatory function of the *Sxl* gene product (CLINE 1984). This gene is controlled throughout development by alternative splicing of its primary transcript (BELL *et al.* 1988). The gene *f l(2)d* is needed for the female-specific splicing of *Sxl* RNA, thus suggesting the involvement of *f l(2)d* in the positive autoregulatory pathway of *Sxl* (GRANADINO, CAMPUZANO and SANCHEZ 1990).

The gene *Sxl* produces two temporally separate sets of transcripts. The early set is composed of three transcripts found only around the blastoderm stage, presumably, in female embryos (SALZ *et al.* 1989). The late set is formed by three other transcripts, present in both females and males, which appear slightly later in embryogenesis and persist throughout the remainder of development and in adult life. The three female late transcripts overlap extensively and share most exons, but differ at their 3′ ends. The three male transcripts are similar to their female counterparts, except for the presence of an additional exon (exon 3), which contains a translation stop codon; consequently, the male late transcripts give rise to presumably inactive truncated proteins (BELL *et al.* 1988). Two *Sxl* transcripts are associated with the development of the female germline, one of them being also present in the soma (SALZ *et al.* 1989).

Two sets of *Sxl* mutations have been isolated. One set is formed by loss-of-function mutations, generically named as Sxl^{f}, which are characterized by their reces-

[1] Present address: EMBL, Postfach 10.2209, 6900 Heidelberg, Federal Republic of Germany.

sive female-specific lethal phenotype (CLINE 1978; MARSHALL and WHITTLE 1978; SANCHEZ and NÖTHIGER 1982; this report). The other set is formed by gain-of-function mutations, generically named at Sxl^M, which are characterized by their dominant male-specific lethal phenotype (CLINE 1978; MAINE *et al.* 1985a). Molecular analysis of *Sxl* mutations (MAINE *et al.* 1985; SALZ, CLINE and SCHEDL 1987; this report) has allowed us to establish a correlation between changes in *Sxl* specific functions and DNA alterations.

We have isolated two X-ray-induced (Sxl^{fb} and Sxl^{fc}) and one EMS-induced (Sxl^{fd}) female-specific *Sxl* mutation. Here we report, first, the complementation analysis of these new mutations and other known Sxl^f mutations for the processes controlled by *Sxl*: sex determination, dosage compensation and oogenesis. Second, the DNA map is given of the new Sxl^{fb}, Sxl^{fc} and Sxl^{fd} mutations. Finally, we analyzed the late *Sxl* transcripts of females mutant for different Sxl^f alleles.

MATERIALS AND METHODS

Flies were cultured on standard food at 25°, unless otherwise stated. For a description of the mutations and chromosomes see LINDSLEY and GRELL (1968) and LINDSLEY and ZIMM (1986, 1985, 1987, 1990).

Induction, isolation and genetic mapping of *Sxl* mutants: Sxl^{fb} and Sxl^{fc} were induced by X-ray treatment (3000 rad) of males, while Sxl^{fd} was induced by treatment of males with EMS according to the procedure of LEWIS and BAKER (1968). We used *X* chromosome isogenic strains for mutagenesis. The isolation criteria was the female-specific lethal phenotype of the mutagenized *X* chromosome. To rid the lethal-bearing chromosomes of other deleterious mutations that might have been induced during mutagenesis, each original mutant line was allowed to recombine with chromosomes carrying different marker mutations to control the recombinational events and thus having an initial map position of the new mutations. This initial mapping located the new mutations in the chromosomal region containing the locus *Sxl* (location: 19.2). For a more precise location and to demonstrate if the new mutations represented alleles of *Sxl*, we mapped these mutations relative to *cm* (location: 18.9) and *ct* (location: 20.0), finding that these mutations map to the same location as *Sxl*, and fail to complement *Sxl* mutations. These mutations therefore are new alleles of *Sxl*.

Crosses for complementation analysis: Sxl^{fx}/*Balancer* females were crossed with Sxl^{fz}/Y males. The Sxl^{fz}/*Balancer* daughters were used as control for viability reference. The genotype of the flies was: *y cm* Sxl^{7B0}/*FM7 // cm* $Sxl^{f1}ct^6$/*FM6 // y w* Sxl^{fd}/*FM6 // cm* $Sxl^{fm7M1}ct^6$/*FM7 // cm* Sxl^{fm7M1} ct^6v/*FM7 // y w* Sxl^{M1fm3} ct^6 *v/In(1)*sc^{S1L8R} + *d1 − 49, y* sc^{S1} sc^8 *w* sn^{X2} *B // Sxl*^{M1fm3} ct^6 *v/FM7 //* Sxl^{f2593} *ct sn*^{3}/*FM6 // y* Sxl^{f2593} ct^6 sn^3/*FM6 //* Sxl^{fhv1} *//* Sxl^{fLS} *oc* v^{f36a}/*FM6 // y* Sxl^{fb}/*FM6 // y* Sxl^{fc}/*FM6*. Experimental females obtained in the viability complementation test were used for the fertility and sexual phenotype complementation tests.

Crosses for the interaction of the Sxl^f mutations with *sis-a* and *sc(T4)*: *Df(1)N71, sis-a*$^-$/*FM7//Df(1)svr, y AS-C*$^-$/*FM7 and Df(1)N71, y cho* Sxl^{M1} *sis-a*$^-$/*FM7* females were crossed with males carrying the Sxl^f mutant alleles from the stocks detailed above. Daughters carrying the Balancer chromosome were used as control for viability reference.

Crosses for the interaction of the Sxl^f mutations with the maternal *da* products: *y; d* da^2/*CyO* females were crossed, at 29°, with males carrying the Sxl^f mutations from the stocks detailed above.

Crosses for the clonal analysis of the Sxl^f mutations in the germline: *y w* Sxl^{fd} f^{36a}/*FM6 //* Sxl^{fLS} *oc v* f^{36a}/*FM6 //* Sxl^{f2593} ct^6 sn^3/*FM7 // y* Sxl^{fb} *v* f^{36a}/*FM6* and *y* Sxl^{fc} f^{36a}/*FM6* females were crossed with ovo^{D1} *v/Y* males. The Sxl^f/ovo^{D1} females were crossed to appropriate males to test mosaicism in the germline.

Clonal analysis of Sxl^f mutations in the soma: The irradiation dose was 1000 rad and was supplied by a Philips X-Ray machine at a rate of 300 rad/min. The adult flies were kept in a mixture of ethanol:glycerol (3:1) for several days, and subsequently mounted for analysis under a compound microscope. The sexual phenotype of the marked clones was assessed in sexually dimorphic regions of the fly: sex comb region of foreleg and external derivatives of the genital disc. For a description of the morphology of these structures see BRYANT (1978).

Clonal analysis of Sxl^f mutations in the germline: The progeny of the different crosses were irradiated at 24–48 hr after oviposition. The irradiation dose was 1000 rad applied by a Philips X-ray machine at a rate of 300 rad/min.

The clonal analysis in the germline is based on the use of ovo^{D1}. This is a dominant female-sterile mutation, which affects autonomously the development of the germline, while having no effect on the somatic tissues involved in oogenesis (PERRIMON 1984). Germ cells of the sterile ovo^{D1}/Sxl^f females that suffered X-ray-induced mitotic recombination between their two *X* chromosomes will render, in the next cell division, two daughter cells: one will be homozygous for ovo^{D1}, which will not develop, and the other will be homozygous for Sxl^f, whose capacity to develop is being analyzed. When the irradiated females eclosed, they were mated to appropriately marker stocks to test mosaicism in the germline.

Effect of Sxl^{fc} on the lethal phase of female larvae hemizygous for Shaker: We crossed *y* Sxl^{fc} *Df(Sh)/FM6,* y^2 *w B; Dp(*Sh^+*)/TM1, Me red* females with *y w* Sxl^{fc}/Y; *red/red* males. *Df(Sh)* stands for *Df(1)*JC^{153} and *Dp(*Sh^+*)* stands for *Dp(1;3)*JC^{153}. The female progeny will show the *white* or the *yellow* phenotype if they are, respectively, heterozygous or homozygous for Sxl^{fc}. In this last case, they will be red^+ if they carry the *Dp(*Sh^+*)* (two doses of Sh^+) and *red* if not (one dose of Sh^+). Thus, the existence of *yellow-red* larvae indicates that the *Df(Sh)/+* females do not die as embryos. The *white* and the *red* phenotype was monitored in the Malpighian tubules, and the *yellow* phenotype in the mouthparts.

Analysis of enzymatic activities: We analyzed the activities of the *X*-linked enzymes fumarase (FUM), 6-phosphogluconate dehydrogenase (6PGDH) and glucose-6-phosphate dehydrogenase (G6PDH) and the activities of the autosomal enzymes alcohol dehydrogenase (ADH) and NADP-isocitrate dehydrogenase (IDH-NADP). We followed the experimental procedure of LUCCHESI and RAWLS (1973) for G6PDH, 6-PGD and IDH-NADP; the experimental procedures of JACOBSON, MURPHY and HARTMAN (1970) for ADH, and the experimental procedure of WHITNEY and LUCCHESI (1972) for FUM. We used a spectrophotometer Kontron Uvicon 810. The larvae were homogenized in a solution described by BELOTE and LUCCHESI (1980). To produce the larvae we crossed *y w* Sxl^{fc}/*FM6* females with *y w* Sxl^{fc}/w^+Y males. The female larvae homozygous for Sxl^{fc} showed the *yellow-white* phenotype, while the Sxl^{fc} heterozygous females showed a wild-type phenotype. The Sxl^{fc} males showed the *yellow* phenotype, while the Sxl^+ males (*FM6*) showed a wild-type phenotype. The *yellow* phenotype was monitored in the mouthparts and the *white*

phenotype in the Malpighian tubules. Females and males were separated by the different size of their gonads.

Analysis of *sgs-4* and *sgs-3* transcripts: Total RNA from each type of larvae was extracted following the experimental procedure of CASE and DANEHOLT (1978). Levels of *sgs-4* and *sgs-3* transcripts was quantitated by the dot-blot technique using as probes pRH0.75 and adm124E8, respectively (MEYEROWITZ and HOGNESS 1982; MCGINNIS, SHERMOEN and BEKENDORF 1983), kindly supplied by S. K. BEKENDORF. RNA blotting and hybridization were performed as described by MANIATIS, FRITSCH and SAMBROOK (1982). To quantify the degree of hybridization we measured the spots of the film with a Molecular Dynamics Computing Densitometer, model 300A.

DNA analysis of *Sxl* mutants: Total nucleic acid from adult males was extracted as described (MEYEROWITZ and HOGNESS 1982) and treated with RNase (2 mg/ml) for 2 hr at 37° (BREEN and LUCCHESI 1986). Restriction digests of genomic DNA were fractionated on 0.7–0.8% agarose gels and subsequently transferred to nitrocellulose filters. The filters were hybridized with ^{32}P-labeled nick-translated DNA probes λS1, λS2A and S1-p15. λS1 and λS2A were kindly supplied by T. W. CLINE. S1-p15 refers to the 4.8-kb fragment (*Xho*I-*Xho*I, coordinates −10.8 to −6) from λS1 that we subcloned in the pBR322 plasmid vector. Hybridization was carried out as described in MANIATIS, FRITSCH and SAMBROOK (1982).

Northern analysis of *Sxl* transcripts: RNA preparation from frozen larvae or adult flies, electrophoretical fractionation of total RNA and blotting to nylon membranes were performed as described elsewhere (MANIATIS, FRITSCH and SAMBROOK 1982; CAMPUZANO *et al.* 1986). Hybridization with [^{32}P]RNA probes was carried out as previously described (GRANADINO, CAMPUZANO and SANCHEZ 1990). Genomic fragments from λS1 (MAINE *et al.* 1985) were subcloned in pGem-1 and pGem-2, and used as templates for preparing the male (g2) and the non-sex specific (h^1) RNA probes, respectively (see Figure 4).

RESULTS

Effect of *Sxl* mutant alleles on female viability and their complementation pattern: The results of the complementation test are shown in Table 1. None of the *Sxl* mutant alleles complements Sxl^{7B0}, which is a deficiency for *Sxl* (SALZ *et al.* 1987). All of the *Sxl* mutations in homozygosis produce variable degrees of lethality in female flies, with the only exception of Sxl^{fhv1}. This is the weakest mutant allele which, by itself, has no effect on female viability (CLINE 1980). Sxl^{f1} and Sxl^{fd} appear as the most extreme mutant alleles, they are lethal with any of the other *Sxl* mutations, except Sxl^{fhv1}. The different viability degree of Sxl^{fhv1} with Sxl^{f1} (2%) and with Sxl^{fd} (35%) qualifies Sxl^{f1} as the strongest mutant allele. Sxl^{fb} and Sxl^{fc} do not complement each other. They do not complement neither Sxl^{f9}. However, Sxl^{fb} and Sxl^{fc} fully complement Sxl^{fLS}, as Sxl^{f9} does. This defines two complementation groups within the *Sxl* gene for female vital functions: one group is defined by the Sxl^{fLS} mutation (*LS* complementation group) and the other is defined by the Sxl^{fb}, Sxl^{fc} and Sxl^{f9} mutations (*bc* complementation group). The rest of the *Sxl* mutations affect both complementation groups albeit with different degrees. Sxl^{fm7M1} and Sxl^{M1fm3} affect the *LS* group more than the *bc* one. On the contrary, Sxl^{fhv1} and Sxl^{f2593} affect the *bc* group more than the *LS* one.

Analysis of dosage compensation in females homozygous for Sxl^{fb} or Sxl^{fc}: The female-specific phenotype of Sxl^{fb} and Sxl^{fc} suggests that females homozygous for these mutations die because of a disarrangement in their dosage compensation process, as is the case with other female-specific lethals at the gene *Sxl* (LUCCHESI and SKRIPSKY 1981). As a first approach, we analyzed the effect of Sxl^{fb} and Sxl^{fc} on dosage compensation in larvae: 70% of the Sxl^{fb} or Sxl^{fc} homozygous individuals die as embryos, and 28% as larvae. Therefore, the test only assays the individuals that survived the early lethal period. We followed three different experimental approaches.

The first experimental approach was one followed by BREEN and LUCCHESI (1986). In Sxl^{fb} or Sxl^{fc} homozygous female larvae, we measured the amount of the *X*-linked transcript coded by the gene *sgs-4*, which is dosage compensated (MCGINNIS, SHERMOEN and BEKENDORF 1983), relative to the amount of RNA encoded by the autosomal gene *sgs-3* (MEYEROWITZ and HOGNESS 1982). These two RNAs are coordinately expressed in salivary glands from the middle of the third larval instar to the prepupal stage. If Sxl^{fb} and Sxl^{fc} alter the dosage compensation process, the level of the *sgs-4* transcript should increase, compared to the *sgs-3* transcript, in the females homozygous for these *Sxl* mutations. The measurement of the amounts of *sgs-4* and *sgs-3* transcripts was carried out by means of the dot-blot technique (for details (see MATERIALS AND METHODS). The results are shown in Figure 1. Females homozygous for Sxl^{fc} have a higher value for the *sgs-4*/*sgs-3* ratio than heterozygous females; however, this difference is not significant ($P > 0.05$). With respect to the Sxl^{fb} mutation, the *sgs-4*/*sgs-3* value of the homozygous females, which is similar to the one found for the males, is significantly ($P < 0.05$) lower than that of the heterozygous females, contrary to expectation if Sxl^{fb} was affecting dosage compensation. This suggests that the Sxl^{fb} and Sxl^{fc} mutations do not increase the transcription of the *X* chromosome in homozygous female larvae.

The second experimental approach consisted in measuring the specific activity of enzymes coded by dosage-compensated *X*-linked genes relative to enzymes coded by autosomal genes. The *X*-linked enzymes tested were FUM, 6PGDH, and G6PDH, and the autosomal enzymes were ADH and IDH-NADP. The analysis was carried out for the Sxl^{fc} mutation. The enzymatic activities were measured in crude extracts of larvae. If Sxl^{fc} was affecting dosage compensation, an increase in the enzymatic activities of the *X*-linked enzymes, compared to the autosomal ones,

TABLE 1

Complementation analysis for the viability of females double heterozygous for different *Sxl* mutant alleles

Sxl allele	*Sxl* allele									
	7B0	*f1*	*fd*	*fm7M1*	*M1fm3*	*f2593*	*fhv1*	*fLS*	*fb*	*fc*
7B0	0[a]									
	(321[b])									
f1	0	0								
	(241)	(561)								
fd	0	0	0							
	(402)	(1647)	(713)							
fm7M1	0	0	0	0.5						
	(256)	(618)	(1118)	(626)						
M1fm3	0	0	0	6	13					
	(352)	(1385)	(1305)	(488)	(137)					
f2593	0	0	0	1	42	24				
	(199)	(393)	(1093)	(529)	(263)	(393)				
fhv1	0	2	35	36	124	75	116			
	(161)	(369)	(341)	(704)	(495)	(304)	(486)			
fLS	0	0	0	0	0	73	87	0		
	(228)	(383)	(1930)	(236)	(310)	(264)	(228)	(858)		
fb	0	0	0	12	30	9	45	101	6	
	(210)	(488)	(1108)	(237)	(312)	(266)	(142)	(438)	(366)	
fc	0	0	0	11	75	21	68	125	1	5
	(147)	(252)	(1850)	(237)	(291)	(447)	(226)	(327)	(424)	(254)
f9		0						98	2	3
		(298)						(249)	(290)	(205)

[a] Percentage of experimental females with respect to control sister females.
[b] Number of control flies.

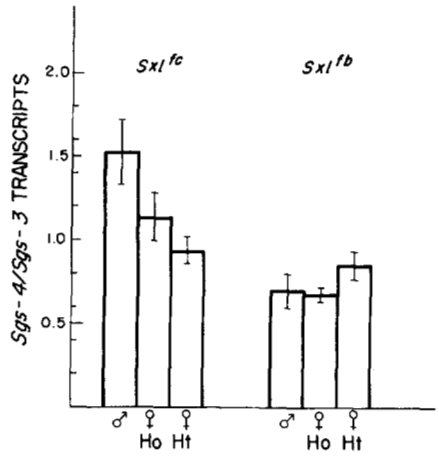


FIGURE 1.—Analysis of the level of *sgs-4* and *sgs-3* transcripts in male and female larvae homozygous (Ho) or heterozygous (Ht) for Sxl^{fb} or Sxl^{fc}. The bars represent the 95% confidence intervals. The experiment was performed four times. A statistical analysis of the results showed nonsignificant differences for the *sgs-4/sgs-3* ratio between the four replicas of the experiment. We then pooled all the data to get a better estimate for the *sgs-4/sgs-3* ratio value.

should be expected, in consequence of a hypertranscription of the two *X* chromosomes. The results are shown in Figure 2. No significant ($P > 0.05$) differences were observed between the four genotypes (homozygous and heterozygous females and males) for FUM and G6PDH. On the contrary, significant ($P < 0.05$) differences were observed between these four genotypes for 6PGDH; however, the reduced level of that enzyme in homozygous compared to heterozygous females is contrary to expectation if Sxl^{fc} was affecting dosage compensation. All the results are the same regardless of which autosomal enzyme (ADH or IDH) was used for reference. Thus, also with this experimental test we have not detected alterations in the dosage compensation process of female larvae homozygous for Sxl^{fc}.

The two sets of experiments that we performed are based on the capacity to quantitate certain products (transcripts or enzymes) by means of *in vitro* tests (hybridization with a probe or enzymatic reactions). The fact that we did not detect significant changes in the level of *X* chromosome transcription, caused by the *bc* mutations, cannot be attributed to a limited sensitivity of the experimental test that we used, since they have been successfully applied to determining variations in the level of *X* chromosome transcription due to mutations affecting dosage compensation (LUCCHESI and MANNING 1987). We still performed a third experimental test. This is an *in vivo* test based on the capacity of the organism to survive depending on the level of *X* chromosome transcription. This test takes advantage of the haploinsufficient character of the *X*-linked gene *Shaker* (*Sh*): both *Df(Sh)/Y* males and *Df(Sh)/+* females die as embryos (FERRUS *et al.* 1990). J. L. DE LA POMPA and A. FERRUS (personal communication) found that the lethal phase of *Df(Sh)/+* females is retarded to larval stage if a hypertranscription

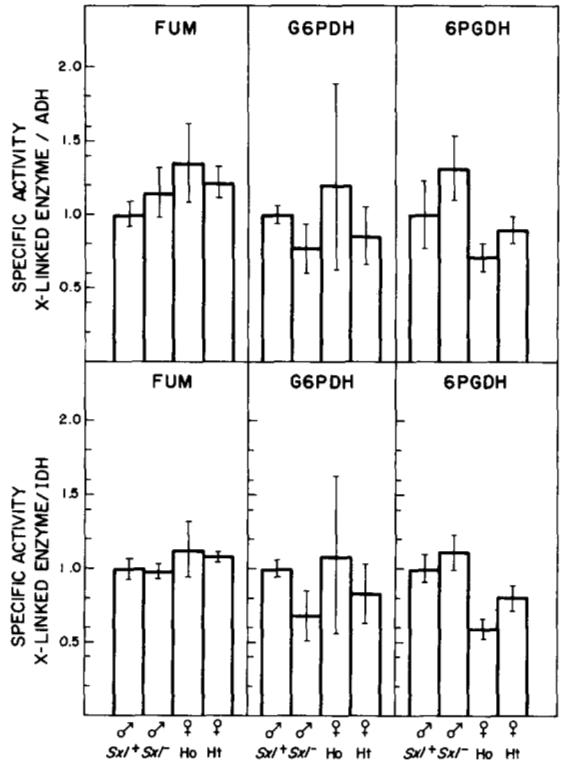


FIGURE 2.—Analysis of specific activities of *X*-linked compensated and autosomal enzymes in male and female larvae homozygous (Ho) or heterozygous (Ht) for Sxl^{fc}. The bars represent the 95% confidence intervals. The experiment was performed three times. A statistical analysis of the results showed nonsignificant differences for the specific activity of *X*-linked enzyme/autosomal enzyme ratio value between the three replicas of the experiment. We then pooled all the data to get a better estimate for these ratio values.

of the *X* chromosomes was assured. They found that Sxl^{f1} *Df(Sh)*/Sxl^{fhv1} Sh^+ females die as larvae, because the Sxl^{f1}/Sxl^{fhv1} constitution causes a hypertranscription of the *X* chromosomes (LUCCHESI and SKRIPSKY 1981), so that more Sh^+ product is produced from the single Sh^+ allele. Our reasoning was that if the Sxl^{fc} mutation is affecting dosage compensation, an increase of the *X* chromosome transcription will occur, and then the lethal phase of *Df(Sh)*/+ females homozygous for Sxl^{fc} is expected to be retarded to the larval stage. We set up crosses to produce females of genotype Sxl^{fc}*Df(Sh)*/Sxl^{fc} Sh^+, which can be identified as the larval stages (see MATERIALS AND METHODS). We did not find any of these female larvae among a total of 685 larvae that we analyzed. Thus, homozygosity for Sxl^{fc} does not change the lethal phase of *Df(Sh)*/+ females, which still die as embryos.

Within the limits of resolution of the techniques that we have applied, we conclude that the Sxl^{fb} and Sxl^{fc} mutations seem to have no obvious effect on late dosage compensation.

Effect of *Sxl* mutant alleles on female sexual development and their complementation pattern: Sxl^{fb} and Sxl^{fc} fully complement Sxl^{fLS}. All the viable flies

TABLE 2

Clonal analysis of *Sxl* mutations following irradiation at 24–48 hr of development

	yellow-forked clones in forelegs			*yellow-forked* clones in external terminalia		
Sxl allele tested	No. of forelegs	Female phenotype	Male phenotype	No. of terminalia	Female phenotype	Male phenotype
Sxl^{fd}	298	0	6	100	0	9
Sxl^{fb}	781	19	0	413	27	0
Sxl^{fc}	1112	21	0	456	21	0

The genotypes of the irradiated females were: *y* Sxl^{fd} $f^{36a}/M(1)O^{sp}$, *y* $Sxl^{fb}f^{36a}/M(1)O^{sp}$ and *y* Sxl^{fc} $f^{36a}/M(1)O^{sp}$.

double heterozygous for Sxl^{fb} or Sxl^{fc} and the other *Sxl* mutations develop as wild-type females, with the exception of Sxl^{fm7M1}/Sxl^{fb} and Sxl^{fm7M1}/Sxl^{fc} flies, which occasionally present a male spot in the fifth or sixth tergites. Moreover, Sxl^{fm7M1} or Sxl^{M1fm3} homozygous flies survive as males and Sxl^{f2593} flies as intersexes, while $Sxl^{fh}/^{v1}$ produce female flies (MARSHALL and WHITTLE 1978; CLINE 1980; 1984; this report). None of the alleles that produce sexual transformation fully complements each other. Thus, they define a single complementation group for *Sxl* functions involved in female sexual development.

Females homozygous for Sxl^{f1}, Sxl^{fLS} or Sxl^{fd} are not viable. The clonal analysis of Sxl^{f1} (CLINE 1979) and Sxl^{fLS} (SANCHEZ and NÖTHIGER 1982) has revealed that these two mutations affect the female sexual development. We have carried out the clonal analysis of Sxl^{fd}. All clones homozygous for this mutation developed male structures instead of female ones, thus demonstrating that Sxl^{fd} does also affect the female sexual development (Table 2).

The Sxl^{fb} and Sxl^{fc} homozygous flies that survive are normal females. It is possible, however, that we are missing the effect of Sxl^{fb} and Sxl^{fc} on sexual development due to its association with a lethal phenotype, or to complementation in the case of viable combinations with other alleles. To assess the role of Sxl^{fb} and Sxl^{fc} in sexual determination, we have carried out their clonal analysis. None of the clones homozygous for these mutations displayed sexual transformation (Table 2), contrary to the observation with other female-specific *Sxl* mutations analyzed so far. A similar result has been reported for Sxl^{f9} (T. CLINE, unpublished results, cited in MAINE *et al.* 1985b). These results could indicate that either Sxl^{fb} and Sxl^{fc} do not affect the *Sxl* feminizing functions, or that they affect *Sxl* functions only needed at the beginning of development, when the state of activity of *Sxl* is defined. For this reason, we carried out the clonal analysis of Sxl^{fb} and Sxl^{fc} by irradiation at the blastoderm stage. All of the Sxl^{fb} clones (8 in forelegs and 14 in the external terminalia) and all of the Sxl^{fc} clones (9

in forelegs and 14 in the external terminalia) developed female structures.

It can be argued that we are losing some of the Sxl^{fb} or Sxl^{fc} clones and only the "escaper" ones survive, those that for unknown reasons express higher levels of Sxl^{+} activity and consequently develop female structures. We believe that this is not the case, since even clones homozygous for the very strong amorph mutation Sxl^{f1} (CLINE 1979) or for a deficiency of *Sxl* (Sxl^{7BO}, induced at blastoderm, our unpublished results) can survive, differentiating male structures. Moreover, the frequency of clones homozygous for Sxl^{fb} or Sxl^{fc} does not differ significantly ($P > 0.05$) from those homozygous for Sxl^{fd}, a stronger mutation (this report). We also irradiated at 24–48 hr of development female larvae of genotype $yf^{36a}/M(1)O^{sp}$, finding that the frequency of *yf* clones in the forelegs of these females is similar to that found in the clonal analysis of Sxl^{fb}, Sxl^{fc} and Sxl^{fd} (data not shown). We conclude that the *bc* mutations do not affect the *Sxl*-feminizing functions.

The Sxl^{f2593} mutation appears to reduce all the somatic functions in a temperature-dependent fashion (MARSHALL and WHITTLE 1978; CLINE 1984). Interestingly, the temperature-sensitive period of the Sxl^{f9}/Sxl^{f2593} combination occurs early in development (CLINE 1985). Sxl^{f9} seems to affect the early *Sxl* functions (MAINE *et al.* 1985b; CLINE 1985). Sxl^{f2593} may also specifically affect these *Sxl* functions, and its effect on late *Sxl* functions may be a consequence of the alteration of early functions needed for *Sxl* stable activation (SALZ *et al.* 1989). We carried out a clonal analysis of Sxl^{f2593} to test if this mutation affects the late *Sxl* functions. We irradiated *y* Sxl^{f2593} sn^{3}/f^{36a} females at 24–48 hr of development. We focussed the analysis on the forelegs considering only the *yellow-singed//forked* twin clones. The *yellow-singed* partner is homozygous for Sxl^{f2593}. We found a total of 22 *y* sn^{3}/f^{36a} twin clones. The *y* sn^{3} partner always developed sex combs. These were formed by pure male teeth, or a mixture of pure male and true intersexual teeth. These results demonstrate that Sxl^{f2593} directly affects the late *Sxl* functions required continuously during development of the female cells.

In summary, mutations at the *bc* complementation group do not affect the *Sxl*-feminizing functions, contrary to mutations at the *LS* complementation group.

Effect of the *Sxl* mutant alleles on female fertility and their complementation pattern: The Sxl^{f1} mutation autonomously affects the development of the germline, while Sxl^{fm7M1} and Sxl^{M1fm3} do not (CLINE 1983, 1984; SCHÜPBACH 1985; STEINMANN-ZWICKY, SCHMID and NÖTHIGER 1989; NÖTHIGER *et al.* 1989). We carried out the clonal analysis of Sxl^{fLS}, Sxl^{fd}, and Sxl^{f2593} in the germline. The results are shown in Table 3. Germ cells homozygous for Sxl^{fd} or Sxl^{fLS} do not develop into oocytes, while germ cells homozygous for Sxl^{f2593} give rise to functional eggs. Table 3 also presents the data on the clonal analysis of Sxl^{fb} and Sxl^{fc} in the germline. It could be argued that the Sxl^{fb} or Sxl^{fc} homozygous females that survived were escapers that expressed high levels of Sxl^{+} function and consequently were fertile. Germ cells homozygous for Sxl^{fb} or Sxl^{fc}, however, are able to produce functional eggs.

TABLE 3

Clonal analysis of *Sxl* mutant alleles in the germline

Genotype of homozygous clones	No. of females tested	No. of females with homozygous clones
Sxl^{fd}	1126	0
Sxl^{fLS}	1309	0
Sxl^{f2593}	849	22
Sxl^{fb}	725	6
Sxl^{fc}	530	5

The genotypes of the irradiated females were: *y* $Sxl^{fd}f^{36a}/ovo^{D1}v$, *y* $Sxl^{fLS}oc$ *v* f^{36a}/ovo^{D1} *v*, Sxl^{f2593} $ct^{6}sn/ovo^{D1}v$, *y* $Sxl^{fb}f^{36a}/ovo^{D1}$ and *y* $Sxl^{fc}f^{36a}/ovo^{D1}$. The different frequency of females with homozygous clones for Sxl^{fb} or Sxl^{fc} relative to Sxl^{f2593} is presumably due to the map position of the markers used for the clonal analysis (see MATERIALS AND METHODS), and to the different irradiation conditions used for the clonal analysis of Sxl^{fb} and Sxl^{fc}, as a consequence of using a different X-ray machine.

The fertility test was carried out with those females which did not present a sexual transformation big enough to disregard the possibility of its fertility. All the viable combinations of Sxl^{fb} or Sxl^{fc} with the other *Sxl* alleles are fertile, except with Sxl^{fm7M1}. Sxl^{fhv1} has a similar behavior but in combination with Sxl^{fm7M1} produces some females which are fertile and others which are sterile.

Table 4 shows the analysis of the gonads and the internal derivatives from the genital disc of sterile females, as well as transformed ones, carrying different *Sxl* mutations. The first three allelic combinations have testis, though atrophied, showing undifferentiated cells unable to produce sperm. These aberrations are not due to the absence of the *Y* chromosome, because *XO* males show normal testis that produce nonmotile elongated sperm. There are cases in which the yellow sheath typical for testis does not cover the testis completely, but just the apical region of them. The internal genital structures are male-like, which are indistinguishable from the normal male ones. Some Sxl^{fLS}/Sxl^{f2593} females lack gonads, and others show female-like genital structures.

The Sxl^{fb}/Sxl^{fm7M1} and the Sxl^{fc}/Sxl^{fm7M1} females lack gonads. The Sxl^{fhv1}/Sxl^{fm7M1} females may present two, one or no ovaries in a 18:3:13 rate. The fertile females always contain two ovaries, while the sterile females contain one or two ovaries which look normal, but oviposition never takes place, even though these fe-

TABLE 4

Analysis of the gonads and the internal derivatives from the genital disc of females carrying different *Sxl* mutations

Genotype	No. of flies	Gonads			Internal derivatives from the genital disc		
		Female-like	Male-like	Absent	Female	Male	Female and male[a]
Sxl^{f2593} Sxl^{M1fm3}	10	0	20	0	0	10	0
Sxl^{f2593} Sxl^{f2593}	6	0	12	0	0	6	0
Sxl^{fLS} Sxl^{f2593}	6	0	6	6	2	2	2
Sxl^{fhv1} Sxl^{fm7M1}	74	90[b]	0	58	74	0	0
Sxl^{fb} Sxl^{fm7M1}	12	2[c]	0	22	12	0	0
Sxl^{fc} Sxl^{fm7M1}	13	0	0	26	13	0	0

[a] Both types of tissues coexist in the same fly.
[b] One among the 90 flies contained some male gonadal tissue.
[c] Both females contained some male gonadal tissue.

males are fertilized. These three allelic combinations contain female-like genital structures.

In summary, Sxl^{fb}, Sxl^{fc} and Sxl^{f2593} do not severely affect the *Sxl* functions required in the germline to follow the oogenic pathway, while Sxl^{fLS} and Sxl^{fd} do so.

Female-specific lethal synergistic interaction between *Sxl* mutant alleles and mutations at *sisterless-a*, *scute* or *daughterless* genes: As mentioned above, the state of activity of *Sxl* is defined by the X:A signal and the maternal *da* product. As a characterization test for the *Sxl* mutant alleles we have analyzed the response of these alleles to alterations in either the X:A signal or in the *da* gene.

Figure 3 shows the results of the interaction between the *Sxl* mutant alleles and a deficiency for *sisterless-a* (*sis-a*), the *Df(1)N71*, or a deficiency for *scute* (*sc*), the *Df(1)svr*. As mentioned in the Introduction, both *sis-a* and *sc* genes behave as numerator elements of the X:A signal. In general, both deficiencies present a similar specificity of interaction with the different *Sxl* alleles. Among these we can basically distinguish two groups, according to the strength of their female-specific lethal interaction. The strong group is formed by the *bc* complementation group, together with the Sxl^{f2593} allele and the strongest alleles Sxl^{f1} and Sxl^{fd}. The weak group is formed by the *LS* complementation group and the Sxl^{fm7M1}, Sxl^{M1fm3} and Sxl^{fhv1} alleles. In all cases the replacement of the Sxl^{+} copy by the constitutive Sxl^{M1} allele restores full viability of experimental females (data not shown).

Table 5 shows the results of the interaction between the *Sxl* mutant alleles and a reduced level of maternal *da* product. Females heterozygous for any of the tested *Sxl* alleles, except Sxl^{fLS}, exhibit a reduced viability. These alleles are the same that interact with an altered X:A ratio signal.

Molecular mapping of *Sxl* mutations: Southern blot analysis of genomic DNA from Sxl^{fb} and Sxl^{fc} males digested with different restriction enzymes and probed with λS2A (see Figure 4) displayed no detectable differences in restriction fragment pattern, when compared with wild-type strains *Oregon-R* (*OR-R*) and *Canton-S* (*CS*), and with $yellow^{iso}$ (y^{iso}) (an isogenic strain for the *X* chromosome that was used to induce the Sxl^{fb} and Sxl^{fc} mutations) (data not shown). In contrast, genomic DNA samples from the mutant strains have gross alterations in their restriction fragment pattern when probed with λS1.

Figure 5A shows that the restriction pattern of *OR-R* (lane a) and of y^{iso} (lane e) DNA digested with *Pst*I and hybridized with λS1 is in agreement with the restriction map of MAINE *et al.* (1985a), except an extra band of more than 14 kb (marked with a point) that does not fit with that map and that is also present in flies deficient for *Sxl* (our unpublished data). In *CS* (lane b) the 9.5-kb band is replaced by one of more than 19 kb, due to the presence of the B104 transposable element in *CS* (at position −15) (MAINE *et al.* 1985a and Figure 5A) with at least a *Pst*I restriction size. In the Sxl^{fb} (lane d) and Sxl^{fc} (lane c) mutants, the 9.5-kb *Pst*I fragment is missing, whereas two new fragments appear (marked with an arrowhead) one of 2.0 kb and one of about 13 kb that partially overlaps with the 14-kb band. This modification is consistent with a DNA insertion of at least 5 kb, with a *Pst*I restriction site, located to the right of the *Pst*I site at coordinate −9. The digestion with *Xho*I and hybridization with λS1 (Figure 5B) allows location of the insertion within the 1.8-kb *Xho*I-*Pst*I fragment at coordinates −10.8 and −9, respectively, since the 4.8 *Xho*I-*Xho*I fragment (−10.8, −6) is missing in the mutants. To define better the molecular lesion of Sxl^{fb} and Sxl^{fc} we have used this 4.8-kb *Xho*I-*Xho*I fragment (plasmid S1-p15, see MATERIALS AND METHODS) as a probe. *OR-R* (lane a) and y^{iso} (lane e) DNA digested with *Pst*I and hybridized with S1-p15 (Figure 5C) display the expected fragments of 5.9 and 9.5 kb, while *CS* (lane b) displays the 5.9- and 10-kb fragments. In the case of Sxl^{fb} (lane d) and Sxl^{fc} (lane c) we found the expected 5.9-kb fragment, and a new fragment of 2.0 kb, that appeared when λS1 was used as probe, while the other new fragment of 13 kb is not detectable. This is explained if the 5-kb foreign DNA fragment is inserted very close to the *Xho*I restriction site at coordinate −10.8 and the *Pst*I restriction site of the insertion map next to its left end.

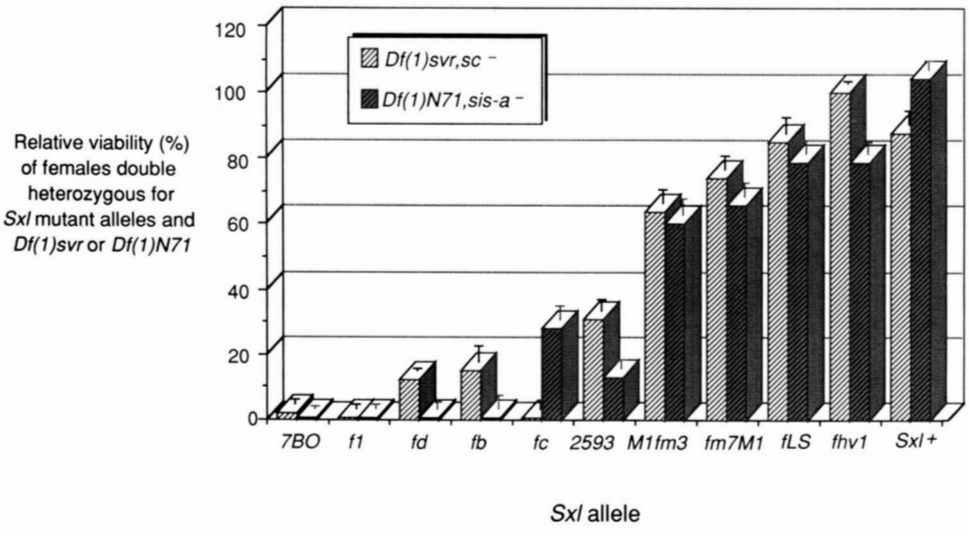


FIGURE 3.—Lethal synergistic interaction between *Sxl* mutant alleles and mutations affecting the X:A ratio signal. For crosses see MATERIALS AND METHODS.

TABLE 5

Analysis of the female-specific lethal synergistic interaction between the maternal product of *daughterless* and the *Sxl* mutant alleles

Sxl mutant allele tested	No. of male flies used for viability reference	Relative viability (%) of Sxl^-/Sxl^+ females
Sxl^{f1}	195	55
Sxl^{fd}	317	73
$Sxl^{f2\text{-}}{}_{593}$	261	55
Sxl^{fb}	521	36
Sxl^{fc}	469	65
Sxl^{fLS}	394	111

The experiment was performed at 29° because of the temperature-dependence for the lethal effects involving the interaction between the maternal *da* product and *Sxl* mutations (CLINE 1980).

The digestion with *Xho*I and hybridization with S1-p15 (Figure 5D) show that in Sxl^{fb} (lane d) and Sxl^{fc} (lane c) the 4.8-kb fragment is replaced by one of 9.5 kb, which overlaps with the 9.2-kb fragment and was hardly detectable in Figure 5B. These results and those obtained by a double digestion with *Pst*I and *Xho*I followed by hybridization with the S1-p15 subclone (Figure 5E) are consistent with the insertion of a foreign DNA fragment of around 5 kb inserted near the *Xho*I restriction site at position −10.8 in the Sxl^{fb} and Sxl^{fc} mutants (see Figure 4). Although Sxl^{fb} and Sxl^{fc} arose in different mutagenesis, both appear to contain a similar insertion.

With respect to Sxl^{fd}, no detectable alterations in the restriction fragment pattern were observed, either with λS1 or with λS2A. Thus, Sxl^{fd} seems to be a "point" mutation.

Analysis of late *Sxl* transcripts in mutants for this gene: We have analyzed by Northern blots the late *Sxl* transcripts from female-specific lethal mutations at the gene *Sxl*. Two *Sxl* probes have been used: the h^1 probe, that contains some of the exons common to both sexes, and the g^2 probe, that contains the male-specific exon (BELL *et al.* 1988) (see Figure 4).

Figure 6A shows that, when the h^1 probe was used, the Sxl^{fc} female larvae (lane 3) present only the three characteristic female *Sxl* transcripts of 4.2, 3.3 and 1.9 kb, while the Sxl^{fc} male larvae (lane 4) present the three *Sxl* transcripts of 4.4, 3.6 and 2.0 kb typical of males. In lanes 1 and 2 are shown the transcripts from female and male *OR-R* larvae, respectively. We must stress that the Sxl^{fc} female larvae present exclusively the female *Sxl* transcripts, as confirmed when the g^2-probe was used (Figure 6B). Male *Sxl* transcripts are only present in the OR-R (lane 4) and the Sxl^{fc} (lane 2) male larvae. With respect to the Sxl^{fLS} female and male larvae, they express the *Sxl* transcripts characteristic of males (Figure 7A, hybridization with the h^1 probe, and Figure 7B hybridization with the g^2 probe). On the other hand, the Sxl^{fc}/Sxl^{fLS} female larvae express the typical female *Sxl* transcripts and none of the male transcripts (Figure 8A, hybridization with the h^1 probe, and Figure 8B hybridization with the g^2 probe). These results agree with the complementation behavior shown by these mutations.

The Sxl^{fd} homozygous females die very soon in development. For this reason, to determine the type of *Sxl* transcripts generated by that mutant allele we have analyzed the transcripts expressed by the Sxl^{fhv1}/Sxl^{fd} female larvae, using as control female larvae homozygous for Sxl^{fhv1}. This mutation is viable in females (CLINE 1980; this report), while in combination with Sxl^{fd} only 30% of the females survive and the rest die as larvae (Table 1). The Sxl^{fhv1} female and male larvae present the female and male *Sxl* transcripts, respectively (Figure 9A hybridization with the h^1 probe, and Figure 9B hybridization with the g^2 probe). However, the Sxl^{fhv1}/Sxl^{fd} female larvae express both female and male *Sxl* transcripts (Figure 9A hybridization with the h^1 probe, and Figure 9B hy-

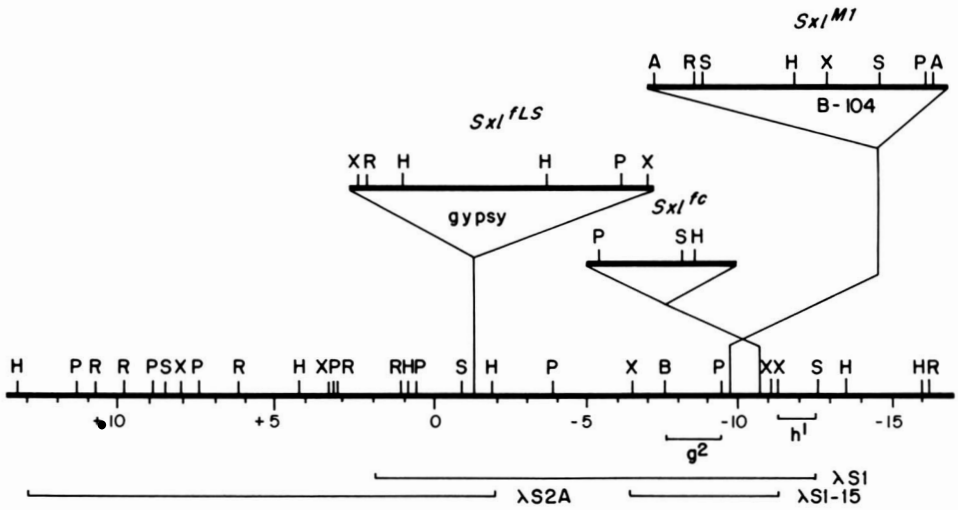
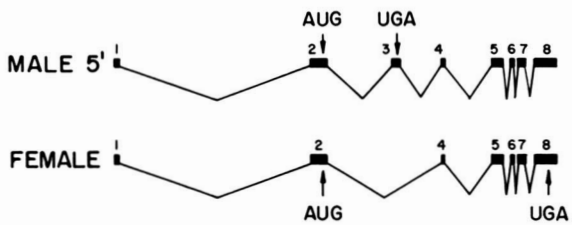


FIGURE 4.—DNA rearrangements associated with Sxl^{fb} and Sxl^{fc} mutations. We followed the restriction map of MAINE *et al.* (1985). Restriction sites are designated as follows: R, *Eco*RI; X, *Xho*I; H, *Hin*dIII; S, *Sal*I; P, *Pst*I. λS1, λS2A and S1-p15 were the probes used for Southern blots. Schematic representation of the male and female *Sxl* cDNAs and the two probes, g^2 and h^1, used for Northern blots (BELL *et al.* 1988).



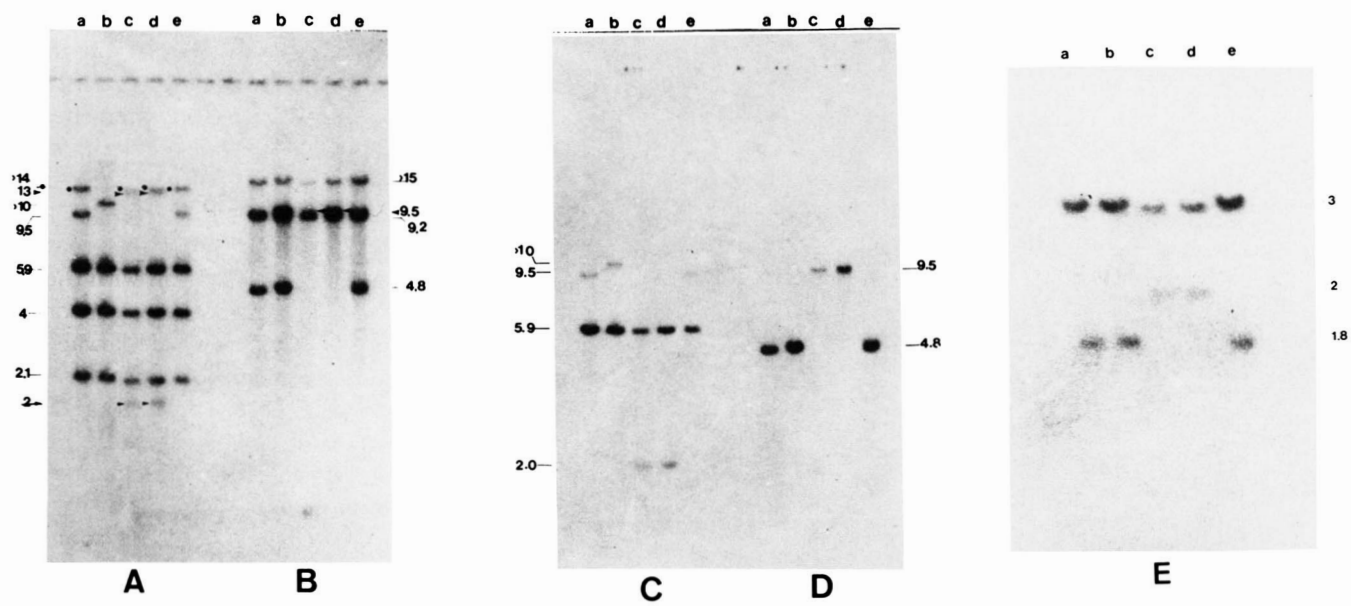


FIGURE 5.—Genomic Southern blots from Sxl^{fb} and Sxl^{fc} mutant flies. Lane a, *OR-R*; lane b, *CS*; lane c, Sxl^{fc}; lane d, Sxl^{fb} and lane e, y^{uo}. A, restriction with *Pst*I and hybridization with λS1. B, restriction with *Xho*I and hybridization with λS1. C, restriction with *Pst*I and hybridization with S1-p15. D, restriction with *Xho*I and hybridization with S1-p15. E, restriction with *Pst*I and *Xho*I and hybridization with S1-p15.

bridization with the g^2 probe). We suggest that the male *Sxl* transcripts come from the Sxl^{fd} mutant allele.

DISCUSSION

The complementation analysis between different *Sxl* mutant alleles shows that the new alleles Sxl^{fb} and Sxl^{fc} belong to a different complementation group from most other previously described *Sxl* alleles: they define together with Sxl^{f9} the *bc* group of alleles, whereas the Sxl^{fLS} allele defines the *LS* group. The rest of the *Sxl* mutant alleles tested in this report, including the new allele Sxl^{fd}, affect both complementation groups albeit with different degrees. Sxl^{fm7M1} and Sxl^{M1fm3} affect more the *LS* group than the *bc* group. On the contrary, Sxl^{fhv1} and Sxl^{f2593} affect more the *bc* group than the *LS* group. Other *Sxl* alleles have been identified that specifically affect the develop-

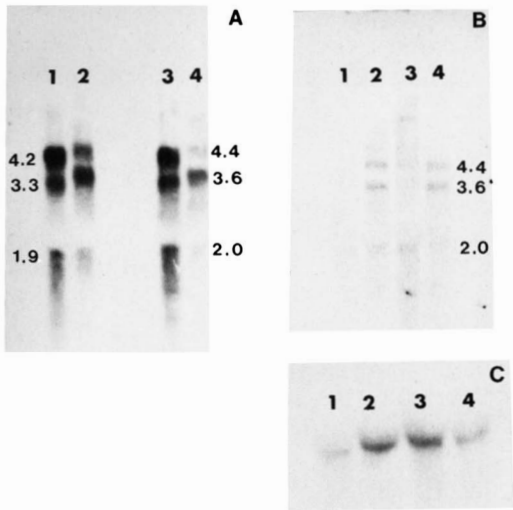


FIGURE 6.—Late *Sxl* transcripts in female larvae mutant for Sxl^{fc}. To generate the larvae we crossed *y cm* Sxl^{fc} homozygous females with males of the same genotype. The ratio of female to male larvae is 0.76 and that of female to male adults is 0.30. Lane 1, wild-type females; lane 2, wild-type males; lane 3, Sxl^{fc} homozygous females; and lane 4, Sxl^{fc} males. *A*, filter probed with h^1; *B*, filter probed with g^2. C, hybridization of filter 6B with the actin probe DM-A3. The distortion in the migration of the lower molecular weight RNAs is due to the presence of rRNA in the RNA preparation.

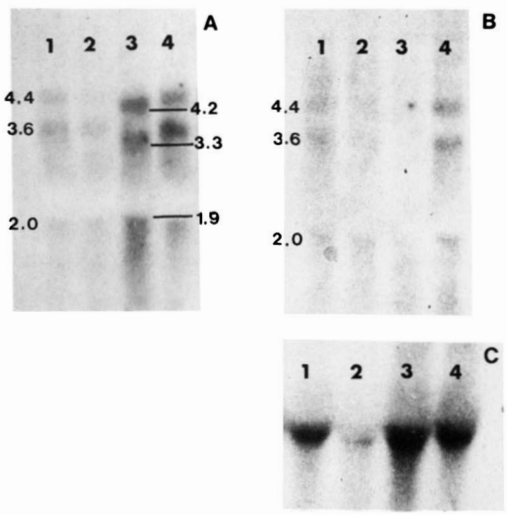


FIGURE 7.—Late *Sxl* transcripts in female larvae mutant for Sxl^{fLS}. To generate the larvae we crossed *y w* Sxl^{fLS} *oc v* f^{36a}/*FM6* females with *y w* Sxl^{fLS} *oc v* f^{36a} males. The mutant larvae were recognized by the *yellow* phenotype of the mouthparts and the *white* phenotype of the Malpighian tubules. Female and male larvae were separated by the different size of their gonads. Lane 1, Sxl^{fLS} homozygous females; lane 2, Sxl^{fLS} males; lane 3, wild-type females, and lane 4, wild-type males. A, filter probed with h^1; B, filter probed with g^2; C, hybridization of filter 7B with the actin probe DM-A3.

ment of the female germline (PERRIMON *et al.* 1986; SALZ *et al.* 1987).

The Sxl^{fLS} mutation results from the insertion of the transposable element gypsy between positions 0 and −2 on the molecular map, within the first intron (MAINE *et al.* 1985). The *bc* mutations result from the insertion of foreign DNA between positions −10 and

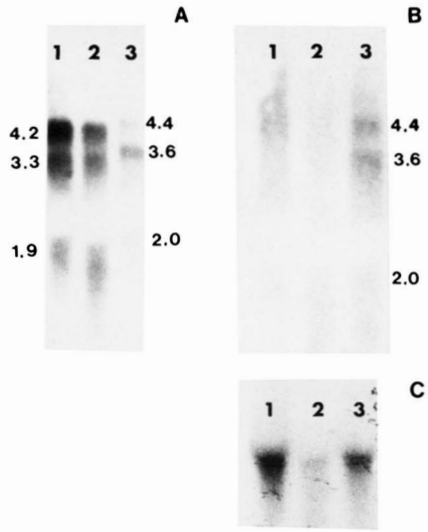


FIGURE 8.—Late *Sxl* transcripts in Sxl^{fc}/Sxl^{fLS} larvae. The cross was *cm* Sxl^{fc} homozygous females mated with *y w* Sxl^{fLS} *oc v* f^{36a} males. Lane 1, Sxl^{fc}/Sxl^{fLS} larvae; lane 2, wild-type females; and lane 3, wild-type males. A, Filter probed with h^1; B, filter probed with g^2; C, hybridization of filter 8B with the actin probe DM-A3.

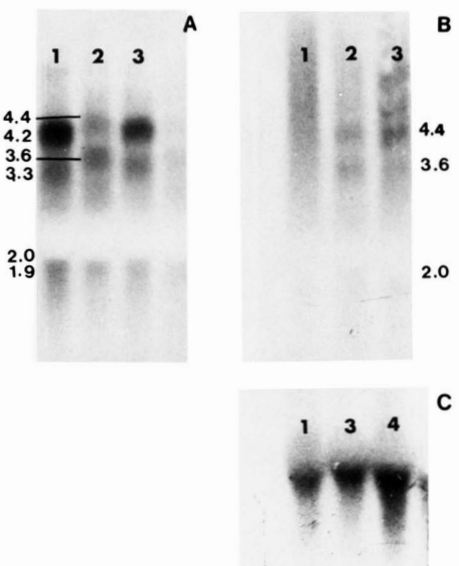


FIGURE 9.—Late *Sxl* transcripts in female larvae mutant for Sxl^{fd} and Sxl^{fhv1}. The crosses to generate the larvae were Sxl^{fhv1} homozygous females mated with either Sxl^{fhv1} males or *y w* $Sxl^{fd}/B^s y$ males. The ratio between Sxl^{fd}/Sxl^{fhv1} females and Sxp^f;kh^{v1} males is 0.95 at the larval stage, while 0.35 at the adult stage. Lane 1, Sxl^{fhv1} homozygous female larvae; lane 2, Sxl^{fhv1} male larvae, and lane 3, Sxl^{fd}/Sxl^{fhv1} female larvae. *A*, Filter probed with h^1; B, filter probed with g^2; C, hybridization of filter 9B with the actin probe DM-A3.

−11 on the molecular map, within the fourth intron (this report). We believe that the two complementation groups are related to the two sets of early and late *Sxl* transcripts, which are responsible for the early and late *Sxl* functions, respectively.

The analysis of the late *Sxl* transcripts in female larvae homozygous for Sxl^{fc} shows that these females express only the *Sxl* transcripts typical of females. Female larvae homozygous for Sxl^{fLS}, on the contrary,

express the *Sxl* transcripts characteristic of males and none of the females. This would explain why Sxl^{fb} and Sxl^{fc} do not affect the processes of somatic sex determination, late dosage compensation and oogenesis (this report), while Sxl^{fLS} does (SÁNCHEZ and NÖTHIGER 1982; MAINE *et al.* 1985b; CLINE 1986; this report). Moreover, the Sxl^{fc}/Sxl^{fLS} female larvae express exclusively the female *Sxl* transcripts, in accordance with the complementation behavior of these mutations: in these females, the Sxl^{fc} allele would provide the late *Sxl* functions, while the Sxl^{fLS} allele would provide the early *Sxl* functions. The fact that Sxl^{fc}/Sxl^{fLS} females express only the late female *Sxl* transcripts indicates that the primary Sxl^{fLS} transcripts are capable of following the female-specific splicing in the presence of normal late Sxl^{+} products from the Sxl^{fc} allele. These female Sxl^{fLS} transcripts might contain a presumably nondetectable alteration by Northern blots, so that they would code for nonfunctional *Sxl* products; since clones homozygous for Sxl^{fLS}, induced during development of $Sxl^{fLS}/+$ larvae, develop male structures instead of female ones (SÁNCHEZ and NÖTHIGER 1982).

There is evidence supporting the sex specificity of the early *Sxl* transcripts (D. COULTER, cited in SALZ *et al.* 1989; TORRES and SÁNCHEZ 1991) The gene *Sxl* seems to contain a sex-specific and a non-sex-specific promoter. The sex-specific promoter would function in females early in development producing the early *Sxl* transcripts, as a consequence of the response of the gene *Sxl* to the X:A ratio signal. The non-sex-specific promoter functions later and throughout development of both females and males giving rise to the primary *Sxl* RNA, that subsequently will be differently spliced in females and males. The early promoter seems to be downstream of the late promoter and located upstream of the male-specific exon (SALZ *et al.* 1989). Then, the production of the early *Sxl* transcripts would be regulated by transcriptional control and subsequent splicing to eliminate the male-specific exon (SALZ *et al.* 1989). The Sxl^{fb} and Sxl^{fc} mutations may affect the early *Sxl* transcripts by interfering with their transcriptional control and/or their splicing. This would explain the female-lethal synergistic interaction between these mutations and the maternal *da* product, and mutations at either *sis-a* or *sc* (Table 5 and Figure 3), which impair early *Sxl* expression.

In this context, the comparison of the Sxl^{fb} and Sxl^{fc} mutations relative to the Sxl^{M} mutations become relevant. The molecular analysis of the Sxl^{M} mutations has revealed the existence of the B104 transposable element inserted around the *Pst*I restriction site at coordinate −9 (MAINE *et al.* 1985a), next to the region where the insertion in the Sxl^{fb} and Sxl^{fc} mutants is located (see Figure 4). However, these mutations present an opposite phenotype. The gain-of-function Sxl^{M} mutations express constitutively the female *Sxl* functions involved in sex determination and dosage compensation, thus causing their dominant male-specific lethal phenotype (CLINE 1978). A possibility is that constitutive *Sxl* expression in these Sxl^{M} mutants is a consequence of *cis*-activation of *Sxl* by promoter and/or enhancer sequences contained in the B104 transposable element (BELL *et al.* 1988). In this respect, it is worth mentioning that the B104 element is specifically transcribed at the beginning of development (SCHERER *et al.* 1982). On the contrary, the Sxl^{fb} and Sxl^{fc} mutations are characterized by their recessive female-specific lethal phenotype, similar to the loss-of-function *Sxl* alleles. Although the nature of the 5-kb insertion of these mutants remains unknown, it may be possible that this insertion disrupts sequences involved in the early activation of *Sxl*. We cannot discard, however, the possibility that these mutations affect the correct splicing of the early *Sxl* RNAs, thus reducing the amount of the correct *Sxl* products.

SALZ *et al.* (1989) suggested that the early transcripts may be involved in directing the first female-specific splicing of the late transcripts. Besides, the early transcripts have to provide the vital *Sxl*-dependent function already operating early in development, such as dosage compensation of the genes expressed at the blastoderm stage (CLINE 1984; GERGEN 1987). Since *bc* mutant female larvae express the late *Sxl* transcript characteristics of females (Figure 6), we propose that these mutations do not prevent the first female-specific splicing of the late *Sxl* transcripts by the early *Sxl* products, but they affect some early female *Sxl* vital function. This function could be responsible for the dosage compensation process required early in development and different from the late dosage compensation process carried out by the *msl* genes (BELOTE and LUCCHESI 1980; CLINE 1984), or it could be a still unknown process.

One possibility is that different early *Sxl* products are responsible for the different early *Sxl* functions: the Sxl^{fb} and Sxl^{fc} mutations would affect the early products involved in the early female *Sxl* vital function, but would not affect the products involved in directing the first female-specific splicing of the late *Sxl* transcripts. Alternatively, in case that both early *Sxl* functions are carried out by common products, the Sxl^{fb} and Sxl^{fc} mutants may produce a low amount of early *Sxl* transcripts, sufficient to direct the first female-specific splicing of the late transcripts, but insufficient for the early female vital function of *Sxl*. The analysis of the early *Sxl* transcripts in the Sxl^{fc} homozygous females will help us to understand the relationship between the early *Sxl* transcripts and the early *Sxl* functions.

The *Sxl* alleles affecting the late *Sxl* functions form

a single complementation group for both sex determination and dosage compensation. However, no correlation exists between the effect of the different *Sxl* alleles (by themselves or in allelic combination) on these processes. Also no correlation is found between the effects of *Sxl* mutations in the germline and soma development. This lack of correlation suggests that the different late *Sxl* functions might be carried out by the different late *Sxl* products, or combinations of them.

Sxl-dependent somatic function for development of the germline: Females of genotype Sxl^{fb}/Sxl^{fm7M1} or Sxl^{fc}/Sxl^{fm7M1} lack gonads. We have observed, however, germ cells at the blastoderm stage and in the larval ovaries of these females (data not shown). Thus, the absence of gonads in these females cannot be attributed to the lack of germ cells, as it happens in females homozygous for loss-of-function mutations at the gene *ovo* (OLIVER, PERRIMON, and MAHOWALD 1987), or in females from mothers homozygous for *oskar* (LEHMANN and NÜSSLEIN-VOLHARD 1986). We cannot discard the possibility that the germ cells die later in development. Germ cells homozygous for Sxl^{fb} and Sxl^{fc} (Table 3), or Sxl^{fm7M1} (CLINE 1984; SCHÜPBACH 1985), give rise to functional oocytes when they are in a wild-type ovary. Then, we believe, that the females of genotype Sxl^{fb}/Sxl^{fm7M1} or Sxl^{fc}/Sxl^{fm7M1} may have affected a *Sxl* function, which is specifically needed for the development of the female gonadal soma, or a somatic function needed for the interaction between the gonadal soma and the germline required for the development of the gonad.

We are grateful to R. DE ANDRÉS and D. MATEOS for their assistance, and to M. CORDERO for her help with the statistical analysis. We are also grateful to J. MODOLELL and S. CAMPUZANO for their comments on the manuscript, and to M. C. PARTEARROYO for typing it. This work was supported by grant PB87-0239 from Comisión Interministerial de Ciencia y Tecnología (CICYT). B. G. and M. T. are recipients of a predoctoral fellowship from the Ministerio de Educación y Ciencia.

LITERATURE CITED

BACHILLER, D., and L. SANCHEZ, 1991 Production of XO clones in XX females of *Drosophila*. Genet. Res. **57:** 23–28.

BELL, L. R., E. M. MAINE, P. SCHEDL and T. W. CLINE, 1988 *Sex-lethal*, a *Drosophila* sex determination switch gene, exhibits sex-specific RNA splicing and sequence similar to RNA binding proteins. Cell **55:** 1037–1946.

BELOTE, J. M., and J. C. LUCCHESI, 1980 Control of X chromosome transcription by the *maleless* gene in *Drosophila*. Nature **285:** 573–575.

BREEN, T. R., and J. C. LUCCHESI, 1986 Analysis of the dosage compensation of a specific transcript in *Drosophila melanogaster*. Genetics **142:** 483–491.

CAMPUZANO, S. L., R. BALCELLS, L. VILLARES, L. CARRAMOLINO, L. GARCIA-ALONSO and J. MODOLELL, 1986 Excess function *Hairy-wing* mutations caused by "gypsy" and "copia" insertion within structural genes of the *achaete-scute* locus of *Drosophila*. Cell **44:** 303–312.

CASE, S. T., and B. DANEHOLT, 1978 The size of the transcription unit in Balbiani rings of *Chironomus tentans* derived from an analysis of the primary transcript and 75s RNA. J. Mol. Biol. **124:** 223–241.

CLINE, T. W. 1978 Two closely-linked mutations in *Drosophila melanogaster* that are lethal to opposite sexes and interact with *daughterless*. Genetics **90:** 683–698.

CLINE, T. W., 1979 A product of maternally-influenced sex-lethal gene determines sex in *Drosophila melanogaster*. Genetics **91:** s22.

CLINE, T. W., 1980 Maternal and zygotic sex-specific gene interactions in *Drosophila melanogaster*. Genetics **96:** 903–926.

CLINE, T. W, 1984 Autoregulatory functioning of a *Drosophila* gene product that establishes and maintains the sexually determined state. Genetics **107:** 231–277.

CLINE, T. W., 1985 Primary events in the determination of sex in *Drosophila melanogaster*, pp. 301–327 in *The Origin and Evolution of Sex*, edited by H. HALVORSON and A. MONROY. Alan R. Liss, New York.

CLINE, T. W., 1986 A female specific lethal lesion in an X-linked positive regulator of the *Drosophila* sex determination gene *Sex-lethal*. Genetics **113:** 641–663.

CLINE, T. W., 1988 Evidence that "*sisterless-a*" and "*sisterless-b*" are two of several discrete "numerator elements" of the X;A sex determination signal in *Drosophila* that switch *Sex-lethal* between two alternative stable expression states. Genetics **119:** 829–862.

ERICKSON, J. W., and T. W. CLINE, 1991 Molecular nature of the *Drosophila* sex determination signal and its links to neurogenesis. Science **251:** 1071–1074.

FERRUS, A., S. LLAMAZARES, J. L. DE LA POMPA, M. A. TANOUYE and O. PONGS, 1990 Genetic analysis of the *Shaker* gene complex of *Drosophila melanogaster*. Genetics **125:** 383–398.

GERGEN, J. P., 1987 Dosage compensation in *Drosophila*. Evidence that *daughterless* and *sex-lethal* control *X* chromosome activity at the blastoderm stage of embryogenesis. Genetics **117:** 477–485.

GRANADINO, B., S. CAMPUZANO and L. Sanchez, 1990 The *Drosophila melanogaster f l(2)d* gene is needed for the female-specific splicing of *Sex-lethal* RNA. EMBO J. **9:** 2597–2602.

JACOBSON, B. K., J. B. MURPHY and F. C. Hartman, 1970 Isoenzymes of *Drosophila* alcohol deshydrogenase. I. Isolation and interconversion of different forms. J. Biol. Chem. **245:** 105–108.

LEHMANN, R., and C. NÜSSLEIN VOLHARD, 1986 Abdominal segmentation, pole cell formation and embryonic polarity requires the localized activity of *oskar*, a maternal gene in *Drosophila*. Cell **47:** 141–152.

LEWIS, E. B., and F. BAKER, 1968 Method of feeding ethyl methanesulfonate (EMS) to *Drosophila* males. Drosophila Inform. Serv. **43:** 193.

LINDSLEY, D. L., and E. H. GRELL, 1968 Genetic Variations of *Drosophila melanogaster*. Carnegie Inst. Wash. Publ. 627.

LINDSLEY, D. L., and G. ZIMM, 1985 The genome of *Drosophila melanogaster*. Part 1: Genes A-K. Drosophila Inform. Serv. **62:**

LINDSLEY, D. L., and G. ZIMM, 1986 The genome of *Drosophila melanogaster*. Part 2: Lethals; maps. Drosophila Inform. Serv. **64:**

LINDSLEY, D. L., and G. ZIMM, 1987 The genome of *Drosophila melanogaster*. Part 3: Rearrangements. Drosophila Inform. Serv. **65:**

LINDSLEY, D. L., and G. ZIMM, 1990 The genome of *Drosophila melanogaster*. Part 4: Genes L–Z, balancers, transposable elements. Drosophila Inform. Serv. **68:**

LUCCHESI, J. C., and J. E. MANNING, 1987 Gene dosage compensation in *Drosophila melanogaster*. Adv. Genet. **24:** 371–429.

LUCCHESI, J. C., and J. M. RAWLS, 1973 Regulation of gene function: a comparison of enzyme activity levels in relation to

gene dosage in diploids and triploids of *D. melanogaster*. Biochem. Genet. **9:** 41–51.

LUCCHESI, J. C., and T. SKRIPSKY, 1981 The link between dosage compensation and sex determination in *Drosophila melanogaster*. Chromosoma **82:** 217–227.

MAINE, E. M., H. K. SALZ, T. W. CLINE and P. SCHEDL, 1985a The *Sex-lethal* of *Drosophila*: DNA alterations associated with sex-specific lethal mutations. Cell **43:** 521–529.

MAINE, E. M., H. K. SALZ, P. SCHEDL and T. W. CLINE, 1985b *Sex-lethal*, a link between sex determination and sexual differentiation in *Drosophila melanogaster*. Cold Spring Harbor Symp. Quant. Biol. **50:** 595–604.

MANIATIS, T., E. F. FRITSCH and Y. SAMBROOK, 1982 *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N. Y.

MARSHALL, T., and J. R. WHITTLE, 1978 Genetic analysis of the mutation *female-lethal* in *Drosophila melanogaster*. Genet Res. **32:** 103–111.

MCGINNIS, W., A. W. SHERMOEN and S. K. BEKENDORF, 1983 A transposable element inserted just 5′ to a *Drosophila melanogaster* glue protein gene alters gene expression and chromatin structure. Cell **34:** 75–84.

MEYEROWITZ, E. M., and D. S. HOGNESS, 1982 Molecular organization of a *Drosophila* puff site that responds to ecdyson. Cell **28:** 165–176.

NÖTHIGER, R., M. JONGLEZ, M. LEUTHOLD, P. MEIER-GERSCHWILER and T. WEBER, 1989 Sex determination in the germ line of *Drosophila* depends on genetic signals and inductive somatic factors. Development **107:** 505–518.

OLIVER, B., N. PERRIMON and A. P. MAHOWALD, 1987 The *ovo* locus is required for sex-specific germline maintenance in *Drosophila*. Genes Dev. **1:** 913–923.

PARKHURST, S. M., D. BOPP and D. ISH-HOROWIEZ, 1990 X:A ratio, the primary sex-determining signal in *Drosophila*, is transduced by helix-loop-helix proteins. Cell **63:** 1179–1191.

PERRIMON, N., 1984 Clonal analysis of dominant female-sterile germline-dependent mutations in *Drosophila melanogaster*. Genetics **108:** 927–939.

PERRIMON, N., D. MOHLER, L. ENGSTROM and A. P. MAHOWALD, 1986 X-linked female-sterile loci in *Drosophila melanogaster*. Genetics **113:** 695–712.

SALZ, H. K., T. W. CLINE and P. SCHEDL, 1987 Functional changes associated with structural alterations induced by mobilization of a P element inserted in the *Sex-lethal* gene of *Drosophila*. Genetics **117:** 221–231.

SALZ, H. K., E. M. MAINE, L. N. KEYES, M. E. SAMUELS, T. W. CLINE and P. SCHEDL, 1989 The *Drosophila* female-specific sex determination gene. *Sex-lethal*, has a stage-, tissue- and sex-specific RNAs suggesting multiple modes of regulations. Genes Dev. **3:** 708–719.

SANCHEZ, L., and R. NÖTHIGER, 1982 Clonal analysis of *Sex-lethal*, a gene needed for female sexual development in *Drosophila melanogaster*. Wilhelm Roux's Arch. Dev. Biol. **191:** 211–214.

SÁNCHEZ, L., and R. NÖTHIGER, 1983 Sex determination and dosage compensation in *Drosophila melanogaster*: production of male clones in XX females. EMBO J. **2:** 485–491.

SCHERER, G., C. TSCHUDI, J. PERERA, H. DELIUS and V. PIRROTA, 1982 B104, a new dispersed repeated gene family in *Drosophila melanogaster* and its analogies with retroviruses. J. Mol. Biol. **157:** 435–451.

SCHÜPBACH, T., 1985 Normal female germ cell differentiation requires the female *X* chromosome to autosome ratio and expression of *Sex-lethal* in *Drosophila melanogaster*. Genetics **109:** 529–548.

STEINMANN-ZWICKY, M., H. SCHMID and R. NÖTHIGER, 1989 Cell-autonomous and inductive signals can determine the sex of the germ line of *Drosophila* by regulating the gene Sxl. Cell **57:** 157–166.

TORRES, M, and L. SÁNCHEZ, 1989 The *scute* (*T4*) gene acts as a numerator element of the X:A signal that determines the state of activity of *Sex-lethal* in *Drosophila melanogaster*. EMBO J. **10:** 3079–3086.

TORRES, M., and L. SÁNCHEZ, 1991 The *sisterless-b* function of the *Drosophila* gene *scute* is restricted to the stage when the X:A ratio determines the activity of *Sex-lethal*. Development (in press).

WHITNEY, J. B., III, and J. C. LUCCHESI, 1972 Ontogenetic expression of fumarase activity in *Drosophila melanogaster*. Insect. Biochem. **2:** 367–370.

Communicating editor: T. SCHÜPBACH