A FEMALE-SPECIFIC LETHAL LESION IN AN *X*-LINKED POSITIVE REGULATOR OF THE DROSOPHILA SEX DETERMINATION GENE, *SEX-LETHAL*

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ABSTRACT

Characterization of a partial-loss-of-function, female-specific lethal mutation has identified an *X*-linked genetic element (*1*-34.3; 10B4) that functions as a positive regulator of *Sxl*, a central gene controlling sex determination in *Drosophila melanogaster*. The name, *sisterless-a*, was chosen both to suggest functional similarities that exist between this gene and another positive regulator of *Sxl*, the maternally acting gene *daughterless* (*da*), and also to highlight an important difference; namely, that in contrast to *da*, it is the zygotic rather than maternal functioning of *sis-a* that is involved in its interaction with *Sxl*. As with *da*, the female-specific lethal phenotype of *sis-a* is suppressed both by $Sxl^{M\#1}$, a gain-of-function mutant allele of the target gene, and, to a lesser extent, by a duplication of Sxl^{+}. Mutations at *sis-a*, *da* and *Sxl* display female-specific dominant synergism, each enhancing the others' lethal effects. The allele specificity with respect to *Sxl* of these dominant interactions indicates that *sis-a* and *da* affect the same aspect of *Sxl* regulation. As with previous studies of *da* and *Sxl*, the masculinizing effects of loss of *sis-a* function are generally obscured by lethal effects, presumably related to upsets in dosage compensation. The masculinizing effects can be dissociated from lethal effects by analysis of triploid intersexes (*XX AAA*) or by analysis of diploid females who are also mutant for autosomal genes known to be required for the transcriptional hyperactivation associated with dosage compensation in males. Analysis of foreleg development shows that intersexuality generated by *sis-a* is of the mosaic type: At the level of individual cells, only male or female development is observed, never an intermediate sexual phenotype characteristic of true intersexes. Sexual development of diplo-*X* germline and somatic clones of *sis-a* tissue generated by mitotic recombination during larval stages is normal, as is the sexual phenotype of homozygous *sis-a* escapers. Considered in their totality, these results indicate that *sis-a* functions early in development to help establish the activity state of *Sxl* and thereby initiate the sexual pathway commitment, rather than functioning later in the processes by which *Sxl* maintains and expresses the sex determination decision.

THE gene *Sex-lethal* (*1*-19.2) plays a central role in Drosophila sex determination and dosage compensation, aspects of fruit fly development that are triggered by a difference in the relative number of *X* chromosomes. Cells with two *X* chromosomes relative to a diploid set of autosomes (*X*:*A* = 1) develop as female, whereas diploid cells with only one *X* chromosome (*X*:*A* =

0.5) develop as male and also hyperactivate most *X*-linked genes to compensate for the fact that males necessarily have one-half the dose of *X*-linked genes than do females. Discovering how a change of such relatively low magnitude in this quantitative parameter can lead with such high fidelity to the many developmental differences that exist between the sexes may have broad implications. Since determination of the activity state of *Sxl* may be the most immediate effect of the *X:A* balance, study of the regulation of *Sxl* early in development would seem likely to increase our understanding of this developmental signal. One early acting positive regulator of *Sxl* has been known for some time—the autosomal gene named *daughterless*. In this report a second specific positive regulator of *Sxl* is described, one that also appears to be early acting.

Sxl acts as a female-specific developmental switch in somatic tissues (reviewed by CLINE 1985; MAINE *et al.* 1986). The gene also has female-specific germline functions, but their relationship to its somatic functions remains to be determined (SCHÜPBACH 1985). Loss-of-function mutations in *Sxl* transform chromosomal females into phenotypic males, but have no adverse effects on normal ("chromosomal") males. Indeed, males that are deleted for this gene are both viable and fertile (MAINE *et al.* 1985). Gain-of-function mutations have the opposite effect: they transform chromosomal males into phenotypic females, but have no adverse effects on normal females. Sxl^+ seems to respond early to the *X:A* balance signal to initiate a developmental commitment to the female-specific pathway in a cell autonomous fashion. It then functions throughout development to maintain this female pathway commitment. Expression of the female pathway commitment occurs through interactions between *Sxl* and a large group of genes downstream of it in a complex regulatory hierarchy (reviewed by BAKER and BELOTE 1983). Analysis of the effects on sexual phenotype of changes in *Sxl* are complicated by the fact that this gene also controls dosage compensation, a cell-vital process. Thus, sex transformations are often masked by the sex-specific lethal effects after which the gene is named. This complication can be overcome in a number of ways, several of which are employed in this study.

Maternal activity of an autosomal gene called *daughterless* (*da*; 2-41.5) is required for proper functioning of Sxl^+ in the zygote (CLINE 1978, 1983a). Decreasing da^+ activity in the maternal germline, just as decreasing the *X:A* balance in the zygote, appears to decrease the probability of the embryo's stably activating the female-specific functions of Sxl^+. Both because it is maternally acting and because it is an autosomal gene that acts as a positive rather than a negative regulator of Sxl^+, *da* itself would not appear to be a candidate for what might be called an *X:A* signal element—one of the genes for which the dose presumably is counted to signal sex chromosome number. Instead, *da* seems to be part of the biochemical machinery built into the egg that allows the developing embryo to sense and respond appropriately to such *X:A* signal elements.

In this paper, a second positive regulator of Sxl^+ is described, a gene named *sisterless-a* (*sis-a*). Experimental results are presented establishing that *sis-a* acts

TABLE 1

Recessive female-specific lethality of *sis-a*

Cross	Progeny genotypes and no. recovered				Relative viability (%)	
	Females		Males		Females	Males
A	*sis-a/sis-a*	*sis-a*$^+$*/sis-a*	*sis-a/Y*	*sis-a*$^+$*/Y*	*sis-a/sis-a* vs. *sis-a*$^+$*/sis-a*	*sis-a/Y* vs. *sis-a*$^+$*/Y*
	0	951	962	1113	<0.1	86
B (controls)	*sis-a/sis-a*$^+$	*sis-a*$^+$*/sis-a*$^+$	*sis-a/Y*	*sis-a*$^+$*/Y*	*sis-a/sis-a*$^+$ vs. *sis-a*$^+$*/sis-a*$^+$	*sis-a/Y* vs. *sis-a*$^+$*/Y*
	500	495	430	494	101	87

Only progeny *non*recombinant for the *v-m* region that includes *sis-a* are included in this table. In the absence of lethal effects, 1:1:1:1 is the expected progeny ratio from both of these crosses. Full genotypes of crosses at 25°: Cross A—*v sis-a m g/+ + + +*; *In(2LR)O, dp*^{lv1}*Cy pr cn*^{2}*/+* ♀♀ × ♂♂ *v sis-a m g/Y*. Cross B—same females as above × ♂♂ *v sis-a*$^+$*m g/Y*.

in the same processes as *da* in the initiation the female sexual pathway commitment. Unlike *da*, however, *sis-a* acts zygotically in this capacity and is *X*-linked.

MATERIALS AND METHODS

Flies were raised in uncrowded conditions on a standard medium described by CLINE (1978). The criterion for survival was eclosion (since in some cases flies are very weak and quickly become stuck on the food surface). Refer to LINDSLEY and GRELL (1968) for mutant designations and descriptions, except as otherwise referenced in the text. The *sis-a* mutation was generated by ethyl methanesulfonate mutagenesis (see ACKNOWLEDGMENTS).

RESULTS

A female-specific recessive lethal mutation at *1*-34.3: The data in Table 1 illustrate the basic *sisterless-a* phenotype: recessive, female-specific lethality of a mutation located between the marker genes *vermillion* (*v*; *1*-33.0) and *miniature* (*m*; *1*-36.1) on the *X* chromosome. Only progeny nonrecombinant for the *v-m* interval are shown in this table. In the experimental cross (A), females heterozygous for *sis-a* were mated to *sis-a* fathers. For the control cross (B), the same genotype of mothers were mated instead to *sis-a*$^+$ fathers. In the absence of lethal effects, equal numbers of all four classes of progeny were expected for both crosses. The experimental cross shows that, at 25°, the viability of homozygous *sis-a* females is less than 0.1% of their heterozygous sisters. In contrast, hemizygous *sis-a* males generated in this cross were as viable as their heterozygous sisters and were nearly as viable (86%) as their *sis-a*$^+$ brothers.

The lethal period at 25° was determined from a collection of 513 fertilized eggs (hatched or discolored after 2 days) from the cross: *sis-a/+* ♀♀ × ♂♂ *sis-a/Y*. Two hundred thirty-four adult males, 123 adult females and 54 dead

TABLE 2

Map position of *sis-a* based on two aspects of the mutant phenotype

	Cross[a]		Progeny phenotypes and no. recovered						Map position[b] and aspect of phenotype that was mapped
			Daughters			Sons			
	Females	Males	Recombinants v^+m	Recombinants $v\ m^+$	Total	Recombinants v^+m	Recombinants $v\ m^+$	Total	
									Recessive female-specific lethality
A	$\frac{v\ sis\text{-}a\ m}{+\ +\ +}$	$\frac{v\ sis\text{-}a\ m}{Y}$	27	19	997	44	33	2152	34.3
									Dominant female-lethal synergism with *da* and Sxl^{fl1}
B	$\frac{v\ sis\text{-}a\ m}{+\ +\ +}$; $\frac{da}{+}$	$\frac{Sxl^{fl1}v\ sis\text{-}a^+\ m}{Y}$	17	12	818	55	66	2344	34.3
Sum of both crosses			44	31	1815	99	99	4496	34.3 (33.8–34.8)[c]

[a] Cross A is described fully in Table 1 (as cross A), and cross B is described fully in Table 3 (as cross C).

[b] Based on the relative proportions of the two recombinant classes of females, normalized to the standard positions of *v* and *m* at 33.0 and 36.1, respectively.

[c] Ninety-five percent confidence interval.

embryos (no pupae) were recovered, indicating that the lethal period spans the embryonic and larval stages, with about one-half of the *sis-a/sis-a* females dying as embryos.

The control cross established that *sis-a* female-specific lethality is a completely recessive character when all other genes in the sex-determination pathway are wild type. Viability of *sis-a* heterozygotes was as high as that of their wild-type sisters. As in the experimental cross, a slight deficit of *sis-a* males relative to their $sis\text{-}a^+$ brothers was observed, but the significance of this small difference is questionable, because it was not observed consistently in other crosses involving *sis-a* (*cf.* Table 3).

The female-lethal effect is somewhat dependent on temperature. At the lower temperature of 18°, some homozygous *sis-a* escapers were observed, but their relative viability was never more than 2% (data not shown). Escapers were never observed at 29°, nor were escapers ever recovered when *sis-a* was hemizygous in females.

An approximate map position for the recessive, female-specific lethal effect of *sis-a* could be deduced from the relative proportions of the two reciprocal classes of *v-m* recombinant daughters from the experimental cross. The top half of Table 2 gives the data. Normalized to the standard values for the map positions of *v* and *m*, the data place *sis-a* at 34.3. Recombination over the *v-m*

interval for the *sis-a* heterozygotes was 4.3 cM (combined data from experimental and control crosses: 273 nonparental per 6311 total), somewhat higher than the nominal value of 3.1 cM—perhaps due to an interchromosomal effect of the second chromosome balancer present in these crosses. Clearly, the *sis-a* mutation does not interfere with recombination.

Since the genetic organization of the region into which *sis-a* falls had been characterized extensively by GEER, LISCHWE and MURPHY (1983), the chromosomal location of this new female-specific lethal could be determined with considerable precision. An efficient method for mapping *sis-a* relative to adjacent vital genes was devised by taking advantage of the fact that *sis-a* fails to complement *X*-chromosome deficiencies that uncover the 34.3 region. The basic scheme was to cross *sis-a* $l(1)^+/sis\text{-}a^+$ $l(1)$ females to *Df(1)*, $sis\text{-}a^-$ $l(1)^-/Y$; *Dp(1;2)*,$sis\text{-}a^+$ $l(1)^+$ males and determine the yield of daughters which survived without receiving the second-chromosome duplication of the *sis-a* region from their fathers. This class should include the $sis\text{-}a^+$ $l(1)^+$ recombinants. The mapping cross was facilitated by marking the $sis\text{-}a^+$ duplication-bearing second chromosome with another duplication that carried the *X*-linked marker, y^+, and by then arranging the cross so that this was the only y^+ allele present. Appropriate flanking markers were also included in the cross. The progeny of each surviving nonduplication-bearing female was examined to ascertain whether she was a true recombinant, rather than an escaper, a matroclinous exception or a rearrangement of the duplication-bearing second chromosome—all rather rare events.

Mapping *sis-a* relative to the GEER, LISCHWE and MURPHY locus-*14* lethal that had been assigned a map position of 34.28, no wild-type recombinants or gene convertants were recovered from among an estimated 54,460 nonduplication-bearing female zygotes. By the statistical method of STEVENS (1942), this places *sis-a* closer than 0.01 cM (95% confidence level) to the locus-*14* vital gene which *sis-a* fully complements. In mapping *sis-a* relative to the adjacent locus-*15* lethal that had been assigned to 34.39, three $sis\text{-}a^+$ $l(1)15^+$ recombinants were recovered from among an estimated 26,030 nonduplication-bearing female zygotes. This result, considered in light of the orientation of the nearby flanking markers, places *sis-a* 0.023-cM centromere distal from locus *15* (95% confidence limits 0.005 and 0.07 cM). Map positions assigned by GEER, LISCHWE and MURPHY indicate 0.11 cM between the vital genes *14* and *15*. In view of the close linkage between *sis-a* and locus *14*, one might therefore have anticipated that the distance between *sis-a* and locus *15* would have been somewhat greater than the observed value. On the other hand, because GEER, LISCHWE and MURPHY did not report confidence limits for their data, this apparent discrepancy may not be meaningful.

Based on the GEER, LISCHWE and MURPHY assignments of genes to chromomeres, these results place *sis-a* in chromomere 10B4. Assignment of *sis-a* to a location immediately adjacent to lethal locus *14* is entirely consistent with the results of complementation tests between *sis-a* and chromosomal deficiencies in the region. Like the locus-*14* lethal, *sis-a* failed to complement *Df(1)N71* (10B4-5 to 10D5), *Df(1)RA37* (10A8 to 10B16) and *Df(1)KA7* (10A9 to

10F10), but did complement *Df(1)HA85* (10C2 to 10F9). *Df(1)N71* was used in the mapping of *sis-a* relative to the adjacent vital loci. Not a single *Df(1)/sis-a* escaper was recovered in these very extensive experiments.

Dominant female-specific lethal synergism of *sis-a* with *da* and $Sxl^{f\#1}$: Because the initial characterization of the chromosome bearing the *sis-a* mutation indicated that it failed to complement fully the recessive female-specific lethal null allele $Sxl^{f\#1}$, it was not immediately apparent that this chromosome carried anything more interesting than simply another *Sxl* allele. The true nature of the situation became apparent when attempts were made to remove an extraneous *X*-linked t.s. lethal that had been coinduced with the *sis-a* mutation and to map the element responsible for female lethality. It soon became apparent that the *Sxl* region of the mutant chromosome was wild type and that the failure to complement $Sxl^{f\#1}$ must be due to dominant lethal synergism between two otherwise recessive, nonallelic female-specific mutations. A precedent for synergistic dominant lethal interactions between functionally related genes controlling sex determination had been established earlier for $Sxl^{f\#1}$ and the female-lethal maternal effect of *da* (CLINE 1980).

Table 3 presents information on some of the synergistic interactions among *sis-a*, $Sxl^{f\#1}$ and *da*. In the absence of lethal effects, equal numbers of all classes of progeny were expected for each of the four crosses. Cross A reflects the original observation that $Sxl^{f\#1}$ and *sis-a* fail to complement in *trans*. The viability of the double heterozygotes (class 1 females) was only 18% of that for their sisters heterozygous for $Sxl^{f\#1}$ alone (class 2 females). Based on the number of males produced in this cross, it could be concluded that $Sxl^{f\#1}$ heterozygous females themselves were fully viable, confirming the recessive character of $Sxl^{f\#1}$ in the absence of other mutations.

Cross B shows that the synergism between *sis-a* and $Sxl^{f\#1}$ is indeed related to that observed previously between $Sxl^{f\#1}$ and *da*, since the female-specific zygotic lethal effect of *sis-a*, like that of $Sxl^{f\#1}$, is enhanced by loss of maternal da^+ activity. The viability of *sis-a* heterozygous daughters of *da* heterozygous mothers (class 1 females) was only 64% of that for their homozygous $sis\text{-}a^+$ sisters (class 2 females).

Strictly speaking, cross B does not establish that the interaction involves a maternal, rather than zygotic, effect of *da*, since relative female viability for the combination was above 50%. That point is established, however, by the data from crosses C and D. The dominant female-lethal interaction among all three mutant genes was much more extreme than that between any pair of mutant genes, reducing relative female viability of the combination to 1% or less. Two "triple-dominant effect" crosses are shown in Table 3. They differ with respect to the parental origin of the wild-type alleles in the progeny. The female-lethal dominant interactions here, and in a variety of other crosses not shown, were consistently more severe when Sxl^+ and/or $sis\text{-}a^+$ alleles were paternally rather than maternally derived. This is reminiscent of an aspect of the dominant interaction between *da* and *Sxl* reported earlier, a situation in which an $Sxl^{f\#1}$ maternal effect *per se* seemed to be ruled out as an explanation for the difference between maternally and paternally derived Sxl^+ alleles

TABLE 3

Dominant female-lethal synergism of *sis-a* with *da* and Sxl^{fm1}

Cross	Nature of female-lethal interaction	Aspects of cross relevant to lethal interaction	Progeny from crosses[a]					
			Females			Males		
			Class 1 genotype and number	Class 2 genotype and number	Viability of class 1 relative to class 2 (%)	Class 1 genotype and number	Class 2 genotype and number	Viability of class 1 relative to class 2 (%)
A	Double-dominant lethal synergism	Mothers da^+; daughters *sis-a*/+ and Sxl^{fm1}/+	$\frac{+\ sis\text{-}a}{Sxl^{fm1}\ +}$ 122	$\frac{+\ +}{Sxl^{fm1}+}$ 684	18	$\frac{sis\text{-}a}{Y}$ 678	$\frac{+}{Y}$ 634	107
B	Double-dominant lethal synergism	Mothers *da*/+; daughters *sis-a*/+ (and Sxl^+)	$\frac{sis\text{-}a}{+}$ 308	$\frac{+}{+}$ 480	64	$\frac{sis\text{-}a}{Y}$ 474	$\frac{+}{Y}$ 530	89
C	Triple-dominant lethal synergism	Mothers *da*/+; daughters *sis-a*/+ and $+/Sxl^{fm1}$ [$sis\text{-}a^+$ patroclinous; Sxl^+ matroclinous]	$\frac{+\ sis\text{-}a}{Sxl^{fm1}\ +}$ 0	$\frac{+\ +}{Sxl^{fm1}\ +}$ 789	<0.1	$\frac{sis\text{-}a}{Y}$ 1161	$\frac{+}{Y}$ 1062	109
D	Triple-dominant lethal synergism	Mothers *da*/+; daughters +/*sis-a* and $+/Sxl^{fm1}$ [Sxl^+ and $sis\text{-}a^+$ matroclinous]	$\frac{+\ +}{Sxl^{fm1}sis\text{-}a}$ 11	(see males class 2)	1		$\frac{+}{Y}$ 1064	

[a] Only progeny that are not recombinant for the *v-m* region that includes *sis-a* are included in this table. In the absence of lethal effects, the expected progeny ratios are 1:1:1:1 (A–C) or 1:1 (D). Full genotypes of crosses at 25°: Cross A—Sxl^+v *sis-a m g*/+ + + + +; *In(2LR)O*, $dp^{lvl}Cy$ *pr* cn^2/+ ♀♀ × ♂♂ *cm* $Sxl^{fm1}ct^6v$ $sis\text{-}a^+m$ *g*/*Y*. Cross B—Sxl^+v *sis-a m g*/+ + + + +; *cl da cn bw*/+ ♀♀ × ♂♂ *v* $sis\text{-}a^+m$ *g*/*Y*. Cross C—same mothers as cross B × ♂♂ *cm* $Sxl^{fm1}ct^6v$ $sis\text{-}a^+m$/*Y* (note: cross B and C mothers were sisters of mothers in cross A). Cross D—*cl da cn bw*/*In(2L+2R),Cy* cn^2 ♀♀ × ♂♂ *cm* $Sxl^{fm1}ct^6$ *sis-a*/*Y*.

(CLINE 1980, p. 922). Results presented in a subsequent section will argue against a maternal effect for *sis-a* being responsible for the maternal/paternal difference here as well.

It is important to establish that this dominant female-lethal synergism does indeed reflect an aspect of the *sis-a* phenotype, rather than some extraneous factor. This presents no difficulty in view of the strength of the lethal synergism with the cross oriented as in C, an arrangement that allowed no escapers. Data presented in the lower half of Table 2 show that the dominant female-specific lethality of *sis-a* for progeny also heterozygous for $Sxl^{f\#1}$ and from mothers heterozygous for *da* maps to the same point as that for the recessive lethal effect of *sis-a*.

A comparison between the behavior of the *sis-a* point mutation and that of a deficiency for the *sis-a* locus [*Df(1)N71*] with respect to the dominant synergistic interaction with $Sxl^{f\#1}$ established that this synergistic interaction is simply a consequence of a decrease in zygotic $sis\text{-}a^+$ activity, rather than some more complicated peculiarity of the mutant allele. At 18°, the viability of $Df(1)N71$, $Sxl^+sis\text{-}a^-/Sxl^{f\#1}sis\text{-}a^+$ females was only 2.8% relative to their $sis\text{-}a^+$ sisters. At the same temperature, the relative viability of $Sxl^+sis\text{-}a/Sxl^{f\#1}sis\text{-}a^+$ females was 84%—considerably above the 18% viability figure for this same genotype at 25° (*cf.* Table 3, cross A). Combined with the information mentioned earlier regarding the greater viability of homozygous *vs.* hemizygous *sis-a* females, this result establishes that the *sis-a* point mutant is a partial (hypomorphic), rather than complete (amorphic), loss-of-function allele and adds to the evidence that the phenotype for *sis-a* is moderately heat-sensitive.

Suppression of *sis-a* female-specific lethality by mutant *Sxl* alleles: The synergistic female-lethal interactions described above indicated that *sis-a* functions are intimately related to those of *Sxl* and *da*. The data in Table 4 extend this conclusion to establish that *sis-a*, like *da*, acts upstream of *Sxl* as a positive regulator. The key element in this analysis is $Sxl^{M\#1}$, a gain-of-function allele that expresses Sxl^+ female-specific functions even in the absence of factors that would normally be required for such expression.

$Sxl^{M\#1}$ was isolated as a suppressor of the female-lethal maternal effect of *da*, yet the first cross in Table 4 shows that it is equally effective in suppressing the recessive, female-specific zygotic lethal effect of *sis-a*. The second cross in this table shows that $Sxl^{M\#1}$ rescues *sis-a* homozygous females even under what would be expected to be particularly deleterious conditions for females in view of the synergism among *sis-a*, *da* and $Sxl^{f\#1}$ described above. In this case, the *sis-a* homozygotes were heterozygous for the female-lethal allele $Sxl^{f\#1}$ and were the progeny of *da/da* mothers. Even without the female-lethal *sis-a* mutation, such daughters would never survive without $Sxl^{M\#1}$. The additional burden of *da* and *Sxl* mutations does reduce the effectiveness of $Sxl^{M\#1}$ somewhat; nevertheless, the suppressor allele still manages to rescue nearly one-half of the females. Although $Sxl^{M\#1}$ counteracts the female lethality of *sis-a*, *sis-a* does not counteract the male lethality of $Sxl^{M\#1}$ (data not shown). This is consistent with expectations.

The third cross shown in Table 4 deals with the interaction between *sis-a*

TABLE 4

Suppression of *sis-a* female-specific lethality by $Sxl^{M\#1}$ and $Sxl^{fm\#7,M\#1}$

Cross	Mothers' genotype with respect to *da*	Progeny genotypes and no. recovered[a]			Viability of $Sxl^{M\#1}$ (*sis-a/sis-a*) daughters relative to reference sons (%)
		Females		Males	
		sis-a/sis-a$^+$ (controls)	*sis-a/sis-a* (experimentals)	Siblings used as viability reference	
A	*da*$^+$	$\frac{Sxl^+ sis\text{-}a^+}{Sxl^+ sis\text{-}a}$	$\frac{Sxl^{M\#1} sis\text{-}a}{Sxl^+ sis\text{-}a}$	$\frac{Sxl^+ sis\text{-}a^+}{Y}$	
		301	263	252	104
B	*da/da*	$\frac{Sxl^+ sis\text{-}a^+}{Sxl^{f\#1} sis\text{-}a}$	$\frac{Sxl^{M\#1} sis\text{-}a}{Sxl^{f\#1} sis\text{-}a}$	$\frac{Sxl^+ sis\text{-}a^+}{Y}$	
		0	233	504	46
C	*da*$^+$	$\frac{Sxl^+ \quad sis a^+}{Sxl^{fm\#7,M\#1} sis\text{-}a}$	$\frac{Sxl^{fm\#7,M\#1} sis\text{-}a}{Sxl^{fm\#7,M\#1} sis\text{-}a}$; $Dp(1;3)Sxl^+/+$	$\frac{Sxl^{fm\#7,M\#1} sis\text{-}a}{Y}$	
		196[b]	83 (65% are sterile)	183	45

[a] In the absence of lethal effects, equal numbers of all classes of progeny listed are expected within each cross. Full genotypes of crosses at 25°: Cross A—*w*^{c}*cm* $Sxl^{M\#1}$*v sis-a m g/Binsinscy*; +/*CyO* ♀♀ × ♂♂ *v sis-a m g/Y*. Cross B—*w*^{c}*cm* $Sxl^{M\#1}$*v sis-a m g/Binsinscy*; *cl da cn bw* ♀♀ × ♂♂ *cm* $Sxl^{f\#1}ct^6$ *sis-a/Y*. *Cross C—cm* $Sxl^{fm\#7,M\#1}ct^6$*v sis-a m g/Binsinscy* ♀♀ × ♂♂ *cm* $Sxl^{fm\#7,M\#1}ct^6$*v sis-a m g/Y* $Dp(1;3)sn^{15a1}$,$Sxl^+/+$. *Binsinscy* is the male-viable, female-sterile balancer: $In(1)sc^{s1L}sc^{8R}$+ *dl-49, y w* $Sxl^+sn^{x2}B$ *sis-a*$^+$.

[b] Females with and without $Dp(1;3)Sxl^+$ could not be distinguished; this number is 50% of the combined total of *sis-a*$^+$ females recovered, since equal numbers of both classes are expected.

and a male-viable, double-mutant allele, $Sxl^{fm\#7,M\#1}$, which has much reduced levels of many of the Sxl^+ activities normally expressed by $Sxl^{M\#1}$. The interaction of this particular mutant allele with *sis-a* was of special interest because of the unusual nature of its interaction with the *da* maternal effect. This *Sxl* allele thus provided a particularly stringent test of the hypothesis that the zygotic effect of the *sis-a* mutation disrupted the same aspect of Sxl^+ functioning as the maternal effect of the *da* mutation.

It had been shown previously that the $Sxl^{fm\#7,M\#1}$ allele could induce the expression of the female-specific functions of an Sxl^+ allele in *trans* among the progeny of *da/da* mothers; however, this mutant allele seemed unable to induce such activity in all somatic tissues (CLINE 1984). This was proposed to be the basis for the fact that daughters that were rescued by $Sxl^{fm\#7,M\#1}$ appeared normal externally, but were invariably sterile because they lacked ovaries. The effectiveness of rescue of daughters by this allele in compound with Sxl^+ was profoundly sensitive to its dose; one dose of $Sxl^{fm\#7,M\#1}$ rescued only 1% of the daughters, and two doses rescued 49%.

Table 4 shows that the interaction between Sxl^+ and $Sxl^{fm\#7,M\#1}$ in zygotes that are homozygous for *sis-a* is strikingly similar to that between these alleles

among the progeny of *da/da* mutant mothers: Two doses of $Sxl^{fm\#7,M\#1}$ in the presence of one dose of Sxl^{+} rescued 45% of *sis-a/sis-a* females. One dose of $Sxl^{fm\#7,M\#1}$ was much less effective—only 0.2% of the *sis-a* mutant females were rescued (data not shown). Even more significant, however, is the fact that 65% of the females rescued by two doses of the mutant *Sxl* allele were sterile with a phenotype indistinguishable from that reported earlier for the rescued progeny of *da/da* mothers. An additional 31% had only one ovary, rather than the normal two. Thus, with respect to this allele-specific interaction with $Sxl^{fm\#7,M\#1}$, the female-lethal maternal effect of *da* and the female-lethal zygotic effect of *sis-a* are extremely similar. The sterility interaction between $Sxl^{fm\#7,M\#1}$ and *sis-a* is, however, somewhat leakier than the interaction with *da* at 25°.

Table 5 presents additional evidence of the remarkable similarity between the female-lethal effects of *da* and *sis-a*. It had been shown that mutations in the maternally acting positive regulator, *da*, are counteracted to a modest extent by increases in the dose of wild-type alleles of the regulated target gene, Sxl^{+}, in the zygote (CLINE 1978). Table 5 shows that Sxl^{+} duplications have a similar effect in combination with mutations in the zygotically acting positive regulator, *sis-a*. Although *sis-a* homozygous females with the normal two doses of Sxl^{+} would not have survived under these conditions, 2% of such mutant females with three Sxl^{+} doses did survive. Viability of *sis-a* mutant females increased to 11% when the number of Sxl^{+} doses was raised to four. Thus, for *sis-a* as well as *da*, increasing the number of Sxl^{+} doses weakly mimics the effect of the gain-of-function allele, $Sxl^{M\#1}$. At 25°, an extra dose of Sxl^{+} is far more effective at rescuing females from *sis-a* than from the *da* maternal effect, suggesting that the effect of *sis-a* on *Sxl* expression is less severe than that of the *da* maternal effect at this temperature. A similar conclusion was suggested in the $Sxl^{fm\#7,M\#1}$ sterility interaction described in the previous paragraph.

***Sxl* allele specificity in dominant female-lethal synergism with *sis-a*:** The dominant synergism presented in Table 3 for *sis-a* and *da* in combination with the null allele $Sxl^{f\#1}$ suggested yet another test of the hypothesis that the maternal effect of *da* and the zygotic effect of *sis-a* disrupt precisely the same aspect of Sxl^{+} functioning. How would the effects of various partial-loss-of-function *Sxl* alleles compare with that of $Sxl^{f\#1}$ in such dominant interactions? It had been shown previously that the dominant interactions between *da* and such hypomorphic *Sxl* alleles were not always those that might be predicted based on the extent to which these mutations appeared to disrupt female-pathway expression functions of *Sxl*; this apparent paradox was resolved by the proposition that the interaction between *da* and *Sxl* involves only the sexual pathway initiation step, not the subsequent steps of sexual pathway maintenance and/or expression (MAINE *et al.* 1986). Thus, an allele might be defective with respect to late functions, but be wild type with respect to the early steps and, thus, wild type with respect to its dominant interaction with the *da* maternal effect (and vice versa).

Would the same pattern of severity hold for the interaction of hypomorphic *Sxl* alleles with *sis-a* as for *da*? Table 6 explores this question with respect to

TABLE 5

Extra doses of *Sxl*$^+$ suppress *sis-a* female-specific recessive lethality

	Female progeny recovered[a]						
	sis-a$^+$/*sis-a* controls			*sis-a/sis-a* experimentals			
Cross	Genotype	No.	Doses *Sxl*$^+$	Genotype	No.	Doses *Sxl*$^+$	Viability relative to controls (%)
A	$\frac{Dp(1;1),Sxl^+Sxl^+sis\text{-}a}{Sxl^+ \quad sis\text{-}a^+}$	743	three	$\frac{Dp(1;1),Sxl^+Sxl^+sis\text{-}a}{Sxl^+ \quad sis\text{-}a}$	14	three	2
B	$\frac{Dp(1;1),Sxl^+Sxl^+sis\text{-}a}{Sxl^+ \quad sis\text{-}a^+}$	2718	three	$\frac{Dp(1;1),Sxl^+Sxl^+sis\text{-}a}{Dp(1;1),Sxl^+Sxl^+sis\text{-}a}$	305	four	11

[a] In the absence of lethal effects, equal numbrs of the two classes of daughters are expected for each cross. Full genotypes of crosses at 25°: Cross A—*y sis-a/Binsinscy* ♀♀ × ♂♂ *Dp(1;1)jnR1-A, cm Sxl*$^+$, *Sxl*$^+$ *v sis-a m g f.* Cross B—*Dp(1;1)jnR1-A, cm Sxl*$^+$,*Sxl*$^+$ *v sis-a m g f/Binsinscy* ♀♀ × ♂♂ *Dp(1;1)jnR1-A, cm Sxl*$^+$,*Sxl*$^+$*v sis-a m g f/Y.*

TABLE 6

Sxl allele-specific viability interactions with _sis-a_ mimic those with _da_

Nature of female-lethal interaction	Aspect of cross relevant to lethal interaction	Cross[a]	Sxl^{fX} allele interacting	Progeny recovered: Controls Genotype	Controls No.	Experimentals Genotype	Experimentals No.	Viability relative to controls (%)
Triple dominant lethal synergism among *da*, *sis-a* and Sxl^{fX}	Mothers *da*/+; daughters *sis-a*/+ and Sxl^{fX}/+	A	Sxl^{+}	*sis-a*/+	188	*sis-a*/*sis-a*	70	37
		B	Sxl^{fLS}	*sis-a*/+	309	*sis-a*/*sis-a*	113	37
		C	Sxl^{fhvm1}	*sis-a*/+	382	*sis-a*/*sis-a*	65	17
		D	Sxl^{f9}	*sis-a*/+	141	*sis-a*/*sis-a*	0	<1
Dominant lethal effect of hypomorphic *Sxl* alleles among progeny of *da*/*da* mothers at semipermissive temperature	Mothers *da*/*da*; daughters Sxl^{fX}/+	E	Sxl^{+}	Sxl^{+}/*Y*	677	Sxl^{+}/+	126	19
		F	Sxl^{fLS}	Sxl^{+}/*Y*	135	Sxl^{fLS}/+	34	25
		G	Sxl^{fhvm1}	Sxl^{+}/*Y*	2247	Sxl^{fhvm1}/+	99	4
		H	Sxl^{f9}	Sxl^{+}/*Y*	298	Sxl^{f9}/+	0	<0.3

[a] Full description of crosses at 25°: Crosses A–D, respectively—*y sis-a/Binsinscy; cl da cn bw/CyO* ♀♀ × ♂♂ Sxl^{+} *v m g f/Y* OR *cm* $Sxl^{fhvm1}ct^{6}$/*Y* OR Sxl^{f9}/*Y* OR *y* Sxl^{fLS} *oc v f/Y*. Crosses E–H, respectively, at 17°—*da b pr mle* (*cl da cn bw* for G) ♀♀ × ♂♂, as in crosses A–D above. For crosses E–H, progeny were scored only from mothers who had been at 17° for at least 6 days; progeny were shifted to 25° after at least 48 hr at the lower temperature (simply to speed up their development). Data for cross G is from CLINE (1980).

three very different well-characterized hypomorphic alleles. $Sxl^{fhv\#1}$ is fully viable and fertile when homozygous, but is female lethal when hemizygous (CLINE 1980). Sxl^{f9} and Sxl^{fLS} are both homozygous female lethal, but Sxl^{f9} appears to be defective only in the sexual pathway initiation step of *Sxl* functioning, whereas Sxl^{fLS} appears to be wild type in those functions, but defective in the later functions of sexual pathway maintenance and/or expression (MAINE *et al.* 1986). $Sxl^{fhv\#1}$ and Sxl^{f9} complement most other partial loss-of-function *Sxl* alleles. The bottom half of this table shows how these three alleles differ with respect to their interaction with *da*. The parameter measured is a dominant effect on the ability of daughters to survive from *da/da* mothers under semipermissive conditions of temperature. Such conditions allowed 19% of Sxl^+/Sxl^+ daughters of *da/da* mothers to survive. The dominant effect of the homozygous viable allele, $Sxl^{fhv\#1}$, reduced daughter viability to 4%. In contrast, the homozygous lethal allele, Sxl^{fLS}, had no dominant effect on daughter viability. The recessive lethal allele, Sxl^{f9}, was the worst of the group; its dominant lethal effect under these conditions killed all daughters.

Data in the top half of Table 6 show that the same pattern of severity holds in the (triple) dominant synergistic interaction of these alleles with *sis-a*: Sxl^{fLS} had no dominant deleterious effect in its interaction with *sis-a* and *da*, whereas the viable allele, $Sxl^{fhv\#1}$, had a significant effect, but one that was much less severe than that of Sxl^{f9}.

Effects of *sis-a* on sexual differentiation: Experiments discussed so far show that $sis\text{-}a^+$ regulates vital functions of Sxl^+, presumably related to dosage compensation, but they do not indicate whether $sis\text{-}a^+$ regulates the nonvital functions of Sxl^+ that are involved in sexual differentiation. Experience with mutations in *da* and *Sxl* has shown that effects on female sexual differentiation often appear to be masked by simultaneous effects on cell growth and organism survival. A likely explanation for this observation is that female cells with sufficiently high levels of *Sxl* vital product functions to grow and differentiate will generally also have sufficiently high levels of *Sxl*'s sexual differentiation functions to generate a normal female phenotype. Three approaches have been used to overcome this complication so that effects on sexual differentiation in females can be monitored.

One approach takes advantage of specific gene interactions that involve elements downstream of *Sxl* that are known to be required in males in order for them to hyperactivate many dosage-compensated *X*-linked loci. By themselves, male-specific lethal mutations in these "hyperactivation" genes have no effect on female sexual phenotype (BELOTE 1983); however, in combination with female-lethal mutations that affect the functioning of *Sxl*, these same mutations can musculinize females (SKRIPSKY and LUCCHESI 1980, 1982; UENOYAMA *et al.* 1982). Analysis of this interaction between male-specific and female-specific lethal mutations at the level of individual cells suggested that these male-specific lethals do not directly masculinize chromosomally female cells. Instead, they appear to rescue diplo-*X* cells that have failed to activate the female-specific functions of Sxl^+ and, thus, have chosen the inappropriate male developmental pathway as a consequence of the female-lethal mutations (see

TABLE 7

Dominant effects of *sis-a* on female foreleg sexual phenotype in genotypes mutant for *da* and *mle*

mle/mle daughters of *da*/+ mothers[a]					
sis-a/+ experimental females (from cross A)			*sis-a*$^+$/+ control female sibs (from cross B)		
No. recovered	Relative viability (%)[b]	Individuals with sexcomb teeth	No. recovered	Relative viability (%)[b]	Individuals with sexcomb teeth
227	84	52% of population	223	116	0% of population

[a] Full genotypes of crosses at 25°: Cross A—*f; msl-2 msl-1 da b mle/CyO; e* ♀♀ × ♂♂ *sis-a/Y; mle/CyO*. Cross B—same mothers as in cross A × ♂♂ *y w spl/Y^{Bs}; mle/SM1,Cy*.

[b] Viability reference in all cases was the *f Cy* male siblings (not shown).

CLINE 1984). If wild type for these hyperactivation genes, such diplo-*X* cells would suffer a genetic imbalance caused by an inappropriate level of dosage compensation. As a consequence, they might not therefore reach the (adult) sexually dimorphic stage, being unable to compete with neighboring cells that had regulated *Sxl*$^+$ properly. By reducing the genetic imbalance suffered by diplo-*X* cells that had improperly regulated *Sxl*$^+$, mutations in downstream genes required for some aspects of dosage-compensated gene hyperactivation would be expected to reduce their competitive disadvantage.

A second approach has been to investigate the effects of mutations in vital sex-determination genes on the sexual phenotype of triploid intersexes, *XX AAA* animals for which the *X*:*A* balance signal has a sexually ambiguous value of 0.67. Such animals generally develop as mosaics of phenotypically male and female cells in proportions that are sensitive to factors that affect *Sxl* functioning. The degree of interspersion of phenotypically male and female cells suggests that sexual ambiguity only exists for such cells quite early in development, after which point the cells become committed to either the male or the female pathway. The extent of genetic imbalance caused by inappropriate dosage compensation levels is expected to be less in the triploid intersex situation than in the normal diploid situation (see CLINE 1983a).

A third approach involves the use of genetic mosaics, generated either by early somatic loss of an unstable *X*-chromosome or by radiation-induced somatic recombination that produces clones of homozygous mutant cells in an otherwise heterozygous animal (CLINE 1979a,b; SANCHEZ and NÖTHIGER 1982). By reducing the fraction of the developing organism that is adversely affected, and/or by reducing the period during development when cells lack vital products, abnormal tissue can survive to differentiate adult structures and thereby express its sexual phenotype.

The first approach, one that involves interactions with male-specific lethal mutations, is illustrated in Table 7 for *sis-a*. Sexual phenotype was monitored in the dimorphic foreleg, the region for which such analysis is most straightforward. Here sexcomb bristles are unambiguously diagnostic of male differentiation. Homozygous *sis-a*$^+$ daughters of heterozygous *da* mothers exhibited no masculine differentiation when homozygous for *mle* (one of the male-specific

TABLE 8

Dominant masculinizing effect of *sis-a* on the phenotype of triploid intersexes (*XX AAA*)

Genotype with respect to *sis-a*	No. of adults recovered[a]			Sexual phenotype of *XX AAA* adults	
	Live	Dead pharates	Total	% of dimorphic structures with some male differentiation[b]	% of dimorphic structures with only male differentiation[c]
sis-a/+; *AAA* (experimentals)	100	38	138	99.9	96.4
+/+; *AAA* (control sibs)	37	50	87	46	13

[a] Progeny from following cross at 25°: *y sis-a/Binsinscy, y w sn sis-a*$^+$ *B* ♀♀ × ♂♂ *y*^{2}; *C(2L)RM,dp; C(2R)RM,px; C(3L)RM,h*^{2}*rs*^{2}; *C(3R),+*. In the absence of viability effects, equal numbers of the two classes of siblings are expected.

[b] Scoring as described in CLINE (1983a) includes morphology of hemitergite 7, hemisternites 6 and 7, analia, external genitalia and foreleg.

[c] Same as for footnote *b*, but (in order to avoid ambiguity) does not include foreleg.

lethal mutations). In contrast, when the daughters were heterozygous for *sis-a*, more than one-half exhibited some male development in their forelegs. As in all previously reported cases of such masculinizing interactions, the intersexual phenotypes generated were of the mosaic type: sexually intermediate cells were never observed (although such intermediates can be generated by other methods). The size of the male tissue patches and the degree of interspersion of male and female tissue was similar to that observed in previous studies of *da* and *Sxl*, indicating a similar (early) period of sexual ambiguity. From the experiment in Table 7, one can conclude that *sis-a* does indeed affect the sexual differentiation functions of *Sxl*. It is worth mentioning that the male-specific lethals do *not* rescue females from the recessive female-specific lethal effects of *sis-a* at the level of the whole organism—yet another similarity between the female-lethal effect of *sis-a* and those of *da* and *Sxl*$^{f\#1}$ (data not shown).

Table 8 illustrates the triploid intersex approach for ascertaining the role of *sis-a* in determining sexual phenotype. The results of this alternative approach are consistent with those in Table 7. Decreasing the dose of *sis-a*$^+$ alleles from two to one in animals with an *X:A* balance of 0.67 caused a profound shift in the proportion of female and male tissues. With respect to the fraction of structures scored that showed only male development, the proportion shifted from 13% male in the *sis-a*$^+$/*sis-a*$^+$ controls to 96.4% for their *sis-a*/*sis-a*$^+$ experimental sibs. Thus, with respect to its effect on triploid intersex phenotype, loss-of-function mutations at *sis-a* show a dominant masculinizing effect that resembles the effect for *da* and *Sxl*$^{f\#1}$. It is important to note that the effect of *sis-a* on triploid intersex phenotype was strictly zygotic, unlike the effect of *da*, which is maternally determined.

An example of the third approach is shown in Table 9. In this experiment, genetic mosaics for *sis-a* were generated by somatic recombination. Clones of homozygous mutant *sis-a* tissue were founded in a *sis-a*/+ background during

TABLE 9

Differentiation of *sis-a/sis-a* somatic clones generated by mitotic recombination in a *sis-a/+* background by irradiation during early larval stages

		Areas of adult exterior examined for clones									
		Foreleg sexcomb region			Analia			External genitalia			
Class of female irradiated[a]	Genotype of clone	No. of clones	No. of structures scored	Sexual phenotype of clones	No. of clones	No. of structures scored	Sexual phenotype of clones	No. of clones	No. of structures scored	Sexual phenotype of clones	Average clone frequency per structure (%)
A (experimentals)	*y sis-a*	26	266	Female	11	125	Female	10	125	Female	9.1
B (controls)	*y*	21	296	Female	11	135	Female	17	135	Female	8.7
C (controls)	*y* $Sxl^{fm\#7,M\#1}$	20	264	Male	16	132	Male	22	132	Male (all but one)	11

[a] Class A—+ + *M(1)o/y sis-a* +; class B—+ *M(1)o/y* +; and class C—+ + *M(1)o/y* $Sxl^{fm\#7,M\#1}$ + individuals, respectively, were exposed to approximately 1500 rad of gamma radiation 48–72 hr (@25°) after eggs were laid (late first to mid second instar larval stage). Surviving adults were dissected, mounted and scored under a compound microscope for the presence of phenotypically yellow clones in the structures indicated.

the early larval stages of development in the anlagen for the imaginal foreleg and terminalia. The experimental design employed *M(1)o* to maximize the size of the clones induced at this developmental stage (MORATA and RIPOLL 1975). In contrast to the situations illustrated by Tables 7 and 8, in this experiment there was no masculinizing effect of *sis-a*. Control clones generated in two crosses run in parallel with the *sis-a* experimentals established that the apparent lack of effect of *sis-a* on chromosomally female tissue's sexual phenotype was not due to some effect of the mutation on cell viability or to a scoring artifact: The frequency of *sis-a* mutant clones generated was no lower than that of *sis-a*$^+$ female clones or of clones masculinized by the sex-transforming mutation $Sxl^{fm\#7,M\#1}$.

There is a fundamental difference between the experiments illustrated in Tables 7 and 8 and that in Table 9 with respect to the time of action of *sis-a*$^+$ that was assayed. The change in cell genotype that was induced by somatic recombination in the experiment of Table 9 took place after the embryonic and early larval stages. Thus, if *sis-a*$^+$ affects sexual development only by acting on *Sxl*$^+$ early in its sexual pathway initiation functions, and not later in its sexual pathway maintenance and/or expression functions, one would not expect the sexual phenotype of the chromosomally female clones to be affected by the mitotic recombination event. In contrast, the phenotype of the animals in Tables 7 and 8 is expected to be dependent on interactions with *Sxl* that occur early in development.

Tests for recessive maternal effects of *sis-a* reveal none: With *da*, it is the maternal genotype that is important in zygotic *Sxl* regulation, whereas in the characterization of *sis-a* reported so far, only the zygotic genotype has been important. On the other hand, the experiments described above could not have revealed a low-magnitude maternal contribution to the *sis-a*$^+$ activity in the zygote, one too small to show up as a dominant maternal effect by the mutant allele. A more sensitive assay for a maternal effect would involve a comparison between the progeny of mothers that are homozygous for *sis-a* and the progeny of mothers that carry *sis-a*$^+$ alleles; however, the female-specific recessive lethality of *sis-a* obviously complicates the task of studying progeny from *sis-a/sis-a* mothers. Two approaches to surmount this complication are presented in Table 10. One employs $Sxl^{M\#1}$, and the other employs germline mitotic recombination.

$Sxl^{M\#1}$ rescues *sis-a/sis-a* females. The presence of $Sxl^{M\#1}$ in the germline of homozygous *sis-a* females is not expected to interfere with the analysis of *sis-a* maternal effects, because $Sxl^{M\#1}$ appears to have no maternal effect of its own and seems not to be constitutive in the germline (CLINE 1978, 1980, 1983b). The results of cross A in Table 10 show that *sis-a*/+ (*Sxl*$^+$) daughters of *sis-a/sis-a* mothers rescued by $Sxl^{M\#1}$ are fully viable; thus, this cross gives no indication of any female-lethal recessive maternal effect for *sis-a*. The design of cross B in Table 10 takes advantage of dominant female-lethal synergism between loss-of-function mutations at *Sxl* and *sis-a* to establish a more sensitive assay that would be expected to reveal even a very weak recessive maternal effect of *sis-a*. The viability of daughters that were simultaneously heterozygous

TABLE 10

***sis-a* has no female-lethal maternal effect, even when homozygous**

Cross[a]	Maternal genotype with respect to *sis-a*	Progeny recovered (only relevant classes shown)				
		Class 1 daughters (experimentals)			Class 2 siblings (used as viability reference)	
		Genotype	No.	Relative viability (%)	Genotype	No.
A	*sis-a/sis-a* germline and soma (mothers with $Sxl^{M\#1}$)	$\frac{Sxl^{+}sis\text{-}a}{Sxl^{+}sis\text{-}a^{+}}$	463	109	$\frac{Sxl^{M\#1}sis\text{-}a}{Sxl^{+}\ sis\text{-}a^{+}}$	425
B	*sis-a/sis-a* germline and soma (mothers with $Sxl^{M\#1}$)	$\frac{Sxl^{f\#1}sis\text{-}a}{Sxl^{+}\ sis\text{-}a^{+}}$	244	38	$\frac{Sxl^{M\#1}sis\text{-}a}{Sxl^{+}\ sis\text{-}a^{+}}$	643
C	*sis-a/sis-a* recombinant germ cells in a *sis-a*/+ soma	$\frac{Sxl^{+}sis\text{-}a}{Sxl^{+}sis\text{-}a^{+}}$	160	83	$\frac{Sxl^{+}sis\text{-}a}{Y}$	192
D	*sis-a/sis-a* recombinant germ cells in a *sis-a*/+ soma	$\frac{Sxl^{+}sis\text{-}a}{Sxl^{f\#1}\ sis\text{-}a^{+}}$	10	20	$\frac{Sxl^{+}sis\text{-}a}{Y}$	50

[a] Full genotypes of crosses at 25°: Cross A—w^{c}-*cm* $Sxl^{M\#1}v$ *sis-a m g/y sis-a; CyO/+* ♀♀ × ♂♂ *cm* ct^{6}/Y (genotype of progeny with respect to *Sxl* inferred from closely linked *cm* marker). Cross B—w^{c}-*cm* $Sxl^{M\#1}v$ *sis-a m g/cm* $Sxl^{f\#1}ct^{6}$*sis-a; CyO/+* ♀♀ × ♂♂ *cm* ct^{6}/Y (genotype of progeny with respect to *Sxl* inferred from closely linked ct^{6} marker allele). Cross C—+ $ovo^{D1}v$ +/*y* + + *sis-a* ♀♀ (exposed to 1200 rad gamma rays as 48- to 72-hr larvae) × ♂♂ *y cm* $ct^{6}sn$ *v/Y*. Progeny arising from mitotic recombination centromere distal to *sis-a* are excluded (nine *y v* animals). Cross D—same as for cross C, except for males *y z* $Sxl^{f\#1}sn$ *f/Y*.

for $Sxl^{f\#1}$ and *sis-a* and that were progeny from *sis-a/sis-a* mothers was 38%. This value for viability is actually higher than that (18%) presented in Table 3, cross A, for females simultaneously heterozygous for $Sxl^{f\#1}$ and *sis-a* but daughters of $sis\text{-}a^{+}$ mothers instead. One might be tempted to interpret the difference between the 38% and 18% viability figures as evidence for an ameliorating maternal effect of $Sxl^{M\#1}$, one too weak to have shown up in previous tests; however, differences of this magnitude are well within the range of values that one observes for double-dominant synergism between *sis-a* and $Sxl^{f\#1}$ in different wild-type genetic backgrounds (data not shown).

Crosses C and D avoid any complication by $Sxl^{M\#1}$ in the assessment of possible *sis-a* maternal effects. On the other hand, their design limits them to detecting maternal effects that are germline autonomous and relatively late-acting in the germline (*da* satisfies both criteria). In these crosses, homozygous *sis-a* germ cells were generated by mitotic recombination in heterozygous *sis-a* larvae which, in the absence of such germline mitotic recombination, are sterile due to ovo^{D} (see BUSSON *et al.* 1983). The results of cross C show that *sis-a/sis-a* germ cells generated in this way are viable and produce fully viable *sis-a*/+ daughters. Cross D shows that the products of such homozygous *sis-a* germ cells can even support the development of daughters that are doubly heterozygous for $Sxl^{f\#1}$ and *sis-a*. The viability of such heterozygous daughters (20%) is not significantly different from that (18%) for the same genotype of daughters from heterozygous *sis-a* germ cells (Table 3, cross A). In summary, in none of these four crosses was there any indication of a maternal effect for *sis-a*.

DISCUSSION

A new *X*-linked hypomorphic female-specific lethal mutation, *sisterless-a* (*sis-a*) has been characterized that identifies a second positive regulator of the Drosophila sex determination switch gene, *Sex-lethal* (*Sxl*). *Sis-a* is located at map position 34.3, at or very near chromomere 10B4. It is less than 0.01 cM from a gene that is vital for both sexes, named "locus 14" by GEER, LISCHWE and MURPHY (1983).

A positive regulatory role of *sis-a* upstream of Sxl^+ was established by studies of the phenotypic interactions between the mutant *sis-a* allele and two opposite types of sex-specific mutant *Sxl* alleles. Loss-of-function *sis-a* and *Sxl* mutant alleles individually are recessive in their female-specific lethal effects; however, if they occur together in the same female, they are semidominant. More significant than this dominant synergism, however, is the observation that the female-specific lethality of *sis-a* is suppressed by the gain-of-function dominant male-specific lethal allele $Sxl^{M\#1}$. $Sxl^{M\#1}$ expresses female-specific Sxl^+ functions even in the absence of the signals that are normally required for female development (CLINE 1979a, 1983a, 1984).

$Sxl^{M\#1}$ was isolated not as a suppressor of *sis-a*, but rather as a suppressor of a mutation at *daughterless*, an autosomal locus shown previously to act upstream of *Sxl* as a positive regulator. Suppression by $Sxl^{M\#1}$ is just one of many similarities between *sis-a* and *da* presented here. Loss-of-function mutations in *da*, like those in *sis-a*, are recessive in their female-lethal effects only so long as females have a wild-type dose of Sxl^+ alleles. Moreover, the combined dominant effect of mutations at all three loci is even more deleterious to females. Defects at *sis-a*, like the *da* maternal effect (under nonpermissive conditions), cause embryonic lethality, the result expected considering that $Sxl^{f\#1}$ (a null allele) is an embryonic lethal for females. Both *sis-a* and *da* may only affect Sxl^+ functioning in somatic tissues; however, this conclusion must remain tentative pending the isolation and characterization of null alleles of both genes.

The effect of growth temperature on female viability is yet another similarity between *da* and *sis-a*. A large fraction of female offspring can survive the *da* maternal effect at low temperatures (CLINE 1976). *Sis-a/sis-a* escaper females are also recovered only at lower temperatures, although their viability even at the most permissive temperatures is never very high. Comparisons between the *da* hypomorphic allele and a da^- deficiency with respect to temperature effects (see CLINE 1980) suggest that the early positive regulation of Sxl^+ in females may be inherently heat-sensitive. This could be responsible for the weakly heat-sensitive behavior of *sis-a* and could be a contributing factor in the much more strongly heat-sensitive maternal effect of *da*.

On the other hand, there is an important difference between *sis-a* and *da* with respect to when the gene is expressed to control Sxl^+ activity. For da^+, it appears to be only the expression of the gene during oogenesis that is required subsequently for the expression of the female-specific functions of Sxl^+ in the zygote (CLINE 1980). In contrast, it is strictly the zygotic functioning of $sis\text{-}a^+$ that is required for proper zygotic functioning of Sxl^+. The name "sisterless" was chosen to highlight both the similarities and differences between

these two regulatory loci. The "a" designation was added because there are reasons to believe that the new *X*-linked female-lethal described here is part of a polygenic system, the elements of which act additively to control Drosophila sexual development through actions on *Sxl* (T. W. CLINE, unpublished results).

Because *Sxl* is such a functionally complex gene, it was important to determine just how far the similarities might extend between *sis-a* and *da* in their control of *Sxl* activities. Defects in functioning of the maternal *da* gene can have consequences for diplo-*X* cell growth and differentiation in progeny up through metamorphosis; however, maternal da^+ activity appears to be required only early in development in connection with the functioning of *Sxl* to initiate the female sexual pathway commitment. *Sxl* itself then functions throughout development to maintain and express the pathway choice, but these functions do not appear to require da^+ activity. *Sis-a*, like the *da* product, appears only to function during these early sexual pathway choice steps. An important part of the studies that led to this conclusion regarding *da* relied on the availability of a variety of female-specific lethal alleles with specific functional defects (CLINE 1984, 1985; MAINE *et al.* 1986). Four particularly useful alleles were Sxl^{f9}, $Sxl^{f\,hv\#1}$, Sxl^{fLS} and $Sxl^{fm\#7,M\#1}$. The remarkable similarity between *sis-a* and *da* in their interactions with these four unusual *Sxl* alleles is one of the strongest pieces of evidence that *da* and *sis-a* control similar aspects of *Sxl* functioning.

Sxl^{f9} and $Sxl^{fhv\#1}$ appear to be defective primarily in functions that are required early to establish stably the female-specific expression mode of Sxl^+. This is the step at which the *X*:*A* balance signal acts, the step that requires maternal da^+ activity, and a step that appears to require a positive autoregulatory Sxl^+ product activity. In its interactions with *da*, the phenotype of Sxl^{f9} is nearly the same as that of a *Sxl* null allele, but in all other respects examined involving later functions, the allele looks nearly wild type. The homozygous viable allele $Sxl^{fhv\#1}$ seems hardly defective at all, yet it is clearly abnormal with respect to the interaction with *da*. Both of these mutants display dominant female-lethal synergism with *sis-a*. This behavior contrasts with that of Sxl^{fLS}, which appears to be wild type with respect to these early steps in which the sexual pathway choice is made, but is clearly defective in later steps required either for the maintenance or expression of the initial sexual pathway commitment. In its interaction with either *da* or *sis-a*, Sxl^{fLS} appears to be wild type.

A close functional relationship between *da* and *sis-a* is shown in a somewhat different way by the unusual phenotype of $Sxl^{fm\#7,M\#1}$. $Sxl^{fm\#7,M\#1}/Sxl^+$ daughters can survive the normally lethal *da* maternal effect, but the daughters rescued by this doubly mutant allele lack ovaries and, thus, are sterile. The sterility is clearly a result of the *da* maternal effect, since genetically identical daughters from mothers carrying a da^+ allele are fully fertile. This "grandchildless-like" maternal effect of *da* is shown here to be mimicked by the interaction between $Sxl^{fm\#7,M\#1}$ and *sis-a*. $Sxl^{fm\#7,M\#1}$ rescues females that are homozygous for *sis-a* and would otherwise die, but as with *da*, the rescued females lacked ovaries.

Sterility in both cases appears to result from a defect in the expression of the Sxl^+ allele carried by these individuals in situations where either one of these two positive regulators of Sxl^+ are defective.

Rare *sis-a/sis-a* escapers exhibit a normal female phenotype; moreover, homozygous *sis-a* diplo-*X* clones induced by mitotic recombination in heterozygous larvae differentiate as phenotypically normal female cells during metamorphosis. These observations might seem to argue against a role of this gene in sex determination *per se*. Studies with *da*, however, have illustrated the fallacy in such logic for genes with cell-vital functions, particularly those that appear to control other genes in a probabilistic fashion early in development. Except in special situations, escaper daughters that survive the *da* maternal effect likewise show no unambiguous signs of masculinization; nevertheless, *da* has been shown to be involved in regulating the sex determination functions of *Sxl* (CLINE 1983a, 1984). The sex-transforming consequences of the *da* maternal effect were masked by cell and organism lethal effects until experimental situations were designed that minimized complications from cell lethal effects arising from upsets in dosage compensation.

The approaches that revealed the masculinizing effects of *da* are shown here also to work with *sis-a*. This loss-of-function allele exhibits a strong dominant masculinizing effect on the sexual phenotype of triploid intersexes, *XX AAA* animals with a sexually ambiguous *X:A* balance of 0.67. Masculinizing effects are also exhibited by *sis-a* in diploid females in combination with mutations in autosomal male-specific lethal genes, mutations that interfere with the hyperactivation of dosage compensated *X*-linked loci, but do not by themselves affect sexual differentiation. Although the sex transformations observed with *sis-a*, like those with the *da* maternal effect, can be incomplete overall, at the level of individual cells (assayed in the foreleg) they appear always to be all-or-none (male or female). The phenotype of these intersexes strongly suggests early effects on the process of sexual pathway choice in both cases. The lack of sex transformation in the somatic recombination study is also indicative of an early time of action for $sis\text{-}a^+$.

In all respects reported here, *sis-a* appeared to be female-specific in its effects; however, the fact that the single allele characterized is hypomorphic limits the conclusions that can be drawn at this point regarding the sex-specificity of this gene's functions. Like *da*, it is clearly central in controlling a gene, *Sxl*, which deficiency analysis shows is female-specific, at least with respect to viability and fertility. On the other hand, *da* itself is involved in other vital processes that are not sex-specific. Indeed, clonal analysis of null *da* alleles has shown that this gene is required zygotically at least as late as the third larval instar, regardless of sex, for the growth of somatic cells (C. CRONMILLER and T. W. CLINE, in preparation). Isolation of a null *sis-a* allele is obviously a high priority.

Although the work reported here stressed the similarities between *sis-a* and *da* in their interaction with *Sxl*, there may be a fundamental difference in the regulatory functions of these two genes. The next paper in this series will present evidence that the most likely role for *sis-a* is as one of the elements

for which the dose establishes the numerator of the *X:A* balance signal itself. In contrast, maternal *da* gene function seems required in order for Sxl^+ to respond properly to this developmental signal, but *da* cannot itself be an analogous dose-sensitive "denominator" signal element. It is not yet even clear whether the *sis-a* locus is truly a gene in the sense of an entity that generates a product; it is possible that *sis-a* may function only as a site for regulatory product binding. In any event, further analysis of *da* and *sis-a* at the genetic and molecular level seems bound to increase our understanding of the nature of the *X:A* balance signal that controls Drosophila sexual dimorphism.

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