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Abstract

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Sex determination in *Drosophila*: the X-chromosomal gene *liz* is required for *Sxl* activity

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In *Drosophila*, females require products of the gene *Sxl* for sex determination, dosage compensation and fertility. I show here that the X-chromosomal gene *liz*, located in 4F1 to 4F11 and previously called *fs(1)1621*, provides maternal and zygotic functions necessary for *Sxl* activity in germ line and soma. In XX animals, the mutation Sxl^{M1} which was reported to express the female-specific functions of *Sxl* constitutively can rescue all phenotypes resulting from lack of *liz* product. XY animals carrying Sxl^{M1} and lacking maternal or zygotic *liz* activity survive as males with some female traits. A stock was constructed in which the females are *liz* Sxl^{M1}/*liz* Sxl^{M1} and males *liz* Sxl^{M1}/*Y*. This shows that Sxl^{M1} is not truly expressed constitutively in animals with an X:A ratio of 0.5, but requires activity of *liz* for initiation or maintenance.

Key words: dosage compensation/*Drosophila*/germ line/sex determination

Introduction

In *Drosophila melanogaster* the primary signal for sex determination is quantitative. The number of X chromosomes is compared to the number of autosomes: with an X:A ratio of 1 the female pathway is chosen, with a value of 0.5 the zygotes develop as males. The key gene *Sex-lethal* (*Sxl*) transduces this quantitative signal into differential gene activity (Cline, 1978, 1983a). Around blastoderm stage, *Sxl* is irreversibly activated in females and left inactive in males (Sanchez and Nöthiger, 1983). *Sxl* activity is required for the differentiation of female sexual structures and for cells to choose the female mode of dosage compensation. As a consequence of its presence, the two X chromosomes are transcribed at a low level, whereas without *Sxl* activity, they are hyperactive which is characteristic of the single X of males and which is lethal to XX flies (Lucchesi and Skripsky, 1981; Gergen, 1987). XX tissue that lacks *Sxl* activity due to the loss-of-function mutation Sxl^{fl}, develops male structures (Sanchez and Nöthiger, 1982). (For general reviews about sex determination see Baker and Belote, 1983; Nöthiger and Steinmann-Zwicky, 1985.)

The nature of the primary signal, the X:A ratio, is still elusive. To activate *Sxl*, maternal activity of the gene *da* is required (Cronmiller and Cline, 1987). In two instances, evidence for zygotic positive regulators of *Sxl* has been published. These pointed to region 3E8 to 4F11 (Steinmann-Zwicky and Nöthiger, 1985) and to the gene *sisterless-a* (*sis-a*) located in 10B (Cline, 1986). The mutation *sis-a* was shown to be a female-specific lethal. A constitutive mutation of *Sxl*, Sxl^{M1} (Cline, 1978, 1979), however, rescues mutant females which suggests that *sis-a* activity is required for Sxl^{+} activation (Cline, 1986). We have identified region 3E8 to 4F11 on the following grounds (Steinmann-Zwicky and Nöthiger, 1985). (i) Genotype *X/Dp 1A to 7D* differentiates female structures in clones and is lethal to whole animals. Genotype *Df 3E8 to 4F11/Dp 1A to 7D*, however, produces males, some of which survive. We concluded that two doses of region 3E8 to 4F11 were required for cells to choose the female pathway. (ii) Heterozygous animals of genotype *Df 3E8 to 4F11*/Sxl^{fl} are often lethal or develop as intersexes of a mosaic type. Some male features appear in flies that are, however, mostly female. These observations indicated that an element within region *Df 3E8 to 4F11* and *Sxl* were part of the same network, namely the regulation of sex determination. Lethality was interpreted as a consequence of improper dosage compensation. (iii) Duplicating region 3E8 to 4B1 was found to be lethal to males. Some males carrying the mutation Sxl^{fl}, however, were found to survive with such a duplication. This suggested that the duplication activated the Sxl^{+} gene to a level that is lethal to males. Sxl^{fl}, however, did not rescue all the males carrying the duplication as would have been predicted with a simple model of one gene activating Sxl^{+} (M.Steinmann-Zwicky, unpublished).

Here I show that region 3E8 to 4F11 does indeed contain at least one major sex determining locus. It is located within region 4F1 to 4F11, not within 3E8 to 4B1. The gene *liz*, previously identified as necessary for female fertility (*fs(1)1621*, Gans *et al.*, 1975), also provides maternal as well as zygotic activity which is required for survival and differentiation of females. Epistatic relationships between *liz* and loss-of-function or gain-of-function alleles of *Sxl* show that liz^{+} is needed for initiation and/or maintenance of Sxl^{+}, in germ line and soma. The name *liz* stands for Elizabeth I of England, who shares some of the mutant's characteristics.

Results

The analysis of region 3E8 to 4F11 involved three steps. First, a characterization of its interactions with *Sxl*; second, mapping by deficiencies the effects described; and third, an analysis of specific mutations within the defined region for interactions with *Sxl*.

Interactions with Sxl

Maternal and zygotic effects of Df 3E8 to 4F11. To test for interactions among the three elements *Df 3E8 to 4F11*, Sxl^{fl} and *sis-a* and to distinguish between maternal and zygotic effects, I analysed a series of crosses. Table I shows that females of genotype *Df 3E8 to 4F11/FM7* produce substantially fewer daughters than expected when crossed with males

Table I. Interaction between *Df 3E8 to 4F11* or *Df 3F-4F* and Sxl^{fl} or *sis-a*

father	+/Y			Sxl^{fl}/Y			*sis-a*/Y		
mother	♀	♂	♀/♂	♀	♂	♀/♂	♀	♂	♀/♂
Df 3E8 to 4F11	426	–	1.45	3	–	[0.01]	213	–	[0.50]
FM7	474	294	1.61	173	536	[0.32]	490	428	[1.14]
Df 3E8 to 4F11				–	–	[–]			
bi ct				4	320	[0.01]			
Df 3F to 4F				–	–	[–]			
cho				18	447	[0.04]			

Progeny of the different crosses were counted and the sex ratio was calculated as the fraction of females with either the deficiency or the homologous X chromosome to males. Males with the deficiency do not survive. Boxed sex ratio values point to female-specific lethality, underlined numbers indicate that these females were sterile. The balancer chromosome *FM7* is semi-lethal so that all sex ratio values of crosses involving mothers of genotype *Df 3E8 to 4F11/FM7* are too high. Thus, the interaction between *Df 3E8 to 4F11* and Sxl^{fl} or *sis-a* is even stronger than it appears from these crosses. The chromosome carrying Sxl^{fl} was marked with *y w cm ct f*, and *sis-a* was linked to *y*. *Df 3F to 4F* is *Df(1)cho 19, y w*.

Table II. Zygotic interaction between *Df 3E8 to 4F11* and Sxl^{fl} or *sis-a*

father	*Df 3E8 to 4F11*/Y			*Df 3F to 4F*/Y		
maternal chromosome	♀ Df	♂ Y	♀/♂	♀ Df	♂ Y	♀/♂
+	245	205	1.20			
Sxl^{fl}	87	187	[0.47]	34	113	[0.30]
sis-a	81	105	[0.77]			

Females were of genotype *y cho* or *y cho cm* Sxl^{fl} *ct f/FM7* or *y cho sis-a/FM7*. Males carried in addition to the X-chromosomal deficiency a duplication on the second chromosome: genotype *Df 3E8 to 4F11/Y*; $T(1;2)w^{64b13}$ /+ or *Df 3F–4F/Y*; $T(1;2)w^{64b13}$/+. Progeny with a balancer chromosome were not scored. Progeny with a duplication, identified by a cho^+ eye colour, are not shown. See also legend to Table I.

carrying Sxl^{fl}. A lethal zygotic effect can be seen, since progeny of genotype *Df 3E8 to 4F11/*Sxl^{fl} are rare. The few survivors are sterile and show male traits such as a few sex comb teeth or male pigmentation on the abdomen (described in Steinmann-Zwicky and Nöthiger, 1985). Ovaries are present and filled with eggs that, however, show abnormal chorion appendages which are often fused and enlarged (Figure 1a). A maternal effect of the deficiency is also revealed: whereas sisters of genotype *FM7/*Sxl^{fl} are expected as frequently as males carrying *FM7*, less than half that number is found. Both the zygotic and the maternal effects appear even more clearly if the mothers carry other X chromosomes than the balancer *FM7*. A second deficiency that I induced on a different chromosome (see below) shows that the effects observed correlate with the absence of the region analysed, and are not due to some other defect on the chromosome carrying *Df 3E8 to 4F11*.

The reciprocal cross Sxl^{fl}*/FM6* × *Df 3E8 to 4F11/Y* again shows the zygotic lethality of genotype *Df 3E8 to 4F11/*Sxl^{fl}, now displayed in the absence of a maternal effect of the deficiency (Table II). All 87 survivors were sterile and most of them had some male transformations. A cross involving a different, overlapping deficiency gave

Table III. Sxl^{M1} rescues the zygotic phenotype resulting from an interaction between *Df 3E8 to 4F11* and Sxl^{fl}

father		Sxl^{fl}/Y	
mother	♀ Sxl^f	♂ Y	♀/♂
Df 3E8 to 4F11 Sxl^{M1}	211	–	[1.09]
FM7	205	193	[1.06]
Df 3E8 to 4F11 Sxl^{M1}	393	–	[0.38]
cm ct	152	1021	[0.15]
Df 3E8 to 4F11 Sxl^{M1}	179		[0.66]
bi ct	149	271	[0.55]

The *Df 3E8 to 4F11* Sxl^{M1} chromosome was also marked with *cm*. Fathers were of genotype *y w cm* Sxl^{fl} *ct f/Y*. See also legend to Table I.

similar results. This shows that all three phenotypes observed here – lethality, sterility and the appearance of male traits – are due to the zygotic lack of some gene activity within region 3E8 to 4F11. Maternal lack of gene activity can, however, also be lethal to females.

Crosses involving the mutation *sis-a* reveal some zygotic lethal and maybe a slight maternal lethal effect of the deficiency (Tables I and II). Females of genotype *sis-a/Df 3E8 to 4F11*, however, are fully fertile, which shows that this mutant combination is less deleterious than combining the deficiency with Sxl^{fl}. Control crosses with wild-type males show no skewed sex ratio among progeny.

Thus, the most striking dominant zygotic and maternal effects of the deficiency are visualized in a cross that produces daughters carrying only one functional Sxl^+ allele. Obviously there is an interaction between region 3E8 to 4F11 and the gene *Sxl*, indicating that both elements must regulate the same pathway. The results, however, do not tell us which element controls which or if they are ordered in a hierarchical series.

Sxl^{M1} rescues the zygotic effect of Df 3E8 to 4F11. The lethality of females of genotype *Df 3E8 to 4F11/*Sxl^{fl} can be explained if we postulate that the Sxl^+ allele present on the chromosome carrying the deficiency is either not activated or not sufficiently activated. Replacing this wild-type allele by the constitutive mutation Sxl^{M1} should then rescue the females. Table III shows that indeed females of genotype *Df 3E8 to 4F11* Sxl^{M1}*/*Sxl^{fl} are rescued. In all three crosses these females were fertile, had no male transformations and appeared slightly more frequently than their sisters. If a constitutive mutation rescues the effect of a lack of function mutation in another gene, the former gene must act after the latter gene (Baker and Ridge, 1980; Hodgkin, 1980). The observation that Sxl^{M1} rescues the zygotic lethal and the sex transforming effect of the deficiency suggests that an element within region 3E8 to 4F11 is involved in the activation of Sxl^+ in females. In the absence of two zygotic regions 3E8 to 4F11, Sxl^+ is not properly activated, which becomes visible in females carrying only one functional Sxl^+ allele.

From the results shown in Table III, we see that the maternal effect is partly, but not totally, rescued by Sxl^{M1}. A partial rescue seems to take place in daughters that themselves carry Sxl^{M1}, and maybe also to a lesser degree in their sisters.

Table IV. Mapping by deficiencies the region that interacts with *Sxl*

		ec	cho	mdl	bi	rb	peb	ecl	fl(1)302	ovo	rg	region interacting with Sxl	breakpoints
Df(1)HC	244	—	—	—	—	—	—	—	—	—	—	—	3E8-4F11
Df(1)RC	40	+	+	+	—	—	—	—	—	—	—	+	4B1-4F1
Df(1)cho	3	—	—	+	+	+	+	+	+	+	+	+	3C-3F
	5	—	—	+	+	+	+	+	+	+	+	+	3D-4A
	10	—	—	+	+	+	+	+	+	+	+	+	
	2	—	—	—	+	+	+	+	+	+	+	+	3E-4A
	6	—	—	—	+	+	+	+	+	+	+	+	1F-4B
	7	—	—	—	+	+	+	+	+	+	+	+	2E/F-4B
	8	—	—	—	+	+	+	+	+	+	+	+	3C-4A
	24	—	—	—	+	+	+	+	+	+	+	+	
	25	—	—	—	+	+	+	+	+	+	+	+	
	23	—	—	—	—	—	—	—	—	+	+	+	3E/F-4F
	19	+	—	—	—	—	—	—	—	—	—	—	3F-4F
Df(1)rb	23	—	—	—	—	—	—	+	+	+	+	+	
	34	—	—	—	—	—	—	+	+	+	+	+	
	29	+	+	+	—	—	—	—	—	+	+	+	
	35	+	+	+	—	—	—	—	—	+	+	+	
	48	+	+	+	—	—	—	—	—	+	+	+	
	27	+	+	+	—	—	+	+	+	+	+	+	
	42	+	+	+	—	—	+	+	+	+	+	+	4B-4C
	44	+	+	+	—	—	+	+	+	+	+	+	
	50	+	+	+	—	—	+	+	+	+	+	+	
	14	+	+	+	—	—	—	—	—	—	—	+	
	13	+	+	+	+	—	—	—	—	+	+	+	
Df(l)ovo	15	—	—	—	—	—	—	—	—	—	—	—	
	44	+	+	+	—	—	—	—	—	—	—	—	
	41	+	+	+	+	—	—	—	—	—	—	—	
	4	+	+	+	+	+	+	+	+	—	—	+	
	14	+	+	+	+	+	+	+	+	—	—	+	

The bars show the extent of the deficiencies. The symbol '+' stands for the wild-type state of a gene or for a region being present.

Mapping the region that interacts with Sxl

To map the region that interacts with *Sxl*, I induced a series of deficiencies that uncover parts of region 3E8 to 4F11. For their isolation I used three markers: *cho* located at the distal end; *rb* in the middle; and *ovo* at the proximal end of 3E to 4F. Females carrying the various putative deficiencies were crossed to males mutant for one of several recessive genetic markers known to be located in the region (for a description of the markers used see Materials and methods). The results, summarized in Table IV, show the size of each deficiency. They also made it possible to order the genetic markers relative to each other. In some cases the break-points were determined by cytological studies of polytene chromosomes.

The first two lines show deficiencies whose effects were described before: the former deletes the region that interacts with Sxl^{fl}, the latter does not (Steinmann-Zwicky and Nöthiger, 1985). All but four of the newly induced deficiencies did not show the lethal interaction with Sxl^{fl}. *Df(1) cho 19* and *Df(1) ovo 15*, which uncover most of the analysed region, as well as two smaller deficiencies, *Df(1) ovo 44* and *Df(1) ovo 41*, clearly show both the maternal and the zygotic effect described in the previous section. Lethality, sterility and sex transformations (sex combs and male pigmentation) were observed with all four deficiencies. Table IV indicates that one gene regulating *Sxl* must be located to the right of *rg*, which is the proximal-most marker used to estimate the size of the various deficiencies. Deleting this gene alone and one *Sxl* allele could lead to the phenotypes scored as the interaction with *Sxl*. Alternatively, the interaction with *Sxl* might only be revealed when several genes located within 3E to 4F are deleted, one of these genes being located to the right of *rg*.

To distinguish between these two possibilities, I started to investigate the effects that several discrete mutations could have in combination with Sxl^{fl}. I chose mutations located within region 3E8 to 4F11 and known to affect only females. Since one of the phenotypes resulting from an interaction with Sxl^{fl} is sterility, I decided to analyse two female sterile mutations *fs(1)1621* (Gans *et al.*, 1975) and *ovo* (Oliver *et al.*, 1987). In an EMS mutagenesis I had isolated one female-specific lethal within region 3E8 to 4F11 (see Materials and methods) that I named *fl(1)302*.

Interactions between mutations specifically affecting females

The three zygotic phenotypes described above, lethality, sterility and male transformations of flies of genotype *Df 3E8 to 4F11/*Sxl^{fl}, were all rescued by the presence of the constitutive mutation Sxl^{M1}. Therefore, mutations in genes whose absence leads to the observed phenotypes in combination with Sxl^{fl} are expected to be rescued by Sxl^{M1} as well. Females of genotype *Df 3E8 to 4F11*Sxl^{M1}*/fl(1)302* were still lethal, and genotype *Df 3E8 to 4F11* Sxl^{M1}*/ovo* gave sterile females with ovaries devoid of any germ cells, as is typical of ovaries homozygous for *ovo* (Oliver *et al.*, 1987). Thus, both mutations *fl(1)302* and *ovo*, being unaffected by Sxl^{M1}, must act downstream of *Sxl* or in a

Table V. Tests for zygotic and maternal effects resulting from an interaction between genes *Sxl*^{fl}, *liz* and *sis-a*

father	+/Y			*Sxl*^{fl}/Y			*liz*/Y			*sis-a*/Y		
mother	♀	♂	♀/♂	♀	♂	♀/♂	♀	♂	♀/♂	♀	♂	♀/♂
liz	243	205	1.19	3	211	[0.01]				188	284	[0.66]
FM3	214	–	1.04	11	–	[0.05]				126	–	[0.44]
Sxl^{fl}	534	282	1.89				168	213	[0.79]	260	539	[0.48]
FM6	482	322	1.50				217	179	1.21	530	417	1.27
sis-a	554	414	1.34	296	719	[0.41]	180	148	1.22			
Binsinscy	422	106	3.98	704	211	3.34	130	37	3.51			

Chromosomes were marked as follows: *liz v, y w cm Sxl*^{fl} *ct f, y sis-a*. Chromosome *FM3* is male lethal; to calculate sex ratios, the number of viable brothers was used. *Binsinscy* is semi-lethal; therefore all sex ratios involving this chromosome are substantially too high. See also legend to Table I.

different pathway. In contrast, females of genotype *Df 3E8 to 4F11 Sxl*^{M1}*/fs(1)1621* turned out to be fully fertile with large, normal looking ovaries. The control genotype *Df 3E8 to 4F11/fs(1)1621* had small ovaries filled with undifferentiated germ cells and only very rare oocysts with nurse cells, as was described for females of genotype *fs(1)1621/fs(1)1621* (Gans *et al.*, 1975; Gollin and King, 1981). Thus, *fs(1)1621* must act upstream of *Sxl*. Therefore, it is a good candidate for a gene required in two copies to ensure proper activation of *Sxl*. When more was known about *fs(1)1621* it was renamed *liz*, which is the name used to designate it in the following sections.

To test for possible zygotic or maternal effects, crosses were performed in which the females carried either one of the mutations *fl(1)302, ovo*, or *liz* and a balancer chromosome, and the fathers were mutant for *Sxl*^{fl}. Both females of genotype *fl(1)302/FM7* and *ovo/FM3* gave a normal number of progeny of both sexes, carrying all chromosomal combinations expected (data not shown). Mothers of genotype *liz/FM3*, however, gave almost exclusively male progeny (Table V). Thus, mutation *liz* has a dominant maternal female-lethal effect that becomes apparent in daughters carrying only one functional *Sxl* allele. A zygotic interaction with *Sxl*^{fl} is also revealed. The few surviving females of genotype *liz/Sxl*^{fl} were sterile and had few spots of male pigmentation on tergites 5 and 6. The reciprocal cross also showed that some flies of genotype *liz/Sxl*^{fl} are lethal (Table V). The survivors were largely sterile: groups of 40 females kept separately produced between 20 and 40 offspring in a period of two weeks. Their ovaries contained eggs in which some chorion appendages were abnormal, as was observed in genotype *Df 3E8 to 4F11/Sxl*^{fl}. Few male spots were seen on tergites 5 and 6, but sex comb teeth were never differentiated. These results show that one gene, *liz*, when mutant, causes several phenotypes that have been found as an interaction between *Df 3E8 to 4F11* and *Sxl*^{fl}. The maternal lethal effect as well as zygotic lethality and some male transformations are all due to lack of *liz* activity. That sex combs and total sterility were not observed in genotype *liz/Sxl*^{fl} can be explained by either of two ways. The mutation *liz* could still provide some gene activity that is lacking in the deficiency, which would lessen the severity of the interaction with *Sxl*^{fl}. Alternatively, a different locus might provide some necessary gene activity. This again would mean that the strongest phenotype results from a cumulative effect of at least three missing genes (*liz, Sxl* and a third gene). If *liz* is the only gene within 3E8 to 4F11 to act upstream of *Sxl*, then it has to be located proximal to *rg* as predicted above. Crossing females carrying the various deficiencies shown in Table IV to males mutant for *liz* gave a clear correlation. Only deficiencies showing the interaction with *Sxl*^{fl} were also sterile *in trans* over *liz*. This in fact places *liz* to the right of *rg* on our deficiency map. The breakpoints of *Df(1)HC 244* and *Df(1)RC 40* tell us that *liz* lies within region *4F1 to 4F11* (Table IV).

It appears that *liz* is the gene whose absence in *Df 3E8 to 4F11* caused such a dramatic maternal effect on daughters carrying an *Sxl*^{fl} mutation. Zygotic lethality and, at least in part, sex transformations and sterility of the surviving *Df 3E8 to 4F11/Sxl*^{fl} females are also due to *liz*. A similar zygotic effect can be seen when females carrying the mutation *sis-a* are crossed to males mutant for *Sxl*^{fl} or vice versa (Table V). Some flies of genotype *sis-a/Sxl*^{fl} are lethal. Surviving females are fertile, a few of them have spots of male pigmentation on tergites 5 or 6. In no case, however, was there evidence for a maternal effect caused by the absence of the gene *sis-a*. When females carrying *liz* are crossed to males mutant for *sis-a*, the maternal effect of *liz* is visible since fewer daughters survive than expected. The reciprocal cross shows that *sis-a* has no maternal effect and that genotype *liz/sis-a* gives fully viable and fertile females.

To test whether the maternal presence of *Sxl*^{M1} alone could have some rescuing effect upon genotype *liz/Sxl*^{fl}, I crossed females of genotype *liz/Sxl*^{M1} to males carrying *Sxl*^{fl}. Many females now survive, but almost all of them carry *Sxl*^{M1}. The sex ratio calculated as the number of females with *Sxl*^{M1} relative to the number of males without *Sxl*^{M1} is 316:502 = 0.63. In this cross, *Sxl*^{M1} definitely seems to rescue females from the maternal effect of *liz* seen in Table V (sex ratio 0.05), but as was the case with the deficiency, this rescue is by no means complete. Only three females survived without the mutation *Sxl*^{M1} which gives a sex ratio of 3:502 = 0.01. Therefore, the maternal presence of *Sxl*^{M1} has no rescuing activity on genotype *liz/Sxl*^{fl}.

The complete cross performed with all the results is represented in Figure 2. Totally unexpected was the finding that males carrying *Sxl*^{M1} can survive if they are also mutant for *liz* (evidence that the 24 *cm* males scored were of genotype *liz Sxl*^{M1}/*Y* is given in the legend to Figure 2). Such males have various abnormalities. Their sex combs are mosaic with male and female bristles, their genitalia are sometimes reduced, sometimes slightly rotated, the sternite 6 is mostly covered with bristles as is typical for females, and a reduced female tergite 7 is sometimes present (Figure

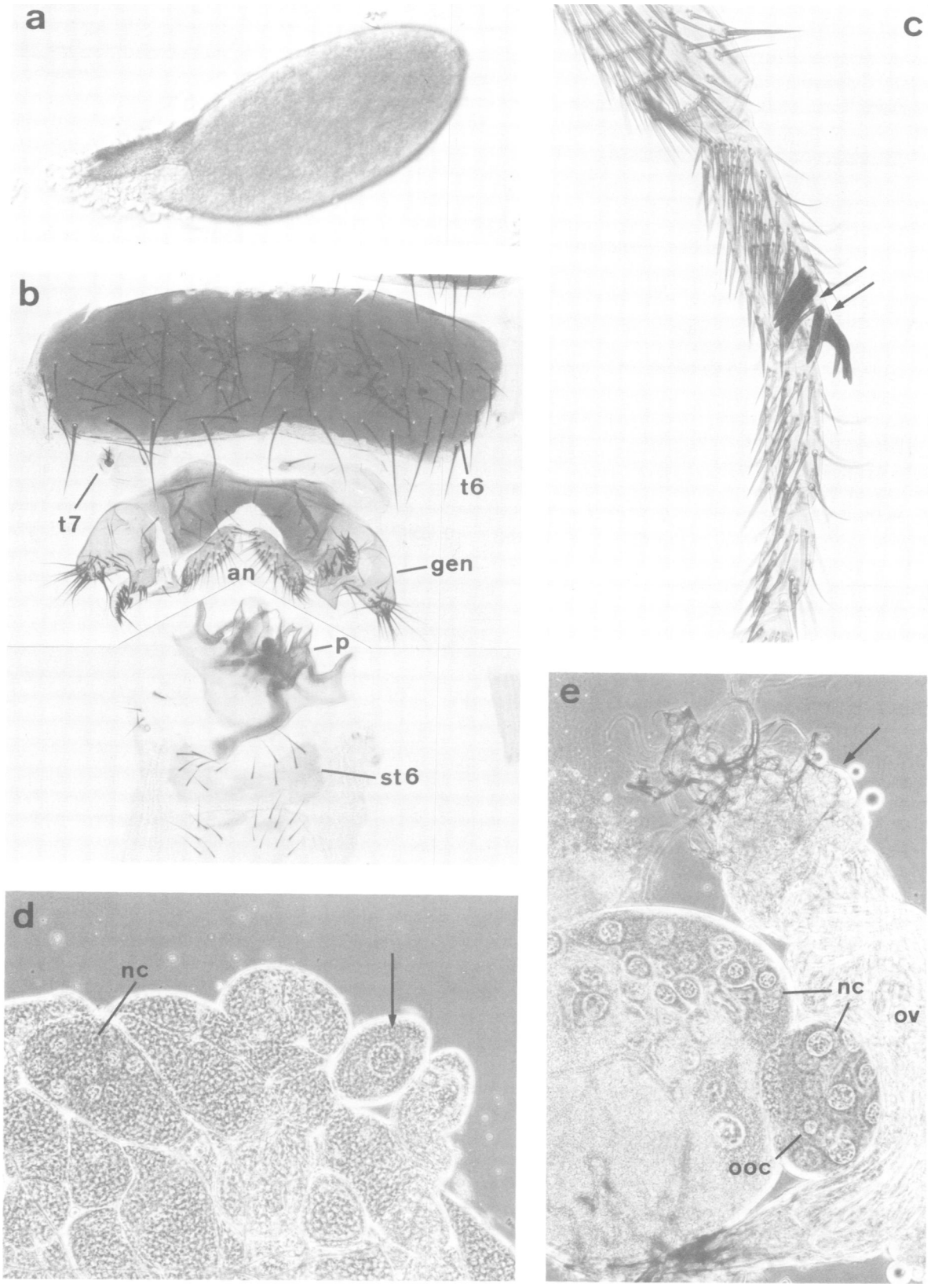

Fig. 1. (**a**) Abnormal chorion appendages of eggs formed by females of genotype *Df 3E8 to 4F11/Sxl*^{fl}. (**b**) *liz Sxl*^{M1}/*Y* males with a sternite six (st6) that is covered with bristles as is typical for females, and with some bristles of a tergite 7 (t7), which is also a female characteristic. Tergite 6 (t6) genitalia (gen) and analia (an) are male and well developed, the penis structure (p) of this individual shows slight defects. (**c**) Mosaic sex comb of *liz Sxl*^{M1}/*Y* males. Dark thick bristles are male, the arrows point to the slender female bristles. (**d**) Ovary of a female of genotype *liz/liz* whose mother was *Df 3E8 to 4F11 Sxl*^{M1}/*liz*. Ovarioles are formed, but these contain mostly undifferentiated germ cells. Only occasionally do egg chambers with a cluster of nurse cells (nc) differentiate. The arrow points to a single nurse cell that can be recognized due to the polytenization of its nucleus. (**e**) Gonads and oviduct (ov) of a female of genotype *Df 3E8 to 4F11 Sxl*^{M1}/*Sxl*^{fl} whose mother lacked *liz* product. The arrow points to an empty undifferentiated gonad; the other gonad contains oogenic cells, nurse cells (nc) and oocytes (ooc), although no ovary has been differentiated.

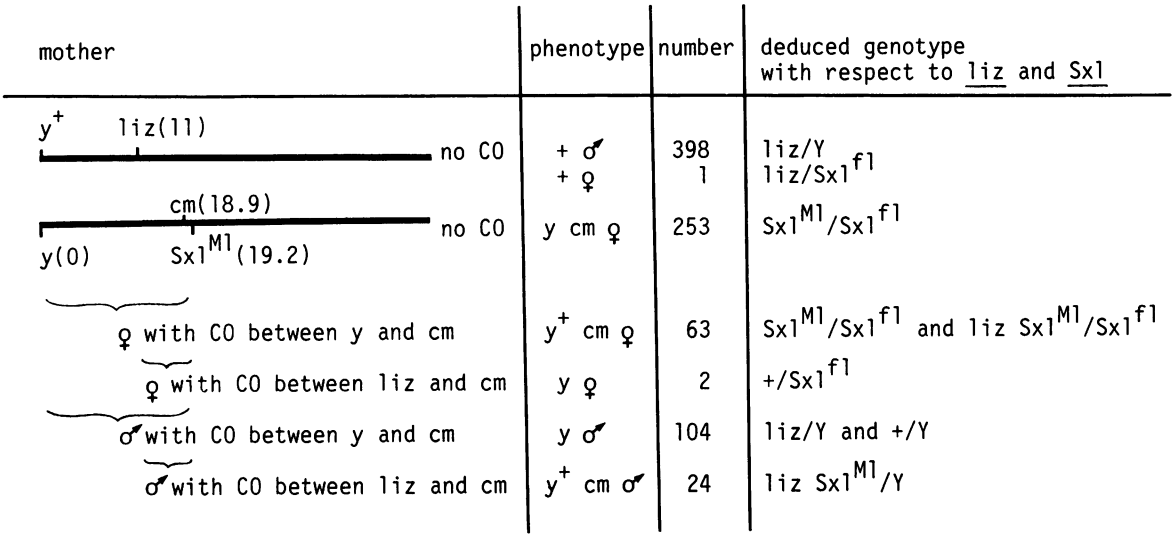

Fig. 2. Progeny of cross *y cm* Sxl^{M1}*/liz* × *cm* Sxl^{fl} *ct f/Y*. The markers are represented at their approximate position on the chromosome. Genetic map positions are given in parenthesis. Brackets mark regions in which a crossing over (CO) has taken place. Sxl^{M1} being lethal to males, 104 *y* males were produced as a result of a CO between *y* and *cm*. y^+ *cm* males can only appear as the reciprocal result of CO in the same interval. Such males were crossed to females mutant for *da*. This cross, kept at 25°C, produced 44 daughters and 70 sons, which shows that Sxl^{M1} is present in y^+ *cm* males and that it can rescue daughters of *da/da* mothers. A control cross with *liz/Y* males gave no female progeny. A CO between *y* and *liz* could not lead to viable *cm* males, since Sxl^{M1} males are lethal. In the interval between *liz* and *cm*, roughly 40 CO events are expected that could lead to double mutant males *liz* Sxl^{M1}*/Y*. 24 *cm* males carrying Sxl^{M1} were observed that can only be explained by such an event.

Table VI. Absence of maternal *liz* product

father	*liz/Y*			*liz* Sxl^{M1}*/Y*			Sxl^{fl}*/Y*		
mother	♀[a]	♂	♀/♂	♀[b]	♂	♀/♂	♀[b]	♂	♀/♂
Df 3E8 to 4F11 Sxl^{M1}	446	(2)	0.95	377	(7)	0.91	120	(4)	0.27
liz	250	469	0.53	408	416	0.98	–	446	–

Chromosomes were marked as follows: *Df 3E8 to 4F11 cm* Sxl^{M1}, *liz v*, *liz cm* Sxl^{M1}, *y w cm* Sxl^{fl} *ct f*.
[a]Females were classified using the marker *v* linked to *liz*. An occasional crossing over might separate the two mutations, but this remains negligible.
[b]Females were identified using *cm*, a marker that is very closely linked to Sxl^{M1}. ( ), these males are *cm* and show intersexual features. They are crossing over products of genotype *liz cm* Sxl^{M1}*/Y*. Other crossing over products are not listed separately. The possibility that the mutation *liz* is hypomorphic was neglected in the text, but has to be kept in mind. See also legend to Table I.

1b and c). Some of the males were fertile and, when crossed with females homozygous for the mutation *da*, rescued daughters which shows that they in fact carry the Sxl^{M1} allele. Viable males carrying the mutation Sxl^{M1} have never been reported before. That mutation *liz* should rescue such males seems at first glance puzzling. An attempt to understand the various effects of *liz* will be made in the Discussion.

Progeny of females lacking maternal liz activity. We have seen that *liz* produces both maternal and zygotic products. The question arose whether females with neither component could nevertheless survive. I crossed females lacking *liz* activity but fertile because of the presence of Sxl^{M1} to males of three different genotypes: males mutant for *liz*, males doubly mutant for both *liz* and Sxl^{M1}, and males carrying Sxl^{fl}. Table VI shows that some females of genotype *liz/liz* and lacking maternal product could survive. They had small ovaries that contained undifferentiated germ cells and few oocysts, which represents the typical *liz* phenotype (Figure 1d). Presence of only one Sxl^+ allele was more deleterious to females than having no *liz* product. In the absence of maternal *liz* activity, genotype *liz*/Sxl^{fl} is lethal. The few surviving females of genotype *Df 3E8 to 4F11* Sxl^{M1}/Sxl^{fl} often have missing legs or tergites and are sterile. Most of them have extremely small gonads that are completely empty. Rarely the gonads contain oogenic stages even though they do not show the typical differentiation of ovaries (Figure 1e). In some cases few ovarioles are found. These contain germ cells that clearly go through oogenesis. Females of the same genotype, but having been provided with maternal *liz* product, have no defects, have normal ovaries and are fertile (Table II). Therefore, in this genotype, maternal *liz* product is required for full viability and for formation of the ovary. A paternal Sxl^{M1} allele was very efficient in compensating for the missing *liz* activity. Females of genotype *liz/liz* Sxl^{M1} were fully viable and fertile although neither maternal nor zygotic *liz* product was available. All other females obtained in Table VI developed slowly and emerged on average two days after their brothers.

Males of genotype *liz* Sxl^{M1}*/Y* could be kept in a stock with females carrying compound X chromosomes. Thus, maternal *liz* product from two wild-type genes is not harmful to these males. Since males of genotype Sxl^{M1}*/Y* are lethal, it is the zygotic activity of liz^+ that kills them. To test whether a zygotic liz^+ gene needs maternal *liz* product to become active, I crossed females of genotype *Df 3E8 to 4F11* Sxl^{M1}*/liz* to males carrying *Df 3E8 to 4F11* and a duplication (Dp) covering the same region. If maternal *liz* is necessary to activate the zygotic *liz* genes, males of genotype *Df 3E8 to 4F11* Sxl^{M1}*/Y; Dp/+* should now be able to

survive although they have a wild-type *liz* gene. These males can be recognized because they carry the mutation *cm*, which is very closely linked to Sxl^{M1} and because they display the marker *Co*, which identifies the duplication. A total of 38 *Co cm* males were found. These males, whose genotype corresponds to $liz^+ Sxl^{M1}/Y$, show all the characteristics of the *liz* Sxl^{M1}/Y males: mosaic sex combs with male and female bristles and female-like sternites. In addition they often had defective tergites and their genitalia seemed to be more often abnormal. Eighty-eight wild-type males were also scored. Males with the marker *cm* are expected to occur as frequently as $+/Y$ males. The observation that fewer of them survive and that these have abnormalities, shows that this genotype is not fully vital. The conclusion, however, can be drawn, that males carrying Sxl^{M1} can survive in the absence of maternal or zygotic *liz* product, and that maternal *liz* product is involved in the activation of zygotic liz^+ genes.

Males carrying a duplication for liz are vital. Males of genotype *Df 3E8 to 4F11/Dp 1A to 7D* can survive or die as pharate adults, whereas genotype *X/Dp 1A to 7D* is not viable in whole flies and differentiates female tissue in clones (Steinmann-Zwicky and Nöthiger, 1985). The former genotype carries one liz^+ gene, the latter two. The question arises whether duplicating liz^+ in XY animals can produce a female signal. If this were the case, males carrying two liz^+ genes should be subvital. If *liz* acts through *Sxl*, males carrying two liz^+ genes and two Sxl^+ genes might be expected not to survive at all. To test this, I crossed females of genotype *Df(1)cho 23, y w* liz^+*/Df(1)HC 244,* liz^-*; Dp 3C to 5A,* $liz^+/+$ to males carrying a *Dp* Sxl^+ on a third chromosome and TM3 as its homologue. Four types of male zygotes are expected to be formed in equal numbers. The numbers of adult males scored were: (i) 145 animals with 2 liz^+ and 1 Sxl^+; (ii) 114 with 2 liz^+ and 2 Sxl^+; (iii) 176 control males with 1 liz^+ and 1 Sxl^+; (iv) 159 with 1 liz^+ and 2 Sxl^+. The males with 2 liz^+ and 1 Sxl^+ were vital, those with 2 liz^+ and 2 Sxl^+ were the least frequent class, but still present in substantial numbers. I conclude that duplicating liz^+ alone does not produce a strong female signal in males. It appears that Dp 1A to 7D contains several genes that, when duplicated, cause cells to choose the female pathway. If, however, region 3E8 to 4F11, that contains *liz*, is not duplicated as well, the female pathway is not chosen.

Discussion

Product of liz is required in females for Sxl^+ *activity*

Maternal and zygotic products of liz^+ act in females to ensure proper sex determination, dosage compensation and oogenesis. The mutation *liz* is defective for these functions: this becomes apparent in females of genotype *liz*/Sxl^{fl} some of which are lethal while the others are sterile and show male characters; in females that carry only one Sxl^+ gene and whose mother was heterozygous for *liz* which are poorly viable; in females homozygous for *liz* which are sterile; and in animals of genotype *liz* Sxl^{M1}/Y which now survive as males, whereas genotype Sxl^{M1}/Y is lethal to whole flies and was reported to be female in clones (Cline, 1979). In my experiments, sex-specific lethality was always taken to reflect upsets in dosage compensation, since it appears as a result of interactions between mutated *liz* alleles and *Sxl* mutations, a gene known to regulate this process.

The mutation Sxl^{M1} can rescue all female-specific defects caused by absence of *liz* product in the germ line and in the soma. This means that *liz* is required for the expression of Sxl^+. But even though *Sxl* product is required in all tissues for female differentiation, females of genotype *liz/liz* are only sterile, and neither lethal nor sex-transformed. Maternal product alone could be sufficient for activating all somatic female functions necessary for sex determination and dosage compensation. Some 50% of females homozygous for the mutation, however, survived in spite of the lack of maternal *liz* activity (Table VI, cross *Df 3E8 to 4F11* Sxl^{M1}/*liz* × *liz*). Although the phenotype of *liz/liz* was not significantly different from that of *Df 3E8 to 4F11/liz* (they both had ovaries filled with undifferentiated germ cells and an occasional oocyst), the mutation *liz* is probably hypomorphic. Females of genotype *Df 3E8 to 4F11*/Sxl^{fl} were definitely more affected than females of genotype *liz*/Sxl^{fl} in their somatic and in their germ line phenotypes. While the former had sex combs and were completely sterile, the latter displayed only weak sexual transformations and could have a few progeny. The mutation *liz* probably still provides soma and germ line with some residual function. Occasional activation of *Sxl* in the germ line can lead to the differentiation of an oocyst or to the differentiation of just one single nurse cell (Figure 1d). A rest of somatic function might be sufficient to keep about half of *liz/liz* daughters derived from *Df 3E8 to 4F11* Sxl^{M1}/*liz* mothers alive as females. This rest of *liz* activity, however, is neither sufficient to kill males of genotype *liz* Sxl^{M1}/Y nor to transform them into females, except for a few cells. Nevertheless, the possibility that *liz* is hypomorphic has to be kept in mind when analysing the data of Table VI. For reasons of clarity, I neglected this in the Results section. An alternative hypothesis that could explain why females with virtually no maternal nor zygotic *liz* activity can survive will be presented later.

Crossing females of genotype *Df 3E8 to 4F11* Sxl^{M1}/*liz* to *liz* Sxl^{M1}/Y males (Table VI) showed that Sxl^{M1} can rescue all daughters (genotype *liz/liz* Sxl^{M1} survived best). Two phenotypes resulting from lack of *liz* activity, however, are only rescued to a small extent. In both cases, females of genotype *Df 3E8 to 4F11* Sxl^{M1}/Sxl^{fl} are affected. If they receive only half the amount of maternal *liz* product many such females die (Table III). If they get no maternal product, the few surviving females have somatic defects and are sterile, most of them having no ovaries, while occasional germ cells are female (Table VI). Obviously, Sxl^{M1} does not express a somatic function that can be activated by maternal *liz* product.

I here want to argue that Sxl^{M1} is not a truly constitutive mutation. I will present evidence that it requires both *liz* product and an X:A ratio of 1 to become stably active, in every cell. That flies carrying Sxl^{M1} are in fact controlled by the X:A ratio can easily be shown. A stock can be constructed, in which all females are *liz* Sxl^{M1}/*liz* Sxl^{M1} and all males are *liz* Sxl^{M1}/Y. The only genetic difference between the two sexes now remains the X:A ratio.

Males of genotype liz Sxl^{M1}/Y *survive because their* Sxl^{M1} *allele is not active*

If Sxl^{M1} is active in males of genotype *liz* Sxl^{M1}/Y, only two different models can provide an explanation for the survival of these males. In the wild-type female, the gene products of liz^+ and Sxl^+ act in parallel to bring about female

differentiation, or liz^+ is activated by Sxl^+ and transmits the female signal. Both models, however, cannot explain why males carrying Sxl^{M1} and a liz^+ allele can survive if there is no maternal *liz* product, nor why Sxl^{M1} can rescue all female-specific phenotypes caused by lack of *liz* product. If, however, Sxl^{M1} is not expressed in males with no maternal or no zygotic liz^+, then all the data can be reconciled and explained with one model which assumes that *liz* acts upstream of *Sxl*.

Is there published evidence that Sxl^{M1} might not always be active? Initially, Sxl^{M1}, that carries a transposable element within the *Sxl* gene (Maine *et al.*, 1985), was believed to express constitutively all female-specific *Sxl* functions because of its ability to rescue daughters of females mutant for *da* (Cline, 1978). Newer results, however, indicate that Sxl^{M1} does not produce female-specific Sxl^+ activity throughout development. Using a hypomorphic mutation of the gene *runt* to monitor dosage compensation, Gergen (1987) could show that Sxl^{M1} remains without effect in male or female embryos at the blastoderm stage, a time at which Sxl^+ is already differentially regulated in the two sexes (Sanchez and Nöthiger, 1983; Gergen, 1987). Although it was shown that tissue of genotype Sxl^{M1}/O can differentiate female-specific structures, 2 out of 7 legs with Sxl^{M1}/O tissue in the sex comb region differentiated male sex comb teeth (Cline, 1979). It is possible that Sxl^{M1} was not active in these cells. Alternatively, the products of Sxl^{M1} might not always be sufficient for dictating the female pathway.

In the germ line, Sxl^+ activity is required for normal oogenesis (Schüpbach, 1985; Salz *et al.*, 1987). My results showing that Sxl^{M1} can rescue a germ line mutant for *liz* confirm such a requirement and indicate that, in the female, Sxl^{M1} expresses this function. Germ cells of genotype Sxl^{M1}/Y, however, can differentiate functional sperm when transplanted into viable males (Cline, 1983b; H.Schmid and M.Steinmann-Zwicky, unpublished results). This means either that Sxl^{M1} is not active in germ cells with only one X chromosome or that Sxl^{M1} activity alone does not dictate the female pathway.

A unifying hypothesis that explains all the results assumes that Sxl^{M1} is controlled by both the X:A ratio and by *liz* product. With an X:A ratio of 0.5, Sxl^{M1} may not become active in every cell, or Sxl^{M1} may not become stably activated. Maternal and zygotic product of liz^+ could help to initiate or maintain expression of Sxl^{M1}. This assumption is supported by the observation that tissue of genotype Sxl^{M1}/O is mostly female with some male cells, whereas genotype *liz* Sxl^{M1}/Y, or Sxl^{M1}/Y but lacking maternal liz^+ product, is male with some female cells. Such a mosaic pattern suggests that Sxl^{M1} needs *liz* product and an X:A ratio of 1 for stable activation.

Thus, Sxl^{M1} can be activated with a specific probability that depends on *liz* product and the X:A ratio. With no *liz* product and an X:A ratio of 0.5, this probability is lowest. With maternal and zygotic *liz* product and an X:A ratio of 0.5 it is significantly higher. Also in females with an X:A ratio of 1 but lacking *liz* product, Sxl^{M1} may not become and remain stably active in every cell, as demonstrated by the cases in which lack of maternal *liz* product was not rescued by Sxl^{M1}. An interesting situation is the few surviving females of genotype *Df 3E8 to 4F11* Sxl^{M1}/Sxl^{fl} (Table VI), most of which had undifferentiated gonads and only very rarely a few ovarioles. They often had missing legs or tergites or other defects. Such a pattern seems to be mosaic, as if most of the somatic cells forming the gonads and some other somatic cells had not activated their Sxl^{M1} allele.

liz and Sxl in the wild-type female

In the germ line of the wild-type female, Sxl^+ genes require zygotic liz^+ activity to be functional. But *liz* is also needed in the soma. Females that carry only one Sxl^+ gene, or one Sxl^{M1} allele, and Sxl^{fl} *in trans* are most heavily affected by the absence of *liz* product or genes (Tables I, II, III and VI). Because females of genotype *Df 3E8 to 4F11* Sxl^{M1}/Sxl^{fl} lacking maternal *liz* product are poorly viable, I have argued that Sxl^{M1} does not express a function that can be activated by maternal *liz*. This function can also be provided by Sxl^+. In fact, in some cases, deleting one Sxl^+ had a more dramatic effect than deleting *liz* (e.g. Table VI, sex ratio 0.27 versus 0.95). Somatic differentiation that is purely female and fully viable is obtained with maternal *liz* product from at least one liz^+ gene and with two Sxl^+ genes or with one Sxl^+ gene and maternal *liz* product from two liz^+ genes as well as two zygotic liz^+ genes. The product of *liz* might participate in the activation of Sxl^+ and/or help to keep the state of activity stable. Two Sxl^+ genes seem to be more active or more stably active than one. Cline (1984) has suggested that Sxl^{M1} can transactivate an Sxl^+ gene present in the same zygote. In my experiments, mutual transactivation between one Sxl^{M1} allele and an Sxl^+ gene or between two Sxl^+ genes could ensure that the functional *Sxl* genes are active and are kept stably active. If only one Sxl^+ and one zygotic liz^+ gene are present (genotype *Df 3E8 to 4F11*/Sxl^{fl}), some cells will become male which leads to mosaic females with patches of male tissue. In the germ line, mosaicism was not observed: cells enter oogenesis, but functional eggs are never laid. Two alternatives can explain this observation. Mosaicism could exist but remain undetected if, in each oocyst, one or more cells out of the 16 present had chosen the male pathway and if this were detrimental to the oocyst. It is also possible that all cells display a low overall activity of *Sxl*, high enough to allow germ cells to complete oogenesis, but not enough to become functional eggs. This latter interpretation might also apply to somatic cells; mosaicism then would indicate that the level of *Sxl* activity is at a threshold, sufficient for some cells, but not for others, to become female. Intermediate levels of *Sxl* activity have been postulated before to explain differences in cell viability of aneuploid tissue (Steinmann-Zwicky and Nöthiger, 1985).

In the germ line, zygotic liz^+ is required for oogenesis. In the soma, *liz* can be mutated, and still 53% of females survive even without maternal *liz* product (Table VI). I have argued that *liz*, being hypomorphic, can still supply some residual activity. An alternative explanation, however, is that the Sxl^{M1} allele present in the maternal germ line was able to transactivate the Sxl^+ gene present on its homologue. In the daughters, this Sxl^+ allele was able to transactivate the paternal Sxl^+ gene, and mutual transactivation could keep the *Sxl* genes active in most cells. Thus, two *Sxl* genes could be kept active even if no liz^+ gene is present. Yet 47% of the females die, and the development of those that survive is delayed, which shows that *liz* has an important function, that cannot be completely substituted by two Sxl^+ genes.

The fact that Sxl^{M1} needs maternal liz^+, and the observation that lack of maternal *liz* kills many females or delays their development, argues for a role of maternal product in the activation of *Sxl*. Cells that do not activate their *Sxl* genes early in development, and that therefore transcribe their X chromosomes at a high rate, are expected to be competed out by the much healthier female cells, or, if too many cells are male, these animals would die. Only lack of zygotic *liz* can produce sexual mosaics with male and female tissue. This suggests that the male cells started their development with *Sxl* activity and thus could survive, but that lack of zygotic *liz* made the activation of *Sxl* unstable. In summary, zygotic liz^+ seems to be required in the female germ line for the initial activation of *Sxl*. Maternal *liz* product is probably involved in the activation of *Sxl* in the soma of zygotes with an X:A ratio of 1. Then, two Sxl^+ genes could keep each other active, in germ line and soma. But two zygotic liz^+ genes are required to stabilize the activation of a single Sxl^+.

Is liz an element of the X:A ratio?

If *liz* participates in the early processes that lead to the activation of *Sxl*, three alternatives are possible. The product of *liz* could be a ubiquitous factor required for *Sxl* expression but with no discriminative function; *liz* could be controlled by the X:A ratio; or *liz* could be an element of the X:A ratio itself. That *liz* is not under direct control of the X:A ratio can be shown with the following argument. Males of genotype *liz* Sxl^{M1}/Y survive. Since males that carry Sxl^{M1} and liz^+ are lethal, liz^+ must be active in these males and this gene expression must be lethal. If liz^+ is or can be expressed in animals with an X:A ratio of 0.5 the gene cannot be under direct control of the X:A ratio. Males of genotype liz^+ Sxl^{M1}/Y can survive in the absence of maternal *liz* product. This suggests that maternal *liz* product is required to activate the zygotic liz^+ genes. The observation that *liz* has an important maternal contribution argues against it being an element of the X:A ratio, which is formed by zygotic elements. But the maternal product does not affect the germ line. In this tissue, *liz* could be an element of the X:A ratio. Thus, at least in the soma, *liz* seems to be an omnipresent factor required for *Sxl* activity in animals with an X:A ratio of 1. But remember that 53% of females could survive in the absence of *liz*, but with two Sxl^+ genes, although they developed slowly.

The gene *liz* is different from all other genes that have been described to act upstream of *Sxl*. Maternal *da* product as well as at least one zygotic $sis\text{-}a^+$ gene are required for the activation of Sxl^+ in the soma; both genes are not needed for female differentiation of the germ line (Cline 1986; Cronmiller and Cline, 1987). In contrast, *liz* has an important function in the germ line. The difference in requirements of *da*, *sis-a* and *liz* is also shown by two further sets of results. Genotype *sis-a*/Sxl^{fl} is poorly viable but fertile, whereas genotype *liz*/Sxl^{fl} shows better viability but is sterile. Females of genotype *liz*/*sis-a* are viable and fertile (Table V). Cline (1984) has shown that of two male viable revertants of Sxl^{M1} tested, one, $Sxl^{M1\ fm7}$, was able to rescue females from the maternal lethal effect of *da*, whereas the other, $Sxl^{fm3\ M1}$, was without effect. Both revertants have lost some of the gene's functions and are now constitutive for an altered, mostly unfunctional product. I have tested both revertants and my results obtained with *liz* show the opposite of what Cline describes for *da*: $Sxl^{fm3\ M1}$ was able to substitute *liz* function whereas $Sxl^{M1\ fm7}$ had no such effect (genotype $Sxl^{fm3\ M1}$/*Df 3E8 to 4F11* is fertile, genotype $Sxl^{M1\ fm7}$/*Df 3E8 to 4F11* is sterile; M.Steinmann-Zwicky, unpublished). Both sets of results indicate that the function of *liz* is required primarily in the germ line, whereas *sis-a* and the product of *da* that is involved in sex determination act only in the soma. The gene *Sxl* appears to be regulated in a tissue-specific manner. A good candidate for another X-chromosomal gene with a similar effect as *liz* was identified within region 11D to 12A1-2. When females carrying a deficiency for this region were crossed to males mutant for Sxl^{fl}, a sex-specific maternal-effect lethality was observed. Surviving female progeny often exhibited patches of male tissue (Belote *et al.*, 1985).

Concluding remarks

In this discussion, I have attempted to explain the results obtained by analysing the effects of a *liz* mutation and several *liz* deficiencies, alone or in combination with loss-of-function or gain-of-function alleles of *Sxl*. Using genetical arguments, I show that the model that fits best is that liz^+ is required for initiation and maintenance of *Sxl* in germ line and soma. The zygotic *liz* genes appear not to be controlled by the X:A ratio; maternal product is involved in their activation. In the germ line, *liz* could be part of the X:A ratio. In the soma and in the germ line it is a factor required for *Sxl* activity.

The gene *liz* alone can neither build nor read the primary signal. It seems, however, to be part of a network in which several genes or gene products interact. The list of distinct X-chromosomal elements that are involved in the activation of *Sxl* keeps growing. From the original insight, that an X:A ratio of 1 is required for *Sxl* activity, we have now progressed: an X:A ratio of 1 (a designation that includes all elements yet undefined), $sis\text{-}a^+$, liz^+ and Sxl^+ itself are involved in the activation of *Sxl*. Each distinct individual element can be present in hemizygous condition in a female and still provide the necessary information. Thus, the system is buffered and is not readily disturbed by single mutations. Deleting two elements in females, e.g. one *liz* gene and one *Sxl* gene, gives females that are marginally viable and that are in part sex-transformed. A single maternal liz^+ gene cannot provide daughters having only one Sxl^+ with the necessary gene activity. The signal seems to be achieved through many components that interact and are balanced in a subtle way. In both germ line and soma, the X:A ratio has to be assessed and *Sxl* has to be activated in females. Identifying elements that contribute to this process will eventually help to understand the primary signal that controls sex determination.

Materials and methods

The flies were raised at 21 °C on standard *Drosophila* medium (cornmeal, agar, sugar, yeast and Nipagin). Unless specified, all chromosomes and mutations used are described in Lindsley and Grell (1968) and Lindsley and Zimm (1985, 1987). For *Df(1)HC244* (referred to in the text as *Df 3E8 to 4F11*) and *Df(1)RC40* see Craymer and Roy (1980). Sex ratios were expressed as the fraction of females to males carrying the same X chromosome. Where these males were lethal, the number of their brothers was taken as a reference.

Inducing deficiencies

Deficiencies were induced by irradiating males with 4000 rad. To isolate chromosomes lacking the gene *cho*, females of genotype *cho shi*/*cho shi*

were crossed to treated males of genotype *y w/Y*. Progeny were raised at 29°C. At this temperature, animals mutant for *shi* (i.e. all males) die which leaves all the females virgin. Female progeny displaying the *cho* phenotype were individually crossed to males with the balancer chromosome *FM7*. Since this balancer carries the mutation w^a, it was possible to distinguish females carrying the treated *y w* chromosome from their sisters with the original *cho* mutation in the next generation. Deficiencies for *rb* were isolated with the same scheme.

Deficiencies for *ovo* were obtained by 'reverting' the dominant female-sterile mutation ovo^{D1} (previously called *Fs(1)K1237*, Busson *et al.*, 1983). Females of genotype *y cm* Sxl^{M1}/*FM6* were crossed to treated males of genotype ovo^{D1}*v/Y*. Groups of 30 females of genotype ovo^{D1}/*FM6* were then kept together with their *FM6/Y* brothers. Vials with progeny showed that the ovo^{D1} allele was lost. They directly gave a stock with the putative reverted chromosome. While deficiencies for *cho* or *rb* were obtained with ease (more than one in 1000 chromosomes tested), it was more difficult to get deficiencies for *ovo*. Among 26 000 chromosomes only five were deficiencies that uncovered at least one of the markers adjacent to *ovo*. Sixteen reversions were defective only for *ovo*: they might be small deletions or point mutations. The paucity of *ovo* deletions and the distribution of the deficiencies isolated (Table IV) suggest that there is a haplo-lethal locus to the right of *ovo*.

Testing deficiencies

The genetic size of the deficiencies was measured by testing them over a series of markers. The mutations *ec, cho, bi, rb, peb* and *rg* were described before. To test for *ovo*, I used the allele lzl^G which was kindly provided by M.Gans. The male diplo-lethal locus was identified in Steinmann-Zwicky and Nöthiger (1985) and further localized with the present experiments. To test whether a chromosome was deleted for this gene, I crossed females with a deficiency to males carrying *T(1;2)*w^{64b13}. This is a second chromosome with the X-chromosomal segment 3C2 to 5A1-2 inserted in 26D (Craymer and Roy, 1980). Deficiencies suspected to extend more distally than 3C2, which therefore would not be covered by the duplication, were crossed to *T(X;Y)B29*, a translocation with a breakpoint in 4C (Steinmann-Zwicky and Nöthiger, 1985). Surviving males would show that the *male diplo-lethal locus* (*mdl*) is deleted.

The interaction with *Sxl* was tested by crossing females with a deficiency to males carrying Sxl^{fl}. The progeny were counted, *trans*-heterozygous females were inspected for sex combs, male terminalia or male pigmentation on the abdomen, and tested for fertility. Groups of 20 flies were kept for at least 20 days together with their brothers carrying the balancer chromosomes *FM7* or *FM6*. Females with deficiencies showing the interaction with *Sxl* never had progeny and laid no eggs.

EMS mutagenesis

A mutagenesis screen was set up to look for female-specific lethal mutations at the male diplo-lethal locus (*mdl*). Individual females carrying a chromosome marked with *rb f* that had been mutagenized with EMS (Lewis and Bacher, 1968), were crossed to males of genotype *Df(1)HC244/Y;* *T(1;2)*w^{64b13}/+. As described in the previous section, this second chromosome carries a wild-type allele of *mdl*. Those chromosomes were kept that were lethal over the deficiency, but viable in males with or without the duplication. Out of 1200 chromosomes tested, one finally remained after numerous retests. It was lethal over a deficiency but fully viable in males; it could marginally survive with the duplication, giving a few males. On the same chromosome, a second mutation (eye structure phenotype) had been induced. It is not allelic to *ec*, was named *echinus-like* (*ecl*) and was localized just to the left of the female-lethal. The female-lethal mutation was shown not to be a defect in *mdl* (the two loci are separated by several other genes, Table IV). It was named *fl(1)302*. Its lethal phase was found to be larval or pupal. The failure to find the mutation that was looked for suggests that deleting *mdl* is lethal to both females and males.

Production of males with 2 liz$^+$ genes

T(1;2)w^{64b13}, the second chromosome with the X-chromosomal segment 3C2 to 5A1, contains both a liz^+ gene and a mdl^+ gene. Therefore, males with an X chromosome and this duplication die. Viable males were obtained that, besides the duplication, carry either of two X-chromosomal deficiencies deleting *mdl*. Large deficiencies were chosen to minimize viability problems due to aneuploidy. *Dp* Sxl^+ is *Dp(1;3)*sn^{13al}. Males of the 4 classes were recognized as follows: (i) *y Sb*; (ii) *y*; (iii) *Sb*; (iv) +. Occasional crossing overs that might link *y* to the deficiency that carries liz^- are rare and can be neglected.

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Note added in proof

In a recent paper published in the September issue of *Genetics*, Oliver, Perrimon and Mahowald (1988) also show that the interaction between Df 3E to 4F and *Sxl* described by Steinmann-Zwicky and Nöthiger (1985) is due to the lack of gene *fs(1)1621*. This leads the authors to conclude that *fs(1)1621* is involved in sex determination. Possible hierarchical relationships are discussed, but not experimentally tested. The locus was renamed *sans fille* (= without daughter), which I think is unfortunate, since the phenotype of the mutation is a female-sterile causing lack of sons as well. The name that I chose, *liz*, refers to the female-specific effects of the mutation, to a maternal effect (Elizabeth I lost her mother in early childhood) and to absence of children.