TWO CLOSELY LINKED MUTATIONS IN *DROSOPHILA MELANOGASTER* THAT ARE LETHAL TO OPPOSITE SEXES AND INTERACT WITH DAUGHTERLESS

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ABSTRACT

A new spontaneous mutation named Sex-lethal, Male-specific #1 (Sxl^{M1}) is described that is lethal to males, even in the presence of an $Sxl+$ duplication. Females homozygous for Sxl^{M1} are fully viable. This dominant, male-specific lethal mutation is on the X chromosome approximately 0.007 map units to the right of a previously isolated female-specific mutation, Female-lethal, here renamed Sex-lethal, Female-specific #1 (Sxl^{F1}). Sxl^{M1} and Sxl^{F1} are opposite in nearly every respect, particularly with regard to their interaction with the maternal effect of the autosomal mutation, daughterless (*da*). Females that are homozygous for *da* produce defective eggs that cannot support female (XX) development. A single dose of Sxl^{M1} enables daughters to survive this *da* female-specific lethal maternal effect. A duplication of the *Sxl* locus weakly mimics this action of Sxl^{M1}. In contrast, Sxl^{F1} and a deficiency for *Sxl*, strongly enhance the female-lethal effects of *da*. The actions of Sxl^{M1} and Sxl^{F1} are explained by a model in which expression of the *Sxl* locus is essential for females, lethal for males, and under the control of a product of the *da* locus. It is suggested that Sxl^{M1} is a constitutive mutation at the *Sxl* locus.

IT has been known for decades that the sexual phenotype of *D. melanogaster* depends on the ratio of X chromosomes to sets of autosomes (BRIDGES 1932). This X:Autosome balance also governs the rate of transcription of most X-chromosome genes in a process known as dosage compensation (see LUCCHESI 1977, for review). X-chromosome genes of a male Drosophila are transcribed at about twice the rate of autosomal or X chromosome genes in a female. Although sex determination and dosage compensation are fundamental processes in Drosophila development, very little is known regarding the genetic or molecular mechanisms by which the X/A ratio is assessed and interpreted. Lethal mutations whose effects depend specifically on the X/A balance might be useful in understanding these processes. Of particular interest with respect to dosage compensation would be those lethals whose effects are not related to sexual physiology *per se*, but which instead interfere with basic cell functions common to a variety of tissue types.

Some of the effects of an unusual autosomal mutation called daughterless appear to depend on the X/A balance in just this way. Daughterless is a tem-

perature-sensitive recessive lethal (*da*, *2*–41.5) with a complex phenotype, the most striking aspect of which is a strong maternal effect that is lethal to the daughters of *da/da* mothers (BELL 1954; SANDLER 1972; MANGE and SANDLER 1973; CLINE 1976). Homozygous mutant females produce defective eggs. If these eggs are produced at 25° and develop as diplo-*X* (female) embryos, they will die before completing development; on the other hand, if such eggs develop as haplo-*X* (male) individuals, their survival can be nearly as high as that of the wild type. Temperature shifts indicate that the maternal effect acts within the first three hours after oviposition (at or prior to the blastoderm stage), though death may occur as late as the pharate adult stage. Studies with genetic mosaics showed that the maternal effect is cell autonomous, in that it disrupts the growth and differentiation of female cells throughout the embryo (including those in the imaginal discs) irrespective of the presence of male cells (CLINE 1976). Mutations that alter the sexual phenotype of diplo-*X* animals do not affect the lethal action of *da* (BELL 1954; COLAIANNE and BELL 1968). It has also been shown that female embryos that would normally die as a result of the *da*-induced egg defect can be rescued by the microinjection of wild-type egg or embryo cytoplasm at the preblastoderm stage (BOWNES, CLINE and SCHNEIDERMAN 1977); consequently the system may be amenable to direct biochemical analysis.

The present report describes the isolation and characterization of a mutation on the *X* chromosome that rescues the daughters of homozygous *da* mothers. This "suppressor" mutation can completely overcome the female-lethal *da* maternal effect at 25°, increasing the viability of daughters by at least $10^4\times$. This mutation, which counteracts a female-specific lethal effect, behaves itself as a male-specific, dominant lethal. More surprising still is the fact that this male-specific lethal is very closely linked to a previously isolated female-specific lethal. The locus containing these two sex-specific lethal mutations has been renamed, Sex-lethal (*Sxl*). The interactions between the two *Sxl* mutations, their wild-type alleles, and the *da* maternal effect, are described and interpreted below.

MATERIALS AND METHODS

Flies were raised in uncrowded conditions on the following medium topped with live yeast: per liter of water, 8.4 g agar, 8.7 g potassium sodium tartrate 4-hydrate, 0.7 g calcium chloride dihydrate, 31.4 g sucrose, 62.7 g glucose, 31.9 g yeast, 76 g cornmeal, and 5.1 ml propionic acid added as a mold inhibitor. The temperature was 25°, unless otherwise indicated. The criterion for survival was eclosion since, in some experiments, a significant fraction of the (often malformed) adult females became stuck on the food surface. Refer to LINDSLEY and GRELL (1968) for mutant designations and descriptions.

RESULTS

Isolation and mapping of a "suppressor" of the daughterless maternal effect

A mutation that counteracted the female-lethal effect of daughterless would be useful in understanding the basis for the *da* sex-specificity. The following approach proved successful for the isolation of such a mutant. Although *da/da* females produce no daughters at 25°, if at least the last two days of oogenesis in

the mutant mothers, and the first 10 to 20 hours of embryonic development in the progeny take place at 18°, *da/da* daughters survive and are fertile. It is therefore possible to maintain a stock homozygous for the *da* mutation. But since the viability of *da/da* daughters in such a stock at 18° is only about 6% or less of that of their *da/da* brothers, there is a strong selective advantage for mutations that increase the viability of the females.

In just such a homozygous *da* stock, a spontaneous mutation arose that increased the viability of *da/da* females to nearly 100% of that of their brothers at 18°, and to more than 80% of that of their brothers at 25°. Preliminary characterization of this altered *da* stock indicated that the suppressor mutation was located on the *X* chromosome somewhat to the left of lozenge and was associated with lethality in hemizygous males. This mutation was named Sex-lethal, Male-specific allele #1, abbreviated Sxl^{M1}. The recovery of this mutant was probably fortuitous. A previous attempt to isolate a suppressor of the female-lethal *da* maternal effect in the F_1 or F_2 generation following X irradiation of males had been unsuccessful (CLINE and L. WYSOCKI, unpublished). Furthermore, a subsequent attempt to isolate additional spontaneous suppressors by the 18° culture technique was not successful, despite the fact that this search was much more extensive than that which produced the first allele.

Table 1 describes the precise mapping of Sxl^{M1} and illustrates a number of important features. Columns 1 and 2 indicate the progeny produced at 25° from females heterozygous for Sxl^{M1} and homozygous for *da*. Among the progeny which were nonrecombinant for the region including the *Sxl* locus (*cm–sn*; 18.9–21.0), only the daughters that received Sxl^{M1} survived (column 2, *cm sn*). The death of the Sxl^{+} daughters shows that the *da/da* mothers continued to produce defective eggs, even though they carried Sxl^{M1}. This indicates that Sxl^{M1} acts in

TABLE 1

Mapping of the Sex-lethal, Male-specific #1 mutation, demonstrating its male-lethal and female-rescuing effects

Progeny phenotype*		Progeny from crosses @ 25° of *cm ct sn/Y* males with: *cm* Sxl^{M1} *sn*/+++; *da/da* females (Cross A)		*cm* Sxl^{M1} *sn*/+++; *da*/+ females (Cross B)		+ Sxl^{M1} ++/*cm* + *ct sn*; *da*/+ females (Cross C)	
		Males (1)	Females (2)	Males (3)	Females (4)	Males (5)	Females (6)
+	+	8507	0†	1754	1856	0	4377
cm	*sn*	0†	2929	0	1726	3877	4078
+	*sn*	153	10	24	30	14	86
cm	+	22	136	6	41	76	94

* To facilitate comparison of the three crosses, phenotype with respect to *ct* is not considered; however, the map position of Sxl^{M1} at 19.2, determined on the basis of *cm-sn* recombination, is entirely consistent with the data for *cm-ct* recombination from cross C. Among recombinant male progeny from cross C, 36 *cm ct* +, 0 + + *sn*, 40 *cm* + +, and 14 + *ct sn* animals were recovered. The numbers for recombinant females were 32, 40, 62 and 46 respectively.

† These values do not include the following exceptional progeny: 12 patroclinous males (*cm ct sn*/O) and five matroclinous females (determined to be *cm* Sxl^{M1}*sn*/+ + +/*Y* based on the fact that their sons were all wild-type with respect to *cm*, *ct* and *sn*).

the daughters to rescue them from the otherwise lethal effects of the maternal *da* mutation. Strictly speaking, then, Sxl^{M1} does not act by directly suppressing the *da* maternal effect, since mothers that carry Sxl^{M1} continue to display a female-lethal maternal effect. Of course, the possibility has not been excluded that Sxl^{M1} must be carried by both the mother and her daughters in order to affect rescue. The daughters that are rescued by Sxl^{M1} are morphologically normal, unlike "escaper" Sxl^{+} daughters, which can survive the *da* maternal effect at lower temperatures (CLINE 1976).

The situation with the male progeny is just the reverse (column 1): all nonrecombinant sons who received Sxl^{M1} died (*cm sn*). Furthermore, the lethal effect of Sxl^{M1} in males was independent of the presence of *da*. This is shown by the data in columns 3 and 4, which indicate the progeny produced in a cross similar to that in columns 1 and 2, except that the mothers were *da*/+ rather than *da/da*. Even in the absence of the maternal effect, the Sxl^{M1} males died (column 3, *cm sn*). As expected, daughters that were wild type with respect to *Sxl* now survived. In addition, most of the Sxl^{M1}/+ females from this second mating (column 4, *cm sn*) also survived, showing that the Sxl^{M1} mutation has little effect on the viability of daughters in the absence of the *da* maternal effect. The slight depression in the viability of these females was due to the marker mutations *cm* and *sn* rather than Sxl^{M1}, as indicated by the fact that the *cm sn* class of females was depressed somewhat even when the orientation of the markers was reversed with respect to Sxl^{M1} (columns 5 and 6).

A more direct indication of the effect of Sxl^{M1} on viability was obtained from a determination of egg-to-adult survival. From the mating described in columns 3 and 4 of Table 1, 933 eggs were collected, from which 645 Sxl^{M1}/+, +/+ and +/*Y* adults developed. Assuming normal chromosome segregation, this represents 92% survival for these three classes of progeny and indicates that their viability was not affected by the Sxl^{M1} mutation. Egg-to-adult survival was also determined at 25° for the progeny of *da/da* females carrying Sxl^{M1}. From 3055 eggs that were collected from a mating of Sxl^{M1}/+; *da cn bw* females to wild-type males, 629 adult males (Sxl^{+}/Y) and 534 adult females (Sxl^{M1}/+) developed, representing 82% and 70% survival, respectively (assuming 100% fertilization). The ratio of female to male progeny in such a mating gives a value for the effectiveness of Sxl^{M1} in rescuing daughters. In this mating, the mutation was 85% effective, while in the mating in columns 1 and 2 of Table 1, it was only 35% effective. This difference between the two experiments was probably caused by the marker mutations, particularly *sn*. This is suggested by a comparison of the viability of various classes of Sxl^{M1}/+ daughters in column 2 of Table 1 to that of the reciprocal classes of sons, a ratio indicating the effectiveness of rescue by Sxl^{M1}. The *cm* + daughters were 89% as viable as their + sn brothers (136/153), a value comparable to that for rescue in the absence of marker mutations. In contrast, the + *sn* daughters were only 45% as viable as their *cm* + brothers (10/22), not significantly different from the 34% value for the nonrecombinant females (2929/8507).

The map position of the mutation causing the male-lethal aspect of the Sxl^{M1} phenotype was 19.2 ± 0.04, calculated from the males in the three crosses in Table 1. This position was determined using the standard value of 18.9 for the position of *cm*. The *cm-sn* distance calculated from the male recombinants in the three crosses was 2.04 map units, very close to the standard value of 2.1; clearly the Sxl^{M1} mutation does not affect recombination in this region. This, along with the fact that the Sxl^{M1} chromosome appears cytologically normal in salivary gland preparations, indicates that the mutation is not associated with a chromosome aberration.

The position of the mutation causing female rescue can be determined from cross A in Table 1. The position is distorted somewhat by the effect of *sn* discussed above. Nevertheless, its position at 19.04 in this cross is not significantly different from that determined by scoring male lethality in the same cross ($p = 0.13$, by 2×2 χ^2). The conclusion that the two aspects of the Sxl^{M1} phenotype are inseparable is also supported by an experiment to be described below.

Effects of duplications and deficiencies of the Sxl *locus*

The conclusions from the mapping of Sxl^{M1} suggested a simple hypothesis for the sex-specific lethal effect of *da* and the dominant action of Sxl^{M1} in overcoming that effect: *Sxl* might be a dosage-sensitive locus on the *X*, such that animals with two copies of the locus (females) would be sensitive to the lethal *da* maternal effect, while animals with one copy (males) might be resistant. If Sxl^{M1} were simply a nonfunctional allele of Sxl^{+}, then $Sxl^{M1}/+$ females would have only a single functional dose of *Sxl* and would therefore survive the *da* maternal effect. If *Sxl* were an essential gene, Sxl^{M1}/Y males would lack a functional copy of the locus and would therefore die. This situation would resemble the interaction between the mutant zeste and the white^{+} locus of Drosophila. The availability of small duplications and deficiencies of the *Sxl* locus made a test of this hypothesis possible. $Df(1)ct^{J6}$ is a deficiency of the *cm-ct* region (bands 6E1 through 7B7), while $Dp(1;3)sn^{13a1}$ is an insertion of the *cm-ct region* (6C12 through 7C9) into chromosome *3* at 79E (LEFEVRE and JOHNSON 1973).

Table 2 illustrates the effect of variations in the dose of the Sxl^{+} locus, both in the presence and absence of the *da* maternal effect. Parents and progeny were kept at 18° during the temperature-critical period for *da*, so that a substantial fraction of the daughters with a normal Sxl^{+} dose would survive the *da* maternal effect (see CLINE 1976). In this experiment, 25% of the daughters with the wild-type Sxl^{+} dose survived the maternal effect (row 1, columns 1 and 7). The effect of varying the dose of Sxl^{+} was just the opposite of that predicted by the hypothesis above: rather than increasing the viability of the daughters of *da/da* mothers, a deficiency for *Sxl* was absolutely lethal to daughters in combination with the *da* maternal effect (row 1, columns 2 and 5). In the absence of the *da* maternal effect, the deficiency had no deleterious effect on female viability (row 2, column 5). Furthermore, it is clear from this experiment that the ability of sons to survive the *da* maternal effect is not due to the fact that they have only one copy of

TABLE 2

Influence of the dose of Sxl+ *on the action of the daughterless maternal effect at 18°*

Genotype of mothers with respect to *da*	*Sxl* dose and phenotype of progeny: Daughters, Normal dose *Sxl*+ *Cy*+,*Me*+ (1)	Daughters, Deficient one Dose *Sxl*+ *Cy*+,*Me* (2)	Sons, Normal dose *Sxl*+ *Cy*+,*Me* (3)	Sons, One extra Dose *Sxl*+ *Cy*+,*Me*+ (4)	Effect of *Sxl*+ dose on progeny viability: Daughters *Sxl*+-deficient Normal (5)	Sons Extra *Sxl*+ Normal (6)	Relative viability of daughters with the normal *Sxl*+ dose: Normal dose ♀♀ Normal dose ♂♂ (7)
da/da	125	0	492	532	<0.008	1.1	0.25
da/+	249	330	268	302	1.3	1.1	0.93

Progeny from crosses of *In(1)dl-49, Df(1)ct*^{j6} *f/Y; Dp(1,3)sn*^{1sa1}*/TM1,Me* males with either *da cn bw/da cn bw* or *da cn bw/In(2L+2R),Cy* females. To facilitate comparison, only the *da/+* progeny are listed. Parents were kept at 17–18°. Progeny were shifted up to 25° 24 to 48 hr after oviposition to complete development.

Sxl+, since in both the presence and absence of the *da* maternal effect, males with two doses of the *Sxl*+ locus survived at least as well as their brothers with only one dose (column 6).

If a deficiency for *Sxl* exacerbated the female-lethal action of the *da* maternal effect, would a duplication of the *Sxl* locus act to ameliorate the lethal effect? The answer is yes, and is indicated in Table 3. From *da/da* mothers at 18°, the relative viability of daughters with the normal two doses of *Sxl*+ was 14% (row 1, column 7). The addition of an extra dose of the *Sxl* locus increased the viability of these daughters more than two-fold (row 1, columns 2 and 5). For the daughters of *da/+* mothers, there was little effect of the extra dose of *Sxl*+ on

TABLE 3

Influence of a duplication of Sxl+ *in the progeny on the action of the daughterless maternal effect*

Genotype of mothers with respect to *da*	*Sxl* dose and phenotype of progeny: Daughters, Normal dose *Sxl*+ *Cy*+,*Me*+ (1)	Daughters, One extra one Dose *Sxl*+ *Cy*+,*Me* (2)	Sons, Normal dose *Sxl*+ *Cy*+,*Me* (3)	Sons, One extra Dose *Sxl*+ *Cy*+,*Me*+ (4)	Effect of *Sxl*+ dose on progeny viability: Daughters Extra *Sxl*+ Normal (5)	Sons Extra *Sxl*+ Normal (6)	Relative viability of daughters with the normal *Sxl*+ dose: Normal dose ♀♀ Normal dose ♂♂ (7)
da/da @ 18°	84	210	616	718	2.5	1.2	0.14
da/+ @ 18°	184	201	179	176	1.1	0.98	1.0
da/da after shift to 25°	0	(7)*	780	804	—	1.0	<0.001
da/+ after shift to 25°	248	243	240	232	0.98	0.97	1.0

Progeny from the crosses of *Dp(1,3)sn*^{1sa1}*/TM1,Me* males to either *da cn bw/da cn bw* or *da cn bw/In(2L+2R),Cy* females. To facilitate comparisons between the experimentals and controls, only the *da/+* progeny are listed. For the 18° data, parents were kept at 17 to 18°, and progeny were shifted up to 25° 48 to 72 hr after oviposition to complete development.

* These daughters were produced within the first 48 hr following the shift of the parents to 25°. No daughters (<0.002) survived after this time.

viability (row 2, column 5). But while the extra dose of Sxl^+ did substantially increase the ability of females to survive the *da* maternal effect at the marginally permissive temperature of 18°, when these same parents were shifted to the nonpermissive temperature of 25°, the extra dose of Sxl^+ was no longer effective at rescuing the daughters (row 3, column 2). Thus, the Sxl^+ duplication only weakly mimics the action of the Sxl^{M1} mutation. The data in Table 3 confirm the conclusion from Table 2 that an extra dose of Sxl^+ had no deleterious effect on male viability, even under conditions (25°) where the *da* maternal effect is severe (column 6).

Table 4 shows the effects of duplications and deficiencies of Sxl^+ on the male-lethal and female-rescuing aspects of the Sxl^{M1} mutation. Column 2 shows that Sxl^{M1} is lethal to males even when they carry a wild-type copy of the *Sxl* locus. This dominant lethal effect of Sxl^{M1} in males contradicts the hypothesis suggested earlier. Column 4 shows that females survive quite well even when Sxl^{M1} is hemizygous. Furthermore, even the Sxl^{M1}/Sxl^- daughters of *da/da* mothers survived in this experiment at 25°, showing that the wild-type Sxl^+ gene has little effect on the ability of Sxl^{M1} to rescue daughters from the *da* maternal effect (column 5).

In order to determine whether females homozygous for Sxl^{M1} would survive, the mutant *mei s332a* (SANDLER *et al.* 1968) was used to induce nondisjunction in $Sxl^{M1}/+$ females at meiosis II and thereby produce $Sxl^{M1}/Sxl^{M1}/Y$ daughters. Since the presence of the *Y* chromosome in these daughters causes them to produce two classes of exceptional progeny in substantial (and equal) numbers (BRIDGES 1916), a value for the relative viability of homozygous Sxl^{M1} females could be obtained. The results of such a study are shown in Table 5 and indicate that Sxl^{M1}/Sxl^{M1} females are at least 67% as viable as their wild-type brothers; this is undoubtedly a conservative estimate, since, as was mentioned earlier, some reduction in female viability may be due to the marker mutations.

TABLE 4

Effects of Sxl+ *dose on the actions of* Sxl^{M1}

Genotype of mothers with respect to *da*	Progeny phenotypes, and progeny genotypes with respect to *Sxl*: Sons (Male-lethal action)		Daughters (Female-rescue action)		Influence of Sxl^- on the relative effectiveness of Sxl^{M1} in rescuing daughters
	B^+ *Me* ♂♂ Sxl^{M1} (1)	B^+ Me^+ ♂♂ Sxl^{M1}; $Dp(Sxl^+)$ (2)	B^+ Me^+ ♀♀ Sxl^{M1}/Sxl^-; $Dp(Sxl^+)$ (3)	B^+ *Me* ♀♀ Sxl^{M1}/Sxl^- (4)	rescue in compound with Sxl^- / rescue in compound with Sxl^+ (5)
da/da @ 25°	0	0	180	104	0.77*
da/+ @ 25°	0	0	341	256	—

Progeny are from crosses of *In(1)dl-49, Df(1)ct^{j6} f/Y; Dp(1,3)sn^{1sa1}/TM1, Me* males to *In(1)sc^{S1L}sc^{8R}* + *dl-49, y sc^{S1}sc^{8}w sn^{X2}B/Sxl^{M1}* females, either *da cn bw/da cn bw* or *da cn bw/* + + + (*i.e.*, Sxl^-/Y; *Dp(Sxl+)Me* males × Sxl^{M1}/B; *da/da*± females. Note that not all classes of progeny are shown.

* To correct for the effects of the markers and Sxl^- that are not related to the interaction with daughterless, this value (column 4 ÷ column 3) is corrected by the (column 4 ÷ column 3) value for the control (progeny from *da/+* mothers).

TABLE 5

Determination of the relative viability of Sxl^{M1}/Sxl^{M1} *females*

Regular progeny		Exceptional progeny		Exceptional ♀♀ (Sxl^{M1}/Sxl^{M1}) / Exceptional ♂♂ (+/Y)
$Sxl^{M1}/+$ (Y) ♀♀	Sxl^{M1}/Y (Y) ♂♂	$Sxl^{M1}/Sxl^{M1}/Y$ ♀♀	+/Y ♂♂	
6420	0	157	233	0.67

Results of a cross of *cm* Sxl^{M1} *ct sn/cm* Sxl^{M1} *ct sn/Y* females to wild-type males.

The functional and genetic relationships between Sxl^{M1} *and Female-lethal*

The location of the male-specific dominant lethal mutation, Sxl^{M1}, at 19.2 on the *X* chromosome was particularly interesting because a female-specific lethal mutation had previously been mapped to nearly the same point (MULLER and ZIMMERING 1960). Males that are hemizygous for this mutation, called Female-lethal, are fully viable, but homozygous *Fl* females never survive. *Fl* is lethal in compound with a deficiency for the locus, but it is viable if the female also carries a duplication of the locus (CLINE, unpublished; T. K. JOHNSON, personal communication). This suggested a hypomorphic or amorphic character for *Fl*. The experiment in Table 6 shows that *Fl* does indeed behave like a deficiency for the *Sxl* locus in its interaction with the *da* maternal effect. Both act as dominant lethals in the daughters from *da/da* mothers at 18°. From the first cross in Table 6, both phenotypes of daughters carry *Fl* in the heterozygous conditions, but the

TABLE 6

Effect of Female-lethal on the survival of daughters from daughterless mothers

Cross* Females × Males	Temperature† °C	Phenotype of progeny: *Sb Ser* ♀♀ (1)	+ ♀♀ (2)	*Sb Ser* ♂♂ (3)	+ ♂♂ (4)	Relative viability of daughters: *Sb Ser* ♀♀ / ++ ♀♀ (5)	++ ♀♀ / ++ ♂♂ (6)
da/da × *Fl/Y*; *Dp(Fl+)/Sb Ser*	25	0	0	1223	1245	—	<0.0008
	18	0‡	308	4713	4799	<0.003	0.064§
Control A:							
da/da × +/*Y*; +/*Sb Ser*	25	0	0	1186	1305	—	<0.0008
	18	65	110	2939	3222	0.59	0.034§
Control B:							
da/+ × *Fl/Y*; *Dp(Fl+)/Sb Ser*	18	588	566	501	511	1.04	1.11

* *da* = *cl da cn bw*; *da*+ = *In(2L+2R)Cy*; *Fl* = *Fl oc ptg v*; *Sb Ser* = *TM3, Sb Ser*; *Dp(Fl+)* = *Dp(1,3)sn*^{13a1}*se*.

† 25 = 24 to 26; 18 = 17 to 18. Parents were kept at the temperature indicated for at least three days prior to the collection of progeny. For the 18° data, progeny were allowed to develop at the lower temperature for at least 24 hr before being shifted to 27° to complete development.

‡ Two *Sb Ser* were recovered in this experiment, but they were subsequently determined to have been +/+/*Y* matroclinous exceptions (*i.e.*, not carrying *Fl*).

§ Relative female viability in the *da/da* matings at 18° would appear to be somewhat lower than in previous reports (CLINE 1976), but this is due to the fact that the data represent the pooling of all progeny produced more than three days after the parents were shifted from 25 to 18°. The maximum value reached for this parameter in the various broods at 18° was 0.26 for the first mating, and 0.058 for Control A.

Sb+ *Ser*+ daughters also carry the *Sxl*+ duplication, which covers the *Fl* locus. Only these daughters survive (column 5, second row). As expected, no daughters of *da/da* mothers survived at 25° (column 6); furthermore, in the absence of the *Fl* mutation (Control A), both phenotypes of daughters were recovered at 18° (fourth row), although the *Sb Ser* animals were somewhat less viable than their wild-type sisters. The *Fl*/+; *Dp(Fl*+*)* daughters from the first cross appear to be somewhat more viable than their +/+ cousins in Control A (compare column 6, second row, with fourth row and see footnote §). With reference to the results from Table 3, this would suggest that *Fl* is hypomorphic rather than amorphic.

Fl was originally isolated as a dominant female-specific lethal. This dominant behavior was subsequently found to depend on unidentified elements of the genetic background. In Table 6 (Control B, columns 5 and 6), there is no suggestion of a dominant lethal effect of *Fl* in the absence of the *da* maternal effect. But it should perhaps be mentioned that the original attempt to set up this experiment with *Fl* introduced from the females rather than the males was unsuccessful due to the very low fertility of females carrying *Fl*, especially when they were also homozygous for *da* (however, the limited results obtained agreed with those of Table 6). It has also been observed that overcrowding can cause *Fl* to exhibit dominant lethal effects.

Like *Sxl*$^-$, *Fl* is viable in females in compound with *Sxl*^{M1}. Consequently, the linkage relationship between these two mutations could be determined as shown in Table 7. From heterozygous mothers, 147 sons were recovered that were recombinant for the flanking markers, *cm*, and *ct*. Each of these males was tested for its genotype with respect to *Fl*. From 145 successful matings, one *cm ct*$^+$ male was found to be wild type with respect to *Fl*. In subsequent tests, it was found that this recombinant *X* chromosome was unable to rescue daughters from the *da* maternal effect. This is a further indication that both the male-lethal and female- rescuing aspects of *Sxl*^{M1} are caused by the same mutation. The *cm ct*$^+$ phenotype of this recombinant male suggests that *Sxl*^{M1} is located approximately 0.007 map units to the right of *Sxl*^{F1} (following STEVENS 1942, the 95% confidence limits were determined to be 0.038 and 0.0017 map units). The map posi-

TABLE 7

Linkage relationship between Sxl^{M1} *and* Fl

	Phenotype of males			
	cm ct	*cm* +	+ *ct*	+ +
	0	45	102	14,614
Genotype with respect to *Fl* and *Sxl*^{M1}: ( ) = assumed, not tested	all (+) *Sxl*^{M1}	43 *Fl* +	101 *Fl* +	all (*Fl*) +
		1 + +		
		1 undetermined (died)	1 undetermined (died)	

Results of a cross between *cm* + *Sxl*^{M1} *ct*/+ *Fl* + + females and wild-type males. The females also carried *sn* + + +/+ *oc ptg v*; *da cn bw*/+ + +.

tion of Sxl^{M1} in this experiment was 19.2 ± 0.04 in (remarkable) agreement with the previous determination (Table 1). The data in Table 7, in combination with those in Tables 1 and 5, illustrate the stable and completely penetrant nature of the male-lethal aspect of the Sxl^{M1} mutation.

DISCUSSION

Although the phenotypes of many Drosophila mutations can be suppressed or enhanced by mutations at other loci, the study of such modifiers has seldom advanced our understanding of the mutations with which they interact. The situation appears more hopeful with the "suppressor" and "enhancer" of daughterless described in this report. Some relationship between the functions of the Sex-lethal locus and those of daughterless is suggested by the phenotypic similarities and genetic interactions between the Female-lethal mutation and the female-lethal *da* maternal effect. But more than this, a direct and indeed dependent functional relationship between these two loci is suggested by the additional fact that a dominant, male-specific lethal mutation, Sxl^{M1}, at the same locus, acts to counteract the female-specific effects of both *Fl* and *da*. The Sex-lethal locus must be involved in controlling vital developmental processes that depend on the dose of the *X* chromosome.

From a genetic standpoint, the Sex-lethal locus is intriguing in that two mutations that are phenotypically opposite in nearly every character are only 0.007 map units apart, well within the range for mutations in the same chromomere. For that reason, I have proposed renaming *Fl* as Sex-lethal, Female-specific allele #1 (Sxl^{F1}) to suggest a pseudoallelic relationship between the male and female specific mutations.

I believe that the effects and interactions of the three mutations, *da*, Sxl^{F1}, and Sxl^{M1}, are most effectively discussed with reference to the simple model illustrated in Figure 1. While the model is admittedly tentative at this point, it has mnemonic as well as predictive value. In this figure, the *Sxl* locus is shown as composed of a regulatory region and a structural region. The structural region of the gene would be expressed (active) in females, but would be inactive in males. The *da* locus would control the synthesis of a factor made by the mother during oogenesis. This "maternal *da* factor" in the egg cytoplasm would be required subsequently by the embryo for the activation of the *Sxl* locus through an interaction at the regulatory region of the gene. Activation of the locus would also depend upon the *X/A* ratio in the embryo, as indicated by the brackets.

Sxl^{F1} would be a lesion in the structural region of the gene, thereby rendering it nonfunctional and killing females. Such a structural lesion would not affect the viability of males, since they would not require the functional product. A mutation at the *da* locus in the mother would have the same ultimate effect as the Sxl^{F1} mutation in the daughter. By reducing the activity of the maternal *da* factor in the egg, the *da* mutation would render the *Sxl* locus nonfunctional by blocking its activation in females. Like the Sxl^{F1} mutation and for the same reasons, this maternal effect would not decrease male viability. Sxl^{M1} behaves

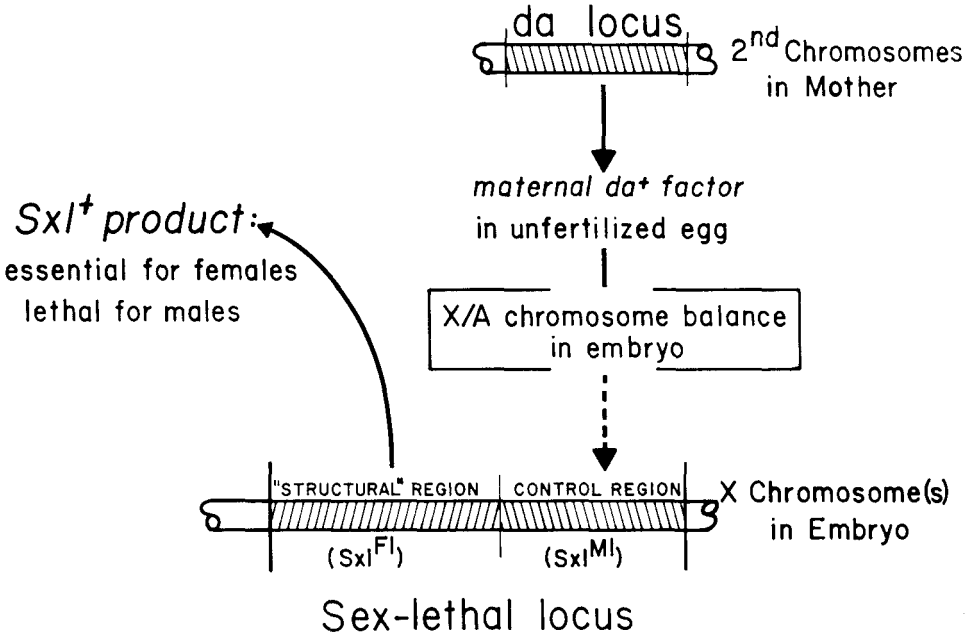

FIGURE 1.—A model to account for the developmental and genetic interactions between the daughterless (*da*) and Sex-lethal (*Sxl*) loci.

like a constitutive mutation in the regulatory region of the *Sxl* locus, altering that region in such a way that the *da* maternal factor would no longer be required to activate the *Sxl* structural region. Sxl^{M1} would thereby allow the daughters of *da/da* mothers to survive. Furthermore, such a mutation should have no deleterious effect on the daughters even of da^+ parents. On the other hand, since an important function of the regulatory region of the *Sxl* gene would be to keep the structural region inactive in males, the constitutive nature of Sxl^{M1} would be lethal to them; they could no longer maintain the gene in the required inactive state. By this model, the dominance of Sxl^{M1} is accounted for, both in rescuing females and in killing males.

Both *da* (MANGE and SANDLER 1973) and Sxl^{F1} have been shown to be hypomorphic on the basis of their interactions with deficiencies. According to the model, reduced activity of the *da* locus causes reduced expression of the *Sxl* locus. A variety of observations support this suggestion of a dependent functional relationship between these two loci. First, mutations that block the expression of either gene have similar developmental effects. Under genetic or environmental conditions that are only marginally permissive for the survival of females, both *da* (CLINE 1976) and Sxl^{F1} (MULLER and ZIMMERING 1960; CLINE, unpublished) can cause female-specific morphological abnormalities in nearly all derivatives of the imaginal discs and histoblasts. Both mutations can cause large duplications and deficiencies of structures, suggesting effects at early developmental stages. Both mutations can cause death of females at any developmental

stage, and under the least permissive conditions both mutations cause embryonic lethality. Although for convenience I have used the term "female" lethal in connection with these mutations, in fact it is not the phenotypic sex of the flies that is important in their action, but rather the X/A balance—the genotypic sex of the flies (BELL 1954; ZIMMERING and MULLER 1961; COLAIANNE and BELL 1968). This is yet another similarity between *da* and Sxl^{F1}.

Second, if the expression of the *Sxl* locus were dependent upon the expression of the *da* gene as proposed, one would expect that the mutations at the two loci would interact synergistically. This is observed to be the case. Under marginally permissive conditions, where Sxl^{F1} alone had little effect on female viability and where a large fraction of Sxl^{+} daughters escaped the *da* maternal effect, the combination of the two mutant effects was absolutely lethal; no $Sxl^{F1}/+$ daughters from *da/da* mothers survived.

Third, a duplication of the Sxl^{+} locus counteracts the female-specific maternal effect of *da*, but only to a limited extent and only under marginally permissive conditions. This is consistent with the suggestion of the model that it is the expression of the Sxl^{+} locus that is the limiting factor for the survival of the daughters of *da/da* mothers.

Fourth, and perhaps most suggestive of a regulatory relationship between the *da* maternal factor and the expression of the *Sxl* locus is the way in which the Sxl^{M1} mutation acts to "suppress" the *da* maternal effect. Sxl^{M1} does not counteract the effect of the *da* mutation by acting in the mother during oogenesis; even though they carry Sxl^{M1}, *da/da* females continue to produce defective eggs that will not support female development at higher temperatures. Instead, Sxl^{M1} acts in the progeny, allowing the daughters to do without the *da* maternal factor. Like Sxl^{M1}, Sxl^{F1} also appears to act primarily in the progeny rather than in the mother. As mentioned above, Sxl^{F1} acts as a dominant female-specific lethal in combination with the *da* maternal effect; however, if these same daughters also carry Sxl^{M1}, then Sxl^{F1} is no longer dominant. Since Sxl^{M1} does not alter the *da* maternal effect per se, Sxl^{M1} can be considered to be a suppressor of the dominant lethal action of Sxl^{F1}. The fact that the effect of the two hypomorphic mutations, *da* and Sxl^{F1}, are counteracted by a third mutation, Sxl^{M1}, suggests a common functional basis for the lethal effects of the first two mutants. This result is also consistent with the proposed pseudo-hypermorphic character of Sxl^{M1} ("pseudo-" because, strictly speaking, Sxl^{M1} is not hypermorphic in females).

In the model proposed above, it was suggested that the Sxl^{M1} region is a regulatory element for the Sxl^{F1} region of the gene. This suggestion was by analogy to the type of gene organization discovered for the rosy locus (CHOVNICK, GELBART and MCCARRON 1977). The 0.007 map unit separation between Sxl^{M1} and Sxl^{F1} is consistent with such an organization. The distance between the rosy structural locus and a *cis*-acting regulatory element was 0.0034 map units in a region of the genome with a somewhat lower rate of recombination per unit DNA length. Polytene chromosome chromomers are though to correspond roughly to individual units of genetic function (reviewed by LEFEVRE 1974). Even the 95% confidence limit for the maximum distance between Sxl^{F1} and Sxl^{M1}, 0.034 map units,

is considerably less than the average distance between chromomeres in the *X* chromosome (0.065 map units. LEFEVRE 1976), suggesting that the two mutations are alterations within the same chromomere. A strong prediction of this model is that Sxl^{F1} mutations should be *cis*-dominant over Sxl^{M1} mutations: the Sxl^{F1} Sxl^{M1} double-mutant male should be viable, and the two mutations in *cis* should be ineffective in rescuing females (in contrast to their effects when *trans*). Preliminary results from a search for gamma-ray induced mutations that allow Sxl^{M1} males to live substantiate this prediction (CLINE, in progress), and indicate that the Sxl^{M1} mutation can serve as an effective tool for the isolation of a variety of new Sxl^{F1} alleles and deletions of the *Sxl* locus. Such double mutants should also greatly facilitate a more precise determination of the linkage relationships between *M* and *F* "alleles."

According to the model, the maternally-synthesized *da* factor in the egg is required to activate the Sxl^+ locus in female embryos, but the specific way in which it accomplishes this is unclear. BOWNES, CLINE and SCHNEIDERMAN (1977) demonstrated that very young female embryos from *da/da* mothers could be rescued by microinjection of wild-type egg cytoplasm. Cytoplasm from unfertilized eggs of *da/da* mothers was ineffective at rescuing; however, an unexpected result from this study was that very young embryos of undetermined sex from *da/da* mothers were also effective at rescuing daughters from these same mothers. To account for this finding, it was hypothesized that the daughters of *da/da* mothers were being rescued by cytoplasm from their brothers; male and female embryos were supposed to differ in their ability to initiate synthesis of some vital substance after fertilization in the absence of the *da* maternal cytoplasmic factor, and that difference would account for the sex specificity of the *da* maternal effect. Such an hypothesis does not relate in any simple way to that proposed in this paper for the role of the *Sxl* locus, but the two hypotheses are not necessarily contradictory, since the activation of the *Sxl* locus must involve a rather complex set of interactions. By the current hypothesis, there remains an important but undefined aspect of the system, namely, the way in which the *X/A* balance is involved in the activation of the *Sxl* locus. Since all wild-type eggs must contain the maternal da^+ factor, but only *XX* embryos are hypothesized to activate the *Sxl* locus, functioning of the factor must depend on the embryo's ability to determine the relative number of *X* chromosomes that it possesses. This is indicated in Figure 1 by the brackets.

What kind of developmental system might specifically require a gene or its product to be active in females, but be inactive or absent in males? It is tempting to speculate that the *Sxl* locus might be involved in the process of *X*-chromosome dosage compensation, perhaps producing a product that inhibits the initiation of transcription at *X* chromosome promotors. Such a role would be consistent with the fact that the *da* maternal effect disrupts a basic aspect of cellular metabolism in a cell-autonomous fashion in response to the *X/A* balance and irrespective of sexual differentiation *per se*. One would expect dosage compensation to be established as soon as embryonic RNA synthesis starts in earnest, the blastoderm stage of development (LAMB and LAIRD 1976; ZALOKAR 1976). This is the time at

which the *da* maternal factor functions, as judged from the temperature-sensitive period, which ends at the blastoderm stage (CLINE 1976). Such a role could easily account for the sex-specific lethal effects of these mutations. Males in which *Sxl* was active would transcribe their single *X* chromosome at the female rate and would therefore produce only half as much *X*-chromosome gene product as they should. On the other hand, inactivation of the *Sxl* gene in females would cause them to transcribe their two *X* chromosomes at the male rate and thereby make twice as much *X*-chromosome gene product as normal. If the *Sxl* locus were involved in dosage compensation, the *da* maternal factor might be a fundamental part of the system by which the embryo determines its *X/A* balance.

The Sxl^{M1} mutation should be particularly useful in analyzing the complex daughterless phenotype. CLINE (1976) proposed that there were at least three functionally separate aspects to the *da* phenotype: a recessive lethal effect in the zygote and two separate effects on oogenesis—one the maternal effect on progeny sex ratio and the other a "sterility" effect on the integrity of the egg membranes. Sxl^{M1} appears to suppress only the female-lethal maternal effect (CLINE, in preparation). A distinction between the various aspects of the *da* phenotype is important, both in understanding the developmental basis for the sex-specific lethality, and in understanding the relationship between *da* and other closely-linked loci that do not exhibit pronounced sex-specific lethal effects, but that do have other interesting features in common with *da* (SANDLER 1977).

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LITERATURE CITED

BELL, A. E., 1954 A gene in *Drosophila melanogaster* that produces all male progeny. Genetics **39**: 958–959.

BOWNES, M., T. W. CLINE and H. A. SCHNEIDERMAN, 1976 Daughters from daughterless mothers—rescuing a female-lethal maternal effect by cytoplasmic transplantation in *Drosophila* embryos. Wilhelm Roux's Arch. **181**: 279–284.

BRIDGES, C. B., 1916 Non-disjunction as proof of the chromosome theory of heredity. Genetics **1**: 1–52. ——, 1932 Genetics of sex in *Drosophila*. pp. 55–93. In: *Sex and Internal Secretions*. Edited by E. ALLEN. Williams and Wilkins, Baltimore.

CHOVNIK, A., W. GELBART and M. MCCARRON, 1977 Organization of the rosy locus in *Drosophila melanogaster*. Cell **11**: 1–10.

CLINE, T. W., 1976 A sex-specific temperature-sensitive maternal effect of the daughterless mutation of *Drosophila melanogaster*. Genetics **84**: 723–742.

COLAIANNE, J. J. and A. E. BELL, 1968 The effect of the doublesex (*dsx*) mutation on the action of daughterless in *Drosophila melanogaster*. Drosophila Inform. Serv. **43**: 155.

LAMB, M. M. and C. D. LAIRD, 1976 Increase in nuclear poly(A)-containing RNA at syncytial blastoderm in *Drosophila melanogaster* embryos. Develop. Biol. **52**: 31–42.

LEFEVER, G., 1974 The relationship between genes and polytene chromosome bands. Ann. Rev. Genet. **8**: 51–62. ——, 1976 A photographic representation and interpretation of the polytene chromosomes of *Drosophila melanogaster* salivary glands. In: *The Genetics and Biology* of Drosophila. Edited by M. ASHBURNER and E. NOVITSKI. Academic Press, New York.

LEFEVRE, G. and T. K. JOHNSON, 1973 Evidence for a sex-linked haplo-inviable locus in the cut-singed region of *Drosophila melanogaster*. Genetics **74**: 633–645.

LINDSLEY, D. L. and E. H. GRELL, 1968 Genetic variations of *Drosophila melanogaster*. Carnegie Inst. Wash. Publ. No. 627.

LUCCHESI, J. C., 1977 Dosage compensation: transcription-level regulation of X-linked genes in *Drosophila*. Amer. Zoologist **17**: 685–693.

MANGE, A. P. and L. SANDLER, 1973 A note on the maternal effect mutants daughterless and abnormal oocyte in *Drosophila melanogaster*. Genetics **73**: 73–86.

MULLER, H. J. and S. ZIMMERING, 1960 A sex-linked lethal without evident effect in Drosophila males but partially dominant in females. Genetics **45**: 1001–1002.

SANDLER, L., 1972 On the genetic control of genes located in the sex chromosome heterochromatin of *Drosophila melanogaster*. Genetics **70**: 261–274. ——, ,1977 Evidence for a set of closely-linked autosomal genes that interact with sex-chromosome heterochromatin in *Drosophila melanogaster*. Genetics **86**: 567–582.

SANDLER, L., D. L. LINDSLEY, B. NICOLETTI and G. TRIPPA, 1968 Mutants affecting meiosis in natural populations of *Drosophila melanogaster*. Genetics **60**: 525–558.

STEVENS, W. L., 1942 Accuracy of mutation rates. J. Genet. **43**: 301–307.

ZALOKAR, M., 1976 Autoradiographic study of protein and RNA formation during early development of *Drosophila* eggs. Devel. Biol. **49**: 425–437.

ZIMMERING, S. and H. J. MULLER, 1961 Studies on the action of the dominant female-lethal *Fl* and of a seemingly less extreme allele, Fl^s. Drosophila Inform. Serv. **35**: 103–104.

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