MATERNAL AND ZYGOTIC SEX-SPECIFIC GENE INTERACTIONS IN *DROSOPHILA MELANOGASTER*

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ABSTRACT

Sex-lethal (*Sxl*) is a vital, *X*-chromosome gene involved in Drosophila sex determination. The most striking aspect of the phenotype of daughterless (*da*), an autosomal maternal-effect mutation, may be explained by effects on the functioning of the *Sxl* gene in the zygote. In this paper, new aspects of interactions between various combinations of *Sxl* and *da* alleles are explored in order to understand better the complex *da* phenotype. The study focuses on the relationship between maternal and zygotic da^+ gene functions, and on the relationship between aspects of the *da* phenotype that are sex-specific and aspects that are not. The $Sxl^{M\#1}$ allele, which counteracts the female-specific maternal effect of *da*, is shown to have no effect on two other aspects of the *da* phenotype (one maternal, one primarily zygotic) that are not sex-specific. The female-lethal *da* maternal effect is shown to kill daughters even when the progeny are entirely wild-type with respect to *da*. Recessive mutant alleles of the two genes can interact synergistically when both are heterozygous with their wild-type alleles, disrupting the development of most of the daughters. Surprisingly, even a deficiency of the da^+ locus can produce a dominant, temperature-sensitive, female-lethal maternal effect. A new class of subliminal Sxl^f alleles is described. These spontaneous mutations can confuse analysis of both *da* and *Sxl* if their presence is not appreciated. Finally, conditions are described that facilitate the study of the Enhancer of daughterless mutation.

MATERNAL-effect mutations identify genes that function in the mother during oogenesis to produce materials that are subsequently required for the normal development of her progeny (representative screens for such mutants are described in KAPLAN *et al.* 1970; BAKKEN 1973; and GANS, AUDIT and MASSON 1975). In the most extreme cases, the phenotype of the progeny is not influenced by their own genotype with respect to the mutation, but depends instead on the genotype of their mothers. Maternal-effect mutations are potentially useful for understanding at the molecular level the control of developmentally regulated genes, including those genes that are involved in the early determinative processes by which cell fates become restricted. Unfortunately, it is generally difficult on the basis of developmental phenotype alone to identify maternal genes whose products may be directly involved in the regulation of specific embryonic genes.

In the case of the autosomal mutation daughterless (*da*; 2–41.5) it may be possible to explain the most striking aspect of this maternal-effect mutant's phenotype in terms of effects on regulation in the progeny of a specific *X*-linked gene.

Above 22°, all diplo-*X* progeny (daughters) of *da/da* mothers die, regardless of paternal genotype with respect to *da*, yet the viability of haplo-*X* progeny (sons) can be quite high, even for individuals that are homozygous for the mutant *da* allele (BELL 1954; SANDLER 1972; MASON 1973; CLINE 1976). Sex-lethal (*Sxl*; *1*–19.2) is a gene which controls sex determination (CLINE 1979 a,b) and perhaps *X*-chromosome dosage compensation as well (CLINE, manuscript in preparation). A relationship between maternal functioning of da^+ and functioning of Sxl^+ in progeny was first suggested by the discovery that a mutant allele, $Sxl^{M\#1}$, could rescue diplo-*X* progeny from the otherwise lethal maternal effect of *da* (CLINE 1978).

The only apparent effect of $Sxl^{M\#1}$ in diplo-*X* progeny is to counteract the *da* maternal effect, but in haplo-*X* progeny, $Sxl^{M\#1}$ acts as a dominant zygotic lethal. A mutant site previously determined by MULLER and ZIMMERING (1960) to cause female-specific zygotic lethality is closely linked to the mutant site causing male-specific lethality. This female-specific allele was initially called Female-lethal, but was renamed $Sxl^{f\#1}$ to indicate its relationship to $Sxl^{M\#1}$ (CLINE 1978). Recent analysis of double-mutant combinations in *cis* and *trans* has confirmed that $Sxl^{f\#1}$ and $Sxl^{M\#1}$ are alleles, despite their radically different phenotypes (CLINE manuscript in preparation).

Previous analysis of the interaction between *da* and *Sxl* led to the hypothesis that the Sxl^+ gene product is essential for development of diplo-*X* tissue, but deleterious to haplo-*X* cells, and that the Sxl^+ gene is therefore normally active only in diplo-*X* (female) animals (CLINE 1978). It was further proposed that the da^+ maternal gene product is required to activate the Sxl^+ gene in daughters following fertilization. Subsequent analysis of the effects of the male-lethal and female-lethal *Sxl* alleles on development of genetically mosaic individuals suggested that the Sxl^+ product causes cells to follow a female-specific morphogenetic sequence, while in the absence of Sxl^+ gene activity, cells follow a male-specific developmental path (CLINE 1979 a,b).

Although daughterless derived its name from its effect on diplo-*X* progeny, the *da* mutation itself is clearly not a sex-specific lethal (SANDLER 1972; MANGE and SANDLER 1973), even though it interacts with a gene, *Sxl*, that is sex specific. At 29°, not even haplo-*X* progeny of *da/da* mothers survive. Furthermore, *da* is a temperature-sensitive zygotic lethal in both sexes, even in the absence of the maternal effect. Based on circumstantial evidence obtained before the discovery of the interaction between Sex-lethal and daughterless, I suggested (CLINE 1976) that the mutant *da* allele might be autonomously pleiotropic (see STERN and TOKUNAGA 1968), interfering with three separate developmental functions: one requiring da^+ gene expression during zygotic development and two others requiring da^+ gene expression during oogenesis.

Pleiotropy may be obvious when it results in a variety of morphological alterations in the adult fly. When each separate developmental effect of a mutation can result in lethality, pleiotropy may be more difficult to recognize and characterize. Only with some appreciation of the possible multiple lethal effects of a mutation, however, is it possible to focus on one aspect of the phenotype, while still using

the crude but efficient experimental measure, viability. The hypothesis of multiple developmental functions for da^+ reconciles the lack of overall sex specificity of the *da* phenotype with the apparently sex-specific function of the Sxl^+ locus. An important aim of the work reported in this paper is to explore further this hypothesis of multiple, vital functions of da^+, and the correlate that the *da-Sxl* interaction involves only the sex-specific maternal function of da^+. In the course of this work, certain additional aspects of the relationship between *da* and *Sxl* were discovered that are important for proper design and interpretation of future studies of these two genes.

MATERIALS AND METHODS

Flies were raised under uncrowded conditions on a medium described by CLINE (1978). The criterion for survival was eclosion. Refer to LINDSLEY and GRELL (1968) for mutant designations and descriptions, except as noted below and in notes to the tables. *Binsinscy* is an *X*-chromosome female-sterile balancer: $In(1)sc^{S1L}sc^{S1R} + In(1)dl\text{-}49$, $y\ sc^{S1}\ w\ sn^{w2}B\ sc^{8}$ see BRYANT and ZORNETZER 1973). Stocks used for the experiment in Table 1 were constructed as follows: (1) a *C(2L), cl da* chromosome was recovered following mating of virgin *cl da cn bw* females treated with 4000 rad of gamma radiation to *C(2L)RM, C(2R)RM* males (HOLM, 1976). (2) $Sxl^M\#1$ was introduced into the *C(2)* stocks by mating virgin $y\ w\ Sxl^M\#1\ sn/y\ w + sn$; *da/da* females treated with 4000 rad of gamma radiation to *C(2L), cl da; C(2R)RM* males and recovering the phenotypically clot-eyed daughters. The markers *y*, *w* and *sn* were eventually removed by recombination, and the stock was maintained homozygous for *da*. (3) The marker *v* was introduced into the *C(2)* stocks by mating virgin *cv v Bx* females maintained for 10 days at 10° (TOKUNGA 1970 a,b) to *C(2L), cl da; C(2R)RM* males, and recovering phenotypically *Bx, cl* sons. The markers *cv* and *Bx* were eventually removed by recombination, and the stock was used to introduce *v* into the $Sxl^M\#1$; *C(2)* stock.

RESULTS

I. *Effect of zygotic* da^+ *gene dose on the daughterless maternal effect*

Recessive maternal-effect mutations have been grouped according to whether a wild-type allele contributed by the sperm can or cannot overcome the effect of mutant alleles in the mother (KAPLAN *et al.* 1970; GANS, AUDIT and MASSON 1975). Rescue of embryos by a paternal wild-type allele indicates that the gene product required for a particular developmental step in the embryo can be provided by either the maternal alleles during oogenesis or by the embryo's own allele(s) functioning after fertilization. Lack of rescue is more ambiguous. It may reflect a strict requirement for only maternal gene expression, with no participation of the embryo's alleles. Alternatively, it may reflect a requirement for both maternal and embryonic gene expression in which neither individually can provide sufficient gene activity for normal development.

Since neither *da/da* nor da/da^+ daughters of *da/da* mothers survive at 25°, the *da* maternal effect appears to be of the nonrescuable type (BELL 1954; SANDLER 1972). The discovery of the temperature-sensitive nature of this mutation allowed a more accurate determination of the effect of zygotic *da* genotype on the ability of daughters to survive the maternal effect (CLINE 1976). Under semipermissive conditions where 7% of *da/da* daughters survived, the presence of a wild-type *da*

allele increased daughter viability to 19%. Thus, the *da* maternal effect appears to be rescuable, but only weakly so. The three-fold viability increase by a paternal da^+ allele is modest indeed when compared to the increase seen with most other rescuable maternal-effect mutants. Rudimentary is good example (COUNCE 1956; CARLSON 1971), the only one for which the molecular basis of the maternal effect is known (NORBY 1970; JARRY and FALK 1974; OKADA, KLEINMAN and SCHNEIDERMAN 1974; RAWLS and FRISTROM 1975).

The three-fold viability increase was observed under conditions where 81% of the daughters died even with one da^+ allele. In order to determine whether zygotic da^+ gene activity was still limiting daughter survival under these semi-permissive conditions, the effects of adding yet another da^+ allele to the embryos was ascertained by the experiment indicated in Table 1.

In the absence of a duplication of da^+, compound second chromosomes were used to produce progeny homozygous for one autosomal allele from mothers homozygous for a different allele. The effect of two wild-type doses of da^+ relative to none in this experiment could then be compared to the effect of a single wild-type dose relative to none in previous experiments.

From the data of Table 1, row 1, it can be seen that even daughters with two doses of the da^+ allele die from the maternal effect at 22°. At 17°, some daughters survive, even those homozygous for *da*. As before, presence of wild-type *da* alleles in daughters at 17° does increase survival, but the difference in viability between the two progeny genotypes in this experiment (55% viability of the "no dose" daughters relative to their sisters with "two doses") is even less than in the previous experiment (30% for "no dose" relative to "one dose"; Table 1 in CLINE 1976). Even with two doses of da$^+$, at least 45% of the daughters fail to complete development. Under these conditions, which are sufficiently permissive to allow a substantial fraction of homozygous mutant daughters to survive, the availability of da^+ maternal gene activity still limits the survival of even da^+ progeny. The data in the third and fourth rows show that the progeny sex ratios are normal in the absence of the maternal effect, and that the two types of compound *2L* chromosomes have no significant effect on progeny viability at these temperatures when the mothers are wild-type for daughterless.

The data in the fifth row of Table 1 are a control of a different sort, and serve to introduce the *da-Sxl* interaction. In this case, the mothers were heterozygous for the *X*-linked, male-specific lethal allele of *Sxl*, which rescues daughters of *da* females. Whereas no daughters survived at 22° without $Sxl^{M\#1}$ (row 1), da^+/da^+ daughters with $Sxl^{M\#1}$ were fully viable relative to their da^+/da^+ brothers (who necessarily did *not* carry $Sxl^{M\#1}$). Curiously, even when the sex-ratio maternal effect of *da* was completely suppressed by $Sxl^{M\#1}$ in the daughters, the recessive lethal effect was significantly greater ($p < .01$; $2 \times 2\ \chi^2$) among progeny of *da/da* mothers than among the progeny of da^+/da^+ mothers (0.75 *vs.* 1.11). Thus, it seems that the magnitude of the recessive lethal effect may depend somewhat on the maternal genotype with respect to *da*, independent of the sex-ratio maternal effect of *da* that is absolutely lethal to Sxl^+ daughters at 22° regardless of their genotype with respect to *da*.

TABLE 1

Effect of da+ *gene dose on progeny survival from* da/da *mothers**

Cross	Maternal genotype with respect to *da*	Temp† °C	Progeny sex and genotype with respect to *da*: Sons *da*+/*da*+	Sons *da*/*da*	Daughters *da*+/*da*+	Daughters *da*/*da*	Recessive lethal effect: Viability of *da*/*da* progeny relative to their +/+ siblings of the same sex ♀	♂	Sex-ratio *da* maternal *effect*: Viability of +/+ daughters relative to +/+ sons
A-1	*da*/*da*	22	600	611	0	0	—	1.02	<0.002
A-2	*da*/*da*	17	495	425	282	156	0.55	0.86	0.57
B-1	*da*+/*da*+	22	781	843	752	831	1.11	1.08	0.96
B-2	*da*+/*da*+	17	298	266	301	302	1.00	0.89	1.01
C	*da*/*da* (& $Sxl^{M\#1}$/*Sxl*+)	22	188	191	213	160	0.75	1.02	1.13

*Progeny were from the following crosses:
A: *v*; *C(2L)*, *cl da*; *C(2R)* ♀♀ × *v*/*Y*; *C(2L)*, +; *C(2R)* ♂♂
B: *v*; *C(2L)*, +; *C(2R)*, + ♀♀ × *v*/*Y*; *C(2L)*, *cl da*; *C(2R)*, ♂♂
C: $Sxl^{M\#1}v$/+*v*; *C(2L)*, *cl da*; *C(2R)*, ♀♀ × *v*/*Y*; *C(2L)*, +; *C(2R)*, ♂♂
Vermilion was included to facilitate the scoring of *cl*, which is otherwise difficult to distinguish from *cl*+ at 17°. A comparison at 29° was not included due to temperature sensitivity of the *C(2L)*, + chromosome obtained from the Bowling Green Drosophila stock center.

† Temperature at which eggs were laid by parents conditioned to the temperature for at least 6 days. Progeny were raised at indicated temperature for at least 5 days, then shifted to 25° to complete development.

In summary, while a da^+ allele in daughters can counteract the maternal effect, it does so to only a very limited extent. Furthermore, the zygotic dose of the da^+ alleles is not what limits the ability of da/da^+ daughters to survive the maternal effect. Put another way, what was discovered to be a recessive lethal effect of *da* among the progeny of da^+ mothers is shown to remain a strictly recessive lethal effect even when the female-lethal maternal effect is severe. The experiments also rule out the possibility that zygotic dominance of the mutant *da* allele is responsible for the death of daughters from *da/da* mothers.

II. *The* Sxl$^{M\#1}$ *allele counteracts just one of three aspects of the* da *phenotype*

The data in Table 2 show that the $Sxl^{M\#1}$ allele, which rescues zygotes from the sex-ratio maternal effect of *da*, has no significant effect on the recessive lethal effect of *da*. The recessive lethal effect is measured as the viability of *da/da* progeny relative to their da/da^+ siblings. At 22°, there is no indication of a recessive lethal effect, but at 29° the relative viability of *da/da* daughters is down to less than 3%, irrespective of the male-lethal *Sxl* allele.

A third aspect of the *da* phenotype is characterized by the fact that homozygous *da* females kept at 29° for several days continue to lay fertilized eggs, but fail to produce progeny of either sex. I referred to this as the female-sterile *da* effect (CLINE 1976). The most straightforward hypothesis for this observation would be that the higher temperature simply exacerbates the lethal maternal effect suffered only by daughters at lower temperatures, so that neither sex of progeny can survive. By this hypothesis, the embryonic function disrupted by the maternal *da* mutation would involve a quantitative rather than qualitative developmental difference between the sexes. But a number of aspects of the earlier study led me to suspect that the situation was not so simple and to propose that there were two separate maternal effects: a sex-ratio effect specifically lethal to diplo-*X* progeny and a female-sterile effect equally deleterious to both sexes of progeny.

The hypothesis of separate maternal effects would be supported if $Sxl^{M\#1}$ were found to counteract only the maternal effect of *da* on the progeny sex ratio, but not the sterility effect lethal to both sexes of progeny at 29°. To test for an effect of $Sxl^{M\#1}$ acting in the mothers on the *da* sterility maternal effect, independent of its effects on progeny sex ratio, one must focus on the survival of Sxl^+/Y sons from *da/da* mothers. Either egg counts or fecundity (with regard to Sxl^+/Y son production per mother per day) can provide a measure of this. To avoid complications arising from the temperature-sensitive recessive lethal aspect of the *da* phenotype, one must focus on da/da^+ sons. This is the purpose of the experiment described in the top panel of Figure 1.

For the experiment in Figure 1, da^+ females (▲) and *da/da* females with (■) and without (●) the $Sxl^{M\#1}$ allele were conditioned to 18° for at least four days prior to the start of progeny collections. Progeny were collected at 18° for three successive days. The numbers of Sxl^+/Y; da/da^+ sons obtained is indicated by the first three data points in the top panel.

$Sxl^{M\#1}/Sxl^+$ females are expected to produce only half as many Sxl^+/Y sons as Sxl^+/Sxl^+ females, regardless of any maternal effects on fecundity. To facili-

TABLE 2

Lack of effect of $Sxl^{M\#1}$ *on the recessive lethal aspect of the* da *phenotype*

Temperature at which eggs were laid and progeny were raised °C	Daughters' genotype with respect to *Sxl* and *da*				Recessive lethal effect of *da*: Viability of *da/da* daughters relative to their *da/da*+ sibs	
	Sxl+/*Sxl*+ *da/da*+	*Sxl*+/*Sxl*+ *da/da*	$Sxl^{M\#1}/Sxl$+ *da/da*+	$Sxl^{M\#1}/Sxl$+ *da/da*	Among *Sxl*+/*Sxl*+ daughters	Among $Sxl^{M\#1}/Sxl$+ daughters
22	279	292	324	295	1.05	0.91
29	584	17	668	18	0.029	0.027

Progeny are from the cross: *Binsinscy/*$Sxl^{M\#1}$*; da cn bw/In(2L+2R)Cy* ♀♀ × sn^3/Y; *da cn bw* ♂♂ (*XXY* matroclinous exceptions are excluded from totals.)

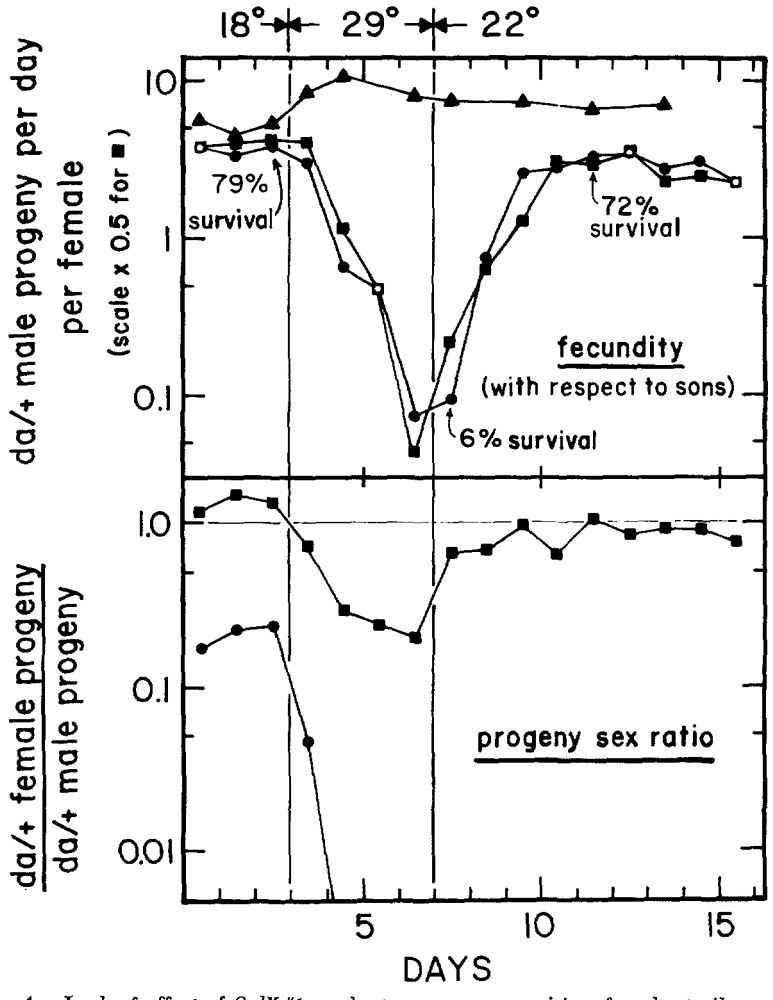

FIGURE 1.—Lack of effect of $Sxl^{M}\#^{1}$ on the temperature-sensitive, female-sterile aspect of the daughterless phenotype. The progeny indicated were obtained from daily transfers of the following adults: Sxl^{+}/Y; *da cn bw/In(2L+2R), Cy* males mated to (▲) 60 wild-type (Oregon-R) females, (●) 307 *da cn bw* females, or (■) 345 $Sxl^{M}\#^{1}/+$; *da cn bw* females. The scale adjustment in fecundity for (■) takes account of the fact that half of the sons in this cross will die due to the dominant, male-lethal effect of $Sxl^{M}\#^{1}$. The measure is also corrected for the day-to-day loss of parental females during the course of the experiment. By the end of the 16th day, 93% (▲), 43% (●), and 54% (■) of the initial number of females remained. "Survival" refers to the percent recovery of adult Sxl^{+}/Y; *da*/+ sons from sample egg collections on the days indicated. The temperatures indicated are those at which the parents laid the eggs and at which the progeny developed. Interpretation of the value for progeny sex ratio requires an understanding of what classes of progeny are expected to survive and why (see text).

tate comparisons between the two types of females, the scale for fecundity for (■) females is adjusted accordingly. Survival of Sxl^{+}/Y; da/da^{+} sons on the third day at 18° was 79%, as determined by egg counts. This is consistent with previous reports that the viability of sons from *da/da* mothers can be quite high (MASON 1973).

On the fourth day, parents were shifted to 29°. Progeny were collected for four days at this temperature; then the parents were shifted back down, but to 22° rather than to the original temperature of 18°. Progeny were collected at 22° for eight days following the shift down. For all data points, progeny were raised at the same temperature at which the eggs had been laid, the temperatures indicated at the top of the figure (with the exception that for the 18° points, progeny were shifted to 25° after three days to complete development, simply to expedite data collection).

The shift from 18° to 29° gave increased fecundity for da^+ control females, but gave a precipitous drop in fecundity for *da/da* mothers, regardless of their genotype with respect to *Sxl*. The drop in fecundity at 29° primarily resulted from a decline in the survival of sons, rather than a decrease in the rate of oviposition. One day after the return to lower temperature, egg to adult survival of Sxl^+/Y; da/da^+ sons from Sxl^+/Sxl^+; *da/da* mothers was only 6%, but it rose to 72% during the next four days at 22°. Clearly there was a maternal effect of *da* on survival of sons, but the effect was not modified by the presence of $Sxl^{M\#1}$ in the mothers.

The failure of $Sxl^{M\#1}$ acting in mothers to overcome the sterility aspect of the *da* phenotype is perhaps not surprising, since it was a zygotic rather than maternal action of $Sxl^{M\#1}$ that counteracted the sex-ratio maternal effect of *da* (CLINE 1978). Nevertheless, the data in the top panel of Figure 1 are important, since they allow a determination to be made of the possible effects of $Sxl^{M\#1}$ acting in zygotes on the sterility aspect of the *da* phenotype. The sex ratio among da/da^+ progeny is the relevant parameter for this determination, indicated in the bottom panel of Figure 1.

To interpret the significance of the progeny sex-ratio data, it is important to note that the $Sxl^{M\#1}/Sxl^+$; *da/da* mothers (■) will produce four types of zygotes in equal numbers; $Sxl^{M\#1}/Y$ sons will invariably die due to the male-lethal effect of $Sxl^{M\#1}$; Sxl^+/Y sons will survive depending on the magnitude of the *da* maternal sterility effect; Sxl^+/Sxl^+ daughters will all die due to the *da* sex-ratio maternal effect, except at low maternal and zygotic temperatures where a fraction can escape; and $Sxl^{M\#1}/Sxl^+$ daughters will survival depending on the extent to which the $Sxl^{M\#1}$ allele can rescue them from both the sex-ratio and the sterility maternal effects of *da*.

Under conditions where none of the Sxl^+/Sxl^+ daughters survive, the sex ratio reflects the ratio of surviving $Sxl^{M\#1}/Sxl^+$ daughters to Sxl^+/Y sons. Under these conditions, the range of values possible for the progeny sex ratio depends upon the relationship between the aspect of the *da* maternal effect that kills sons and the aspect of the *da* maternal effect that is counteracted by $Sxl^{M\#1}$ in daughters. If the effect that is lethal to Sxl^+/Y sons is also suffered by daughters, *and* can be counteracted by $Sxl^{M\#1}$ in these daughters, then the sex ratio among the surviving progeny could exceed 1. On the other hand, if $Sxl^{M\#1}$ counteracts an aspect of the *da* phenotype that is suffered only by daughters (the putative sex-ratio maternal effect), an aspect distinct from a maternal effect on all progeny regardless of sex (the *da* sterility effect, which is manifested by the death of Sxl^+/Y sons), then the sex ratio could never exceed 1. Even if $Sxl^{M\#1}$ were 100% effective at counteract-

ing the sex-specific aspect of the phenotype, daughters would still suffer from the non-sex-specific maternal effect that would be lethal to their brothers as well.

The most significant part of the sex-ratio curves in the bottom panel of Figure 1 is that seen after one day at 29°. After this time, the relative viability of daughters from Sxl^+/Sxl^+ mothers (●) never exceeded zero, even following the subsequent shift down to 22°. Consequently, in this part of the curve, the sex ratio among the progeny of $Sxl^{M\#1}/Sxl^+$ mothers (■) reflects only the viability of $Sxl^{M\#1}/Sxl^+$ daughters relative to their Sxl^+/Y brothers. $Sxl^{M\#1}$ rescued daughters even at the extremely nonpermissive temperature of 29°, though its effectiveness was somewhat reduced compared to that at lower temperatures.

Immediately following the shift down to 22°, the sex-ratio among the progeny of $Sxl^{M\#1}/Sxl^+$ mothers (■) was 0.65, indicating that $Sxl^{M\#1}/Sxl^+$ daughters survived nearly as well as their Sxl^+/Y brothers. Not only was there very little change in this parameter following the shift to 22°, but the value never exceeded 1. During the same period, the viability of the Sxl^+/Y sons increased more than one order of magnitude: from 6% to 72%. Thus, while an increasing fraction of $Sxl^{M\#1}/Sxl^+$ daughters survived the *da* maternal effect after the shift to 22°, the progeny sex ratio remained nearly constant. The result could be explained by increased effectiveness of $Sxl^{M\#1}$ at rescuing daughters, balanced almost exactly by increased survival of Sxl^+/Y sons. A much simpler explanation is that the sex-specific lethal maternal effect was effectively counteracted by $Sxl^{M\#1}$ even immediately after the shift down, but the sterility maternal effect was not. Consequently, the *absolute* number of daughters surviving rose in concert with the increase in Sxl^+/Y son survival, keeping the sex ratio nearly constant and below its maximum value of 1. The sex-ratio above 1 found for the three days at the start of this experiment (■) simply reflects the survival of some Sxl^+/Sxl^+ daughters who escaped the maternal effect a 18°, escapers also evident in the curve for Sxl^+/Sxl^+; *da/da* mothers (●).

III. *A new class of female-specific* Sxl *alleles.*

The dominance properties of *Sxl* and *da* alleles depend strongly on genetic modifiers, some characterized and some not. A novel type of *Sxl* allele has been discovered in the course of investigating such modifiers.

Regardless of genetic background, $Sxl^{f\#1}$ is always lethal to females when homozygous, but when initially characterized, it seemed to behave as a semi-dominant lethal (MULLER and ZIMMERING 1960). ZIMMERING and MULLER (1961) subsequently showed that this dominance was caused by a maternal effect of unidentified modifiers on all three major chromosomes of the stock in which the mutant allele arose. Like $Sxl^{f\#1}$, *da* can also behave in a semidominant fashion, depending on modifiers. MANGE and SANDLER (1973) isolated a *2;3* translocation which they named Enhancer of daughterless, *E(da)*, because it caused *da* to behave as a semi-dominant with respect to its sex-ratio maternal effect. As with $Sxl^{f\#1}$ dominance, the phenotypic effect of *da* with *E(da)* was rather sensitive to both genetic background and specific growth conditions.

While investigating the effect of *E(da)* on survival of daughters carrying $Sxl^{f\#1}$, I found that SANDLER's *E(da)* stock was homozygous for an *Sxl* allele that

is viable and fertile when homozygous, yet lethal when heterozygous with $Sxl^{f\#1}$ or with a deficiency for the *Sxl* locus. Independently, I isolated a line in which *da* appeared to be dominant, much as it was with *E(da)*. The behavior of this line was extremely erratic. The enhancement of the *da* allele was not due to a single, simple genetic factor. Like SANDLER's *E(da)* stock, this stock was also homozygous for the "subliminal" type of female-specific *Sxl* allele. Apparently, in both cases, selection (by the investigators) for strong (dominant) expression of the *da* maternal effect simultaneously resulted in selection for alterations at the *Sxl* locus.

The characteristic phenotype of such *Sxl* alleles is detailed in Table 3 for the allele found in the *E(da)* line, identified here as $Sxl^{fhv\#1}$ (female-specific, homozygous viable allele number 1). The allele is fully viable when homozygous (first row, experimental daughters, viability relative to $Sxl^{fhv\#1}$/Y brothers, 104%), but nearly lethal in compound with the strong $Sxl^{f\#1}$ allele (second row, experimental daughters, viability relative to $Sxl^{fhv\#1}$ brothers, 1%). Those few heteroallelic daughters that survived were often malformed, similar to daughters that escape the *da* maternal effect at low temperatures, or that escape the semidominant effect with *E(da)*. The malformations were also similar to those of $Sxl^{f\#1}/Sxl^{+}$ daughters that escape when genetic background causes semidominance of $Sxl^{f\#1}$. Third leg and sixth sternite defects are most characteristic. Mapping that shows this complementation pattern to be due to a change at the *Sxl* locus is indicated in Table 4. The markers carmine and cut closely flank *Sxl*. The observed *cm-ct* distance was 1.2 map units, compared to the standard value of 1.1 map units, showing that there is no significant effect of the mutant allele on recombination in the interval. The position of $Sxl^{fhv\#1}$ was 19.1, which agrees well with previous mappings of *Sxl* alleles.

In this experiment, the heteroallelic combination was less viable than in Table 3. There is variability in this parameter, though the viability of $Sxl^{fhv\#1}/Sxl^{f\#1}$ individuals is always less than 5% at 29°.

Like $Sxl^{f\#1}$ (CLINE 1978), Sxl^{fhv} alleles reduce the ability of females to survive the *da* sex-ratio maternal effect. This explains why stocks selected for a semidominant *da* maternal effect were likely to carry this class of *Sxl* allele. *cm* Sxl^{+} *ct*/*Y* and + $Sxl^{fhv\#1}$ +/*Y* sons from the mapping cross of Table 4 were mated to Sxl^{+}; *da cn bw* females in order to quantitate this effect. The viability of Sxl^{+}/Sxl^{+} daughters was 12.3% relative to their brothers (2,156) at 17°. The viability of the $Sxl^{fhv\#1}/Sxl^{+}$ daughters under the same conditions was only 4.4% (2,247 brothers). Thus, the subliminal *Sxl* allele caused a 65% reduction in female survival when introduced from the fathers. A third Sxl^{fhv} allele was discovered by chance in a da^{+} stock on the basis of just such a reduction in the survival of daughters from *da/da* mothers. In an attempt to find additional alleles of this type, C. FIELD (unpublished) screened 40 laboratory mutant stocks chosen at random, but failed to discover any.

IV. *Now dominant interactions between* da *and* Sxl

Since relatively minor changes at the *Sxl* locus could be identified on the basis of their interaction with strong Sxl^{f} alleles, or with a strong *da* maternal effect, it seemed possible that previously undetectable weak maternal effects of the *da*

TABLE 3

Complementation analysis of a homozygous viable, female-specific Sxl *allele*

Cross (29°)	Sons	Experimental daughters			Control daughters		
		Sxl genotype (allele combination)		Viability relative to $Sxl^{fhv\#1}$ brothers	*Sxl* genotype (allele combination)		Viability relative to $Sxl^{fhv\#1}$ brothers
	$Sxl^{fhv\#1}/Y$						
A. $Sxl^{fhv\#1}/Binsinscy$ ♀ ♀ × $Sxl^{fhv\#1}/Y$ ♂ ♂	422	*fhv#1/fhv#1*	440	1.04	+/*fhv#1*	425	1.01
B. $Sxl^{fhv\#1}/Binsinscy$ ♀ ♀ × *cm* $Sxl^{f\#1}ct/Y$ ♂ ♂	542	*fhv#1/f#1*	5	0.01	+/*f#1*	466	0.86

TABLE 4

Mapping of a homozygous-viable, female-specific Sxl *allele*

	Parental types				Recombinant types			
Sex of progeny	Males	Males	Females	Females	Males	Males	Females	Females
Phenotype	*cm ct*	*cm*+ *ct*+	*cm ct*	*cm*+ *ct*+	*cm ct*+	*cm*+ *ct*	*cm ct*+	*cm*+ *ct*
Sxl genotype (alleles)	+/*Y*	*fhv#1/Y*	+/*f#1*	*fhv#1/f#1*	—	—	—	—
Number recovered	2468	2541	2165	5*	36	27	23	4

Progeny of the cross at 29°: *cm* + *ct*/+ $Sxl^{fhv\#1}$ + ♀♀ × *cm* $Sxl^{f\#1}$ *ct*/*Y* ♂♂.
Calculated *cm-ct* distance = 1.24 m.u. (90/7269).
Position of *fhv#1* = 19.06 ([4/27 × 1.1] + 18.9).
* Of the five, four died prematurely without laying eggs (probably escapers), while one proved to be a matroclinous exception ($Sxl^{fhv\#1}$/+/*Y*).

locus might be revealed by their consequences for progeny who carry strong Sxl^f mutations. ZIMMERING and MULLER's (1961) earlier discovery that the dominant character of $Sxl^{f\#1}$ depended on an undefined maternal effect was also suggestive.

With respect to its maternal effect on progeny sex ratio, *da* is recessive as long as the progeny are Sxl^+. The data in the A crosses of Table 5 confirm this. The viability of Sxl^+/Sxl^+ daughters did not differ significantly from mothers who did (A-1) or did not (A-2) carry a mutant *da* allele (see column Y). In contrast, the viability of $Sxl^{f\#1}/Sxl^+$ daughters in the A crosses was very strongly dependent on the *da* maternal genotype. The relative viability of $Sxl^{f\#1}/Sxl^+$ daughters (Column X) was only 4% if their mothers were heterozygous for *da*, but was 120% if their mothers were wild type with respect to *da*. Presence or absence of a single *da* allele in the progeny did not seem to matter (6% relative viability for $Sxl^{f\#1}/+$; $da/+$ daughters *vs.* 4% for their $da^+/+$ sisters). Thus, the effect of *da* seems to be strictly maternal. The mothers in crosses A-1 and A-2 were sisters, which minimized extraneous effects of genetic background.

The series A crosses in Table 5 served as a pilot experiment that was followed by a more extensive study that minimized the use of balancer chromosomes and genetic markers and included an investigation of effects of temperature on the dominant maternal effect of *da*. Recall that the recessive *da* maternal effect is temperature dependent (Table 1). The results of this second study—the progeny from cross B— were considerably less dramatic than the results from the series A crosses; nevertheless, they did confirm and extend the initial observations.

Again, relative viability of $Sxl^{f\#1}/Sxl^+$ daughters from da/da^+ mothers was significantly reduced at 29° (57% viability) and 25° (63%), but not at 17° (98%). A control cross (not shown) confirmed that the reduced $Sxl^{f\#1}/Sxl^+$ viability in the cross B progeny was due to the maternal *da* allele, since viability of $Sxl^{f\#1}/Sxl^+$ daughters from $da+/In(2L+2R)$, $Cy\ da^+$ sisters of the mothers used in B was 97% at 25° (622 daughters *vs.* 641 $Sxl^{f\#1}/Y$ sons). Although many $Sxl^{f\#1}Sxl^+$ daughters of da/da^+ mothers in series B survived at the higher temperatures, they were clearly not healthy. Even at 25°, 20% of the $Sxl^{f\#1}/Sxl^+$ daughters of da/da^+ mothers became stuck on the food surface soon after eclosion and died, while only 1% of their Sxl^+/Sxl^+ sisters became stuck. At 17°, in contrast, less than 1% of either genotype became stuck, even several days after eclosion.

Exposure of *da/da* mothers to a higher temperature affects their ability to produce viable daughters even if the daughters themselves develop entirely at a lower temperature; only after several days at 17° do *da/da* mothers begin to produce substantial numbers of daughters that survive to adulthood (CLINE 1976). A similar, though shorter-lasting effect of maternal exposure to higher temperatures was observed for the $Sxl^{f\#1}/Sxl^+$; da/da^+ mothers of cross B in Table 5. Parents that had been conditioned at 29° for many days were shifted down to 17°. The viability of $Sxl^{f\#1}/Sxl^+$ daughters produced during the first day at 17° was 53% (227/361), but rose to 99% (175/176) during the second day. Both groups of daughters developed entirely at 17°, so that the difference in via-

TABLE 5

Dominant interactions between da *and* Sxl$^{f\#1}$

Cross* Genotype of parents with respect to *Sxl* and *da*:		Temp† °C	Progeny genotype with respect to *da*	Progeny genotypes with respect to *Sxl*: Sons		Daughters		% Viability of daughters relative to *Sxl$^{f\#1}$/Y* brothers	
				Sxl$^{f\#1}$/Y	*Sxl+/Y*	*Sxl$^{f\#1}$/Sxl+*	*Sxl+/Sxl+*	*Sxl$^{f\#1}$/Sxl+*	*Sxl+/Sxl+*
								X	*Y*
Sxl+/Y; da+/da+ ♂♂ × ♀♀:									
A–1	*Sxl$^{f\#1}$/Sxl+*	28	+/+	198	(60)‡	8	151	4	76
	da/da+		*da/+*	209	(109)	13	208	6	100
A–2	*Sxl$^{f\#1}$/Sxl+* *da+/da+*	28	+/+	96	(47)	115	86	120	90
B	*Sxl$^{f\#1}$/Sxl+*	29	n.d.	898	1002	511	961	57	107
	da/da+	25	n.d.	1236	1329	776	1241	63	100
		17	n.d.	917	979	896	998	98	109

* Progeny from the following crosses:
A–1: *Sxl$^{f\#1}$ oc ptg v/Binsinscy; da cn bw/In (2L+2R)Cy* ♀♀ × *f/Y* ♂♂.
A–2: Same as above except females *da+/In(2L+2R), Cy.*
B: *cm Sxl$^{f\#1}$ct/+++; cl da cn bw/++++* ♀♀ × *cm ct/Y* ♂♂.
† Temperature at which parents were conditioned for at least 5 days, and at which progeny were laid and developed.
‡ The low value for *Sxl+* sons is due to mutations on the balancer *X* chromosome unrelated to *Sxl.*

bility must reflect effects of temperature on oogenesis. In a reciprocal shift, cross B mothers who had been conditioned to 17° were shifted to 29°, and their progeny were grown entirely at the higher temperature. The relative viability of the $Sxl^{f\#1}/Sxl^{+}$ daughters was 104% (291/279) at 17° the day before the shift. In successive days following the shift to 29° it was 77% (302/391), 64% (189/297); 62% (270/440), 61% (225/371), 56% (173/309), 65% (123/188) and 62% (119/191). Thus viability of the $Sxl^{f\#1}/Sxl^{+}$ daughters dropped substantially as soon as the temperature was raised, and quickly plateaued. This shift up does not allow one to distinguish between temperature effects on zygotes themselves and temperature effects on oogenesis, but the results are consistent with the rapid reduction in daughter viability observed previously in shifts up of *da/da* mothers (see Figure 1 also), and contrast with the effects of temperature shifts on the progeny of da^{-}/da^{+} mothers to be discussed below.

V. *Genetic and environmental conditions that greatly increase expressivity of Enhancer of daughterless*

MANGE and SANDLER (1973) isolated *E(da)* as a mutation (translocation) that caused *da* to exhibit a modest, semi-dominant maternal effect. If mothers carried one dose of *E(da)*, and one dose of *da* either *cis* or *trans* to *E(da)*, then viability of their daughters was reduced by 16 to 24%. In view of the findings reported here of a semidominant maternal effect of *da* alone on $Sxl^{f\#1}/Sxl^{+}$ daughters, an effect that was strongly temperature dependent, it seemed worthwhile to re-examine the *E(da)-da* interaction with respect to maternal and zygotic *Sxl* genotype and with respect to temperature. The data shown in Table 6 indicate that both factors very strongly influence the phenotype of *E(da)*.

Enhancement of the *da* maternal effect by *E(da)* was considerable when parents and progeny were kept at 29°, even when both generations were truly wild type with respect to *Sxl* (recall that previous work with *E(da)* may have been complicated by "subliminal" *Sxl* alleles of the type described above in section III). Relative to sons, only 21% of daughters survived at 29°, but when parents and progeny were kept at 17°, daughters were fully viable (103%). Although the cross does not allow one to distinguish progeny that carry *E(da)* from those that do not, it is clear that both $E(da)/E(da)^{+}$ and $E(da)^{+}/E(da)^{+}$ daughters suffer from the enhanced maternal effect of *da*, since the overall reduction in viability is considerably greater than 50% at 29°.

While Sxl^{+}/Sxl^{+} daughters suffered from the enhanced maternal effect of *da* at 29°, $Sxl^{f\#1}/Sxl^{+}$ daughters were far more sensitive still. Cross B differs from cross A only in that the mothers carried $Sxl^{f\#1}$. None of their daughters survived who received the mutant *Sxl* allele (< 0.1%). It is important to note, however, that their sisters with two functional copies of the *Sxl* locus survived at a rate comparable (36% *vs.* 21%) to that of the Sxl^{+}/Sxl^{+} daughters from cross A. Thus, the grossly deleterious effect of $Sxl^{f\#1}$ is a consequence of its action (or *lack* of action, since it is a hypomorphic or amorphic allele; see CLINE 1978; MARSHALL and WHITTLE 1978) in the zygote, rather than in the mother.

As in cross A, the cross B results depended strongly on the temperature at

TABLE 6

Temperature-sensitive dominance of da *and* $Sxl^{t}\#^{1}$ *with Enhancer of daughterless*

Cross	Genotype of parents with respect to *Sxl*, *da*, and *E(da)* ♀♀		♂♂	Temp* °C	Progeny				Viability of daughters relative to their +/*Y* sibs	
					Sxl$^{t}\#^{1}$/*Y* Sons	*Sxl*+/*Y* Sons	*Sxl*$^{t}\#^{1}$/*Sxl*+ Daughters	*Sxl*+/*Sxl*+ Daughters	*Sxl*$^{t}\#^{1}$/*Sxl*+ Daughters	*Sxl*+/*Sxl*+ Daughters
					(1)	(2)	(3)	(4)	(5)	(6)
A.	+/+;	×	+/*Y*;	29	—	720‡	—	154‡	—	0.21
	da E(da)/++		+/+	17	—	236‡	—	242‡	—	1.03
B.	+/*Sxl*$^{t}\#^{1}$	×	+/*Y*	29	1331	1279	0	463	<0.001	0.36
	da E(da)/++		+/+	17†	636	562	257	624	0.46	1.11
C.	+/*Sxl*$^{t}\#^{1}$	×	+/*Y*	29	631	573	562	571	0.98	1.00
	+/+		+/+	17	399	373	375	405	1.01	1.09

Progeny are from the following crosses:
A. +/*Binsinscy; da E(da) cn bw*/++++ ♀♀ × *cm ct*/*Y* ♂♂.
B. +++/*cm Sxl*$^{t}\#^{1}$ *ct; da E(da) cn bw*/++++ ♀♀ × *cm ct*/*Y* ♂♂.
C. +++/*cm Sxl*$^{t}\#^{1}$ *ct;* +/*CyO* ♀♀ × *cm ct*/*Y* ♂♂.
The females in all three crosses are sisters to minimize possible genetic background effects.
* For the low temperature data, progeny developed at 17° for 2 to 4 days, then were shifted to 25° to complete development.
† Only includes progeny produced at least 4 days after the shift down to 17°.
‡ Only includes progeny receiving the structurally normal *X* chromosomes to facilitate comparison with crosses B and C.

which parents and progeny were raised. At 17°, the viability of even $Sxl^{f\#1}/Sxl^{+}$ daughters increased to 46% and their Sxl^{+}/Sxl sisters were fully viable.

The response of *da E(da)/da*$^{+}$ *E(da)*$^{+}$ mothers and their $Sxl^{f\#}/Sxl^{+}$ daughters to a temperature shift from 29° to 17° was remarkably similar to that found previously for Sxl^{+}/Sxl^{+} daughters of *da/da* mothers. In both cases, several days were required following the shift down before daughter viability reached a maximum. For $Sxl^{f\#1}/Sxl^{+}$ daughters from cross B, relative viability during the first, second and third two-day periods following a shift down was 2.2% (3/137), 22% (38/173), and 41% (73/178). This result indicates that maternal temperature is at least as important a factor as zygotic temperature in determining viability of $Sxl^{f\#1}/Sxl^{+}$ daughters. Recovery of their Sxl^{+}/Sxl^{+} sisters was more rapid, rising from 55% (78/143) immediately before the shift down, to 100% (132/131) from the first two-day collection at 17°.

The results from cross C in Table 6 shown once again that $Sxl^{f\#1}$ is not a dominant lethal at low or high temperatures in the absence of additional *da* or *Sxl* mutations in the mother or the zygote. For this reason, throughout this paper I have used the lower case (*f#1*) to designate what is actually a recessive *Sxl* allele in the absence of genetic modifiers.

VI. *Dominant temperature-sensitive effects of a* da *deficiency*

All aspects of the *da* phenotype are temperature dependent. The most straightforward explanation for these temperature effects is that they reflect temperature sensitivity of the mutant *da* gene product. An earlier paper (CLINE 1976) gave several arguments in favor of the hypothesis that the *da* mutation renders that gene's product thermally unstable. The similarity between the phenotypic effects of temperature on the recessive *da* maternal effect (suffered by Sxl^{+}/Sxl^{+} daughters) and the effects on the dominant *da* maternal effect (suffered by $Sxl^{f\#1}/Sxl^{+}$ daughters) reported here seemed to further support the earlier hypothesis. The discovery of the strong dominant *da* effect allows a more direct test of this hypothesis. If temperature dependence of the dominant effect were due to thermal instability of the mutant *da* gene product, the phenotype of a *da* deficiency should not be temperature sensitive. A deficiency should alter only the level of wild-type *da* gene product, not its thermal character. Thus, the deficiency phenotype at both 17° and 29° should be the same as that of the mutant *da* allele at 29°.

The experiment described in Table 7 shows that the *da* deficiency does exhibit a dominant maternal effect at 29° comparable to that caused by the mutant *da* allele at 29°; however, the maternal effect of the deficiency, like that of the mutant allele, is temperature dependent. With parents and progeny at 29°, only 14% of the $Sxl^{f\#1}/Sxl^{+}$ daughters survived (cross A, first row) from mothers carrying the deficiency. As with the *da* mutation, their Sxl^{+}/Sxl^{+} sisters were fully viable (95%). But at 17°, the viability of both classes of daughters from the deficiency mothers was normal. The control, cross B, showed that the dominant maternal effect at 29° was due to the maternal *da* deficiency. The deficiency and the *da* allele did differ somewhat with respect to the effect of a

TABLE 7

A dominant, temperature-sensitive, female-lethal maternal effect of a deficiency of the da *locus*

Cross ♀♀ × ♂♂	Temp† °C	Progeny genotypes w/respect to *Sxl* — Sons		Daughters		Relative viability of daughters *Sxl*$^{f\#1}$/*Sxl*+ ♀	*Sxl*+/*Sxl*+ ♀
		Sxl$^{f\#1}$/*Y*	*Sxl*+/*Y*	*Sxl*$^{f\#1}$/*Sxl*+	*Sxl*+/*Sxl*+	*Sxl*$^{f\#1}$/*Y* ♂	*Sxl*+/*Y* ♂
		1	2	3	4	5	6
A. *Sxl*+/*Sxl*$^{f\#1}$; × *Sxl*+/*Y*	29	511	539	73	514	0.14	0.95
da^{-}/*da*+	17	410	411	409	410	1.00	1.00
B. *Sxl*+/*Sxl*$^{f\#1}$; × *Sxl*+/*Y*	29	274	282	265	285	0.97	1.01
da+/*da*+	17	508	541	491	540	0.97	1.00
C. *Sxl*+/*Sxl*$^{f\#1}$; × *Sxl*$^{f\#1}$/*Y*	29	586	632	171	—	0.29	—
da^{-}/*da*+	17	573	548	588	—	1.03	—
D. *Sxl*+/*Sxl*+*; × *Sxl*$^{f\#1}$/*Y*		*Sxl*+/*Y*	*Sxl*+*/*Y*	*Sxl*+/*Sxl*$^{f\#1}$	*Sxl*+*/*Sxl*$^{f\#1}$	*Sxl*+/*Sxl*$^{f\#1}$ / *Sxl*+/*Y*	*Sxl*+*/*Sxl*$^{f\#1}$ / *Slx*+/*Y*
da^{-}/*da*+	29	465	247	60	248	0.13	0.53
	17	417	235	441	348	1.06	0.83

Progeny from the crosses:
A. +++/*cm Sxl*$^{f\#1}$*ct; Df(2L)J-der-27*/+ ♀♀ × *cm ct*/*Y* ♂♂.
B. +++/*cm Sxl*$^{f\#1}$*ct*; +/*In(2L+2R), Cy* ♀♀ × *cm ct*/*Y* ♂♂.
C. +++/*cm Sxl*$^{f\#1}$*ct; Df(2L)J-der-27*/+ ♀♀ × *cm Sxl*$^{f\#1}$ *ct*/*Y* ♂♂.
D. +/*Binsinscy*; *Df(2L)J-der-27*/+ ♀♀ × *cm Sxl*$^{f\#1}$*ct*/*Y* ♂♂. The *Binsinscy X* = *Sxl*.+*

To minimize differences in genetic background, all females used for crosses A through D were progeny from the same cross: *Binsinscy*/*cm Sxl*$^{f\#1}$*ct* ♀♀ × +/*Y*; *Df(2L)J-der-27*/*In(2L+2R), Cy* ♂♂. Effects of the deficiency on the development of progeny from this cross could be seen, but they appeared to be unrelated to the *da-Sxl* interaction. Both sexes of deficiency-bearing progeny eclosed 24–48 hr later than non-deficient siblings. Both sexes of deficiency-bearing progeny showed decreased viability (viability relative to nondeficient siblings at 29.5° was 85% for daughters, 52% for sons) but *Sxl*$^{f\#1}$/*Sxl*+; *Df(2L)*/+ daughters were actually more viable than their *Binsinscy*/+; *Df(2L)*/+ sisters (198/162 = 1.22 at 29.5°).

† Temperature at which parents were conditioned for at least 2 days (29°) or at least 4 days (17°), and at which the progeny were raised.

temperature shift. The following values for $Sxl^{f\#1}/Sxl^+$ daughter viability were observed the day immediately preceeding and the five days following a shift of cross A parents from 17° 29°: 101% (165/163) preshift; 104% (70/67); 45% (44/98); 17% (26/157); 12% (11/93); and 19% (15/81). Recall that the viability of $Sxl^{f\#1}/Sxl^+$ daughters of da/da^+ mothers dropped sharply on the very first day following shift up, while in this case (da^-/da^+ mothers) the drop did not occur until the second day. The magnitude of the viability drop after several days was, however, greater than in the previous experiment.

In these studies of the dominant maternal effect, $Sxl^{f\#1}/Sxl^+$ daughters received their single Sxl^+ allele from their fathers. Although the data are not shown, crosses were also performed in connection with the experiments of Tables 5 and 6 in which the daughters received their single Sxl^+ allele from their mothers instead. These daughters were consistently more viable, suggesting a possible functional difference between maternal and paternal Sxl^+ alleles in the young embryo.

This effect can be seen in Table 7, cross C. The mothers in C were identical to those in A, but the fathers in C carried $Sxl^{f\#1}$ rather than Sxl^+. Thus the $Sxl^+/Sxl^{f\#1}$ daughters in C differed from those in A with respect to which parent contributed the Sxl^+ allele. At 29°, the patroclinous Sxl^+ allele allowed only 14% relative viability, (cross A, column 5), while the matroclinous Sxl^+ allele allowed 29% (cross C, column 5). As in A, daughters in C were not affected by the $Sxl^{f\#1}$ allele at 17°.

Despite this consistent difference, the results must be interpreted with caution. It is clear from the data presented in Tables 5 and 6 that the actual magnitude of the dominant maternal effect is sensitive to elements of the genetic background other than *da* and *Sxl*. This is also evident from cross D, Table 7. The viability of $Sxl^+/Sxl^{f\#1}$ daughters was 13% *vs.* 53% at 29° (column 5 *vs.* 6) depending on which specific Sxl^+-bearing *X* chromosome they received. The difference appears to be due to factors on the *X* chromosome distinct from the Sxl^+ gene itself. The daughters from crosses C and D whose viability is indicated in column 5 (29% *vs.* 13%) differ only with respect to whether or not their Sxl^+ chromosome could freely recombine with its homologue outside the *cm-ct* interval (recombinants within the interval were not included in Table 7 since their genotype with respect to *Sxl* was ambiguous). The homologue of the Sxl^+ chromosome in cross D was a balancer (Sxl^{+*}) that prevented recombination.

DISCUSSION

The results presented here allow a distinction to be made between maternal and zygotic functions of the da^+ gene, at least with respect to the sex-specific interaction between *da* and *Sxl*. This distinction has important practical implications, since it may allow one to separate the synthesis of a regulatory factor (in the mother) from its subsequent functioning (in the zygote) during embryogenesis. Furthermore, the zygotic gene regulated in this case is one that causes cells to choose between alternative developmental pathways related to sexual

dimorphism. An understanding of how genes such as these function is critical to our understanding of eukaryotic development and evolution.

While it is easy to imagine how two deleterious mutations can interact non-specifically to further reduce viability, the existence of one mutation that suppresses the deleterious effects of another has generally been taken to indicate a specific functional relationship between the two elements, even when the molecular mechanism of the interaction is unknown (see HARTMAN and ROTH 1973). For this reason, the behavior of $Sxl^{M\#1}$ seems particularly significant, as much for what it does not counteract of the *da* phenotype, as for what it does. Though $Sxl^{M\#1}$ effectively counteracts the diplo-*X* lethal maternal effect of *da*, it does not counteract two other aspects of the *da* phenotype that do not depend on the *X* chromosome: autosome balance: (1) the recessive lethal aspect that reduces the viability of *da/da* zygotes relative to their da/da^+ sibs, and (2) the female sterile aspect that grossly disrupts the structure of eggs of *da/da* mothers, killing progeny very early in development regardless of their *X:A* balance. $Sxl^{M\#1}$ thus permits a distinction to be made between two lethal effects of the *da* mutation: one involving a disruption in a maternal function of da^+ that specifically kills diplo-*X* progeny by interfering with *Sxl*-mediated zygotic processes; the other killing progeny of both sexes by interfering with an essential zygotic function of the da^+ gene.

The distinction between maternal and zygotic functions of da^+ can be inferred somewhat less directly from the interactions between *da* and Sxl^f-type alleles, and from da^+ gene dose effects. Earlier work (CLINE 1978) showed that a (strong) zygotic $Sxl^{f\#1}$ allele enhanced the already severe female-lethal effect of a maternal *da* mutant allele; the present report greatly extends that observation. *da* and *Sxl* mutations, which individually have no noticeable effect on viability, can combine to disrupt development of most daughters. Such strong, synergistic interactions are generally taken to indicate a common functional relationship between two genes, but the synergism observed in this case differs fundamentally from that typically observed with mutations of the maternal-effect, rescuable class where maternal and zygotic alleles of the same gene are thought to participate in the same developmental processes. In the case of *da*, the synergistic interaction is not between maternal and zygotic *da* alleles themselves, but rather between the maternal *da* alleles and the zygotic alleles of a different gene, *Sxl*. There is no equivalent dominant interaction between zygotic *da* and zygotic *Sxl* alleles or between maternal *da* and maternal *Sxl* alleles.

While there is certainly no synergistic interaction between maternal and zygotic *da* alleles, there may be a weak interaction between them. A clear distinction between maternal and zygotic *da* functions is further complicated by the fact that the recessive lethal (zygotic) aspect of the phenotype is consistently more severe for *XX* zygotes than for *XY* zygotes, suggesting a similarity between the recessive lethal and the sex-ratio maternal effect for which the gene was named. MANGE and SANDLER (1973) reasonably took this as evidence for a single, common maternal and zygotic function for *da*. This work suggests, however, that the situation is more complex, and that the complexities are due

to da^+ having several distinct maternal functions, one involving zygotic *Sxl* gene regulation in response to the *X*-chromosome:autosome (*X:A*) balance. SANDLER and coworkers (SANDLER 1970, 1972, 1975, 1977; MANGE and SANDLER 1973; PARRY and SANDLER 1974) have studied the possible role of da^+ in regulating genes in the heterochromatic regions of the Drosophila genome. *XX* and *XY* individuals differ both with respect to their heterochromatin, and with respect to processes that depend on the *X:A* balance. DOBZHANSKY and SCHULTZ (1934) showed that euchromatic regions of the *X* chromosome, not heterochromatic, are important in determining the *X:A* balance. Thus there could be two separate aspects of *da* gene function with effects that differ between the sexes.

SANDLER discovered similarities between *da* and a mutation called abnormal oocyte (*abo*) located just 2.5 cM to its right. He subsequently identified several other nearby genes that might be functionally related to *abo* and *da*. In this connection, it is worth mentioning that $Sxl^{M\#1}$ does not interact with the *abo* maternal effect, and $Sxl^{f\#1}$ interacts little, if at all (CLINE, unpublished observations). This lack of interaction between *abo* and *Sxl* is certainly consistent with the failure of *Sxl* to interact with aspects of even the *da* phenotype that are not sex-specific, since none of the mutations studied by SANDLER have sex-specific effects.

The results of this study bear on the relationship between the maternal *da* locus and the component of the egg that is presumably required for the functioning of the Sxl^+ locus in the embryo (BOWNES, CLINE and SCHNEIDERMAN 1977). The ability of the egg to support diplo-*X* development depends on the dose of maternal da^+ alleles. Furthermore, the effect of temperature on the *da* maternal effect is similar in both homozygous and heterozygous mutant conditions. Combined with previous observations on *da* temperature sensitivity (CLINE 1976), these two facts seem to support the idea that this gene codes for a maternally synthesized factor in the egg that functions after fertilization. The question of whether the product of the mutant gene is more thermolabile than that of the wild-type allele is complicated by the discovery that a deletion of the *da* locus manifests a dominant, temperature-sensitive phenotype not unlike that of the mutant allele.

The results of the present study have implications for future analysis of *da* and *Sxl* mutations. First, synergism between *da* and *Sxl* mutations and temperature sensitivity of the interaction can help in the identification and characterization of functionally related elements like Enhancer of daughterless, even if the effects of these elements on *da* or *Sxl* individually are very weak. Second, complementation analysis of *Sxl* alleles and further studies of their interaction with *da* should take into account possible differences in effectiveness between maternal and paternal *Sxl* alleles in the zygote; such differences should not automatically be ascribed to maternal effects *per se*. Third, quantitative analysis of *da* maternal effects should include characterization of the *Sxl* alleles present to exclude possible complications by subtle *Sxl* mutations of the subliminal type described here. Finally, detailed investigation of *da* effects on progeny morphogenesis should be interpreted with the multiple maternal and zygotic effects of

da in mind. The gene dose studies do suggest that abnormalities exhibited by progeny that carry at least one da^+ allele are unlikely to be related to effects on the zygotic expression of the *da* locus, a point that can somewhat simplify analysis of this pleiotropic mutation. It remains to be determined whether pleiotropy of the daughterless mutation is due to qualitatively different activities of the da^+ gene product (see KIRSCHNER and BISSWANGER 1976) or is, instead, due to a single gene product activity that affects different developmental processes.

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