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Abstract
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Sex determination in \textit{Drosophila}: the X-chromosomal gene \textit{liz} is required for \textit{Sxl} activity

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

\textbf{Key words:} dosage compensation/\textit{Drosophila}/germ line/sex determination

\section*{Introduction}

In \textit{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 \textit{Sex-lethal} (\textit{Sxl}) transduces this quantitative signal into differential gene activity (Cline, 1978, 1983a). Around blastoderm stage, \textit{Sxl} is irreversibly activated in females and left inactive in males (Sanchez and Nöthiger, 1983). \textit{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 \textit{Sxl} activity, they are hyperactive which is characteristic of the single X of males and which is lethal to XX flies (Lucchesi and Skrisky, 1981; Gergen, 1987). XX tissue that lacks \textit{Sxl} activity due to the loss-of-function mutation \textit{Sx}{\textsuperscript{b}}, 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 \textit{Sxl}, maternal activity of the gene \textit{da} is required (Cronmiller and Cline, 1987). In two instances, evidence for zygotic positive regulators of \textit{Sxl} has been published. These pointed to region 3E8 to 4F11 (Steinmann-Zwicky and Nöthiger, 1985) and to the gene \textit{sisterless-a} (\textit{sis-a}) located in 10B (Cline, 1986). The mutation \textit{sis-a} was shown to be a female-specific lethal. A constitutive mutation of \textit{Sxl}, \textit{Sx}{\textsuperscript{M1}} (Cline, 1978, 1979), however, rescues mutant females which suggests that \textit{sis-a} activity is required for \textit{Sxl} activation (Cline, 1986). We have identified region 3E8 to 4F11 on the following grounds (Steinmann-Zwicky and Nöthiger, 1985). (i) Genotype \textit{X/Dp IA to 7D} differentiates female structures in clones and is lethal to whole animals. Genotype \textit{Df 3E8 to 4F11/Dp IA 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 \textit{Df 3E8 to 4F11/Sx}{\textsuperscript{b}} 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 \textit{Df 3E8 to 4F11} and \textit{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 \textit{Sx}{\textsuperscript{b}}, however, were found to survive with such a duplication. This suggested that the duplication activated the \textit{Sxl}{\textsuperscript{+}} gene to a level that is lethal to males. \textit{Sx}{\textsuperscript{b}}, however, did not rescue all the males carrying the duplication as would have been predicted with a simple model of one gene activating \textit{Sxl}{\textsuperscript{+}} (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 \textit{liz}, previously identified as necessary for female fertility (\textit{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 \textit{liz} and loss-of-function or gain-of-function alleles of \textit{Sxl} show that \textit{liz}{\textsuperscript{-}} is needed for initiation and/or maintenance of \textit{Sxl}{\textsuperscript{-}}, in germ line and soma. The gene \textit{liz} stands for Elizabeth I of England, who shares some of the mutant’s characteristics.

\section*{Results}

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

\section*{Interactions with \textit{Sxl}}

\textbf{Maternal and zygotic effects of \textit{Df 3E8 to 4F11}}. To test for interactions among the three elements \textit{Df 3E8 to 4F11}, \textit{Sx}{\textsuperscript{b}} and \textit{sis-a} and to distinguish between maternal and zygotic effects, I analysed a series of crosses. Table I shows that females of genotype \textit{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 Sxltl or sis-a

<table>
<thead>
<tr>
<th>father</th>
<th>Df 3E8 to 4F11/Y</th>
<th>Sxltl/Y</th>
<th>sis-a/Y</th>
</tr>
</thead>
<tbody>
<tr>
<td>mother</td>
<td>0/0</td>
<td>0/0</td>
<td>0/0</td>
</tr>
<tr>
<td>Df 3E8 to 4F11</td>
<td>426.4</td>
<td>1.45</td>
<td>3</td>
</tr>
<tr>
<td>FM7</td>
<td>474.294</td>
<td>1.61</td>
<td>173</td>
</tr>
</tbody>
</table>

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 ratios point to female-specific lethality, underlined numbers indicate that these females were sterile. The balanced chromosome FM7 is semi-lethal so that all sex ratios of crosses involving mothers of genotype Df 3E8 to 4F11/FM7 are too high. Thus, the interaction between Df 3E8 to 4F11 and Sxltl or sis-a is even stronger than it appears from these crosses. The chromosome carrying Sxltl was marked with w cm ct f, and sis-a was linked to y. Df 3E8 to 4F11 is Df(1)cho 19, y w.

Table II. Zygotic interaction between Df 3E8 to 4F11 and Sxltl or sis-a

<table>
<thead>
<tr>
<th>father</th>
<th>Df 3E8 to 4F11/Y</th>
<th>Sxltl/Y</th>
<th>sis-a/Y</th>
</tr>
</thead>
<tbody>
<tr>
<td>mother</td>
<td>0/0</td>
<td>0/0</td>
<td>0/0</td>
</tr>
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<td>Df 3E8 to 4F11</td>
<td>245</td>
<td>205</td>
<td>1.20</td>
</tr>
<tr>
<td>FM7</td>
<td>87</td>
<td>187</td>
<td>0.47</td>
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</table>

Females were of genotype y cho or y cho w cm Sxltl cm 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)w64bI3 i+/+ or Df 3F-4F/Y, T(1;2)w64bI3 i+. 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 Sxltl. A lethal zygotic effect can be seen, since progeny of genotype Df 3E8 to 4F11/Sxltl 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/Sxltl 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 Sxltl/FM6 × Df 3E8 to 4F11/Y again shows the zygotic lethality of genotype Df 3E8 to 4F11/Sxltl, 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 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 on 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 Sxltl. 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 Sxltl allele. Obviously there is an interaction between region 3E8 to 4F11 and the gene Sxltl, 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.

Sxltl rescues the zygotic effect of Df 3E8 to 4F11. The lethality of females of genotype Df 3E8 to 4F11/Sxltl can be explained if we postulate that the Sxltl 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 Sxltl should then rescue the females. Table III shows that indeed females of genotype Df 3E8 to 4F11 Sxltl/Sxltl 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 Sxltl 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 Sxltl in females. In the absence of two zygotic regions 3E8 to 4F11, Sxltl is not properly activated, which becomes visible in females carrying only one functional Sxltl allele.

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

Table III. Sxltl rescues the zygotic phenotype resulting from an interaction between Df 3E8 to 4F11 and Sxltl

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<td>Df 3E8 to 4F11 Sxltl</td>
<td>205</td>
<td>193</td>
<td>1.06</td>
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<td>FM7</td>
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<td>0.38</td>
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</tr>
<tr>
<td>Df 3E8 to 4F11 Sxltl</td>
<td>179</td>
<td>271</td>
<td>1.06</td>
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<tr>
<td>bi ct</td>
<td>149</td>
<td>0.55</td>
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</tr>
</tbody>
</table>

The Df 3E8 to 4F11 Sxltl chromosome was also marked with cm. Fathers were of genotype y w cm Sxltl ct f/Y. See also legend to Table I.

M. Steinmann-Zwicky
Sex determination gene \( i \zeta \) in Drosophila

### Table IV. Mapping by deficiencies the region that interacts with \( Sxl \)

<table>
<thead>
<tr>
<th></th>
<th>ec</th>
<th>cho</th>
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<th>bi</th>
<th>rb</th>
<th>peb</th>
<th>ecl</th>
<th>fl(1)302</th>
<th>ovo</th>
<th>rg</th>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>3E8</td>
<td></td>
</tr>
</tbody>
</table>

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 the latter three markers: cho located at the distal end; rb in the middle; and ovo at the proximal end of 3E8 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 breakpoints 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 \( Sxl \), the latter does not (Steinmann-Zwicky and Nöthiger, 1985). All but four of the newly induced deficiencies did not show the lethal interaction with \( Sxl \). 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 \( Sxl \). 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 \( Sxl \) is sterility, I decided to analyse two female sterile mutations \( fs(1)\text{1621} \) (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, were all rescued by the presence of the constitutive mutation \( Sxl^M \). Therefore, mutations in genes whose absence leads to the observed phenotypes in combination with \( Sxl \) are expected to be rescued by \( Sxl^M \) as well. Females of genotype \( Df \) 3E8 to 4F11/Sxl/Sxl\(^M\)/fl(1)302 were still lethal, and genotype \( Df \) 3E8 to 4F11 \( Sxl^M \)/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 \( Sxl^M \), must act downstream of \( Sxl \) or in a...
different pathway. In contrast, females of genotype Df 3E8 to 4F11 Sxl[\textsuperscript{Ml}]/{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 oocytes 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\textsuperscript{Ml}. 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\textsuperscript{Ml} is also revealed. The few surviving females of genotype liz/Sxl\textsuperscript{Ml} 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\textsuperscript{Ml} 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/sis-a. 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\textsuperscript{Ml}. 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\textsuperscript{Ml} 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\textsuperscript{Ml}. 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\textsuperscript{Ml} 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\textsuperscript{Ml} mutation. Zygotic lethality and, at least in part, sex transformations and sterility of the surviving Df 3E8 to 4F11/Sxl\textsuperscript{Ml} 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\textsuperscript{Ml} or vice versa (Table V). Some flies of genotype sis-a/Sxl\textsuperscript{Ml} 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\textsuperscript{Ml} alone could have some rescuing effect upon genotype liz/Sxl\textsuperscript{Ml}, I crossed females of genotype liz/Sxl\textsuperscript{Ml} to males carrying Sxl\textsuperscript{Ml}. Many females now survive, but almost all of them carry Sxl\textsuperscript{Ml}. The sex ratio calculated as the number of females with Sxl\textsuperscript{Ml} relative to the number of males without Sxl\textsuperscript{Ml} is 316:502 = 0.63. In this cross, Sxl\textsuperscript{Ml} 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\textsuperscript{Ml} which gives a sex ratio of 3:502 = 0.01. Therefore, the maternal presence of Sxl\textsuperscript{Ml} has no rescuing activity on genotype liz/Sxl\textsuperscript{Ml}.

The complete cross performed with all the results is represented in Figure 2. Totally unexpected was the finding that males carrying Sxl\textsuperscript{Ml} can survive if they are also mutant for liz (evidence that the 24 cm males scored were of genotype liz Sxl\textsuperscript{Ml}/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

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Table V. Tests for zygotic and maternal effects resulting from an interaction between genes Sxl\textsuperscript{Ml}, liz and sis-a

<table>
<thead>
<tr>
<th>father</th>
<th>+/Y</th>
<th>Sxl\textsuperscript{Ml}/Y</th>
<th>liz/Y</th>
<th>sis-a/Y</th>
</tr>
</thead>
<tbody>
<tr>
<td>mother</td>
<td>(\varphi)</td>
<td>(\sigma)</td>
<td>(\varphi/\sigma)</td>
<td>(\varphi)</td>
</tr>
<tr>
<td>liz</td>
<td>243</td>
<td>205</td>
<td>1.19</td>
<td>3</td>
</tr>
<tr>
<td>FM3</td>
<td>214</td>
<td>–</td>
<td>1.04</td>
<td>11</td>
</tr>
<tr>
<td>Sxl\textsuperscript{Ml}</td>
<td>534</td>
<td>282</td>
<td>1.89</td>
<td>168</td>
</tr>
<tr>
<td>FM6</td>
<td>482</td>
<td>322</td>
<td>1.50</td>
<td>217</td>
</tr>
<tr>
<td>sis-a</td>
<td>554</td>
<td>414</td>
<td>1.34</td>
<td>296</td>
</tr>
<tr>
<td>Binsirsy</td>
<td>422</td>
<td>106</td>
<td>3.98</td>
<td>704</td>
</tr>
</tbody>
</table>

Chromosomes were marked as follows: liz, v, y w cm Sxl\textsuperscript{Ml} et f, y sis-a. Chromosome FM3 is male lethal; to calculate sex ratios, the number of viable brothers was used. Binsirsy is semi-lethal; therefore all sex ratios involving this chromosome are substantially too high. See also legend to Table I.
Fig. 1. (a) Abnormal chorion appendages of eggs formed by females of genotype Df 3E8 to 4F11/Sx6. (b) liz Sx6M1/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 Sx6M1/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/Sx6M1/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/Sx6M1/Sx6 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 SxM/yliz × cm Sx6° 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. SxM being lethal to males, 104 y males were produced as a result of a CO between y and cm. y+y cm males can only appear as the reciprocal result of CO in the same interval. Such males were crossed to females mutant for a. This cross, kept at 25°C, produced 44 daughters and 70 sons, which shows that SxM is present in y+y cm males and that it can rescue daughters of a males mothers. A control cross with y+y/Y males gave no female progeny. A CO between y and liz could not lead to viable cm males, since SxM males are lethal. In the interval between liz and cm, roughly 40 CO events are expected that could lead to double mutant males liz SxM/Y. 24 cm males carrying SxM were observed that can only be explained by such an event.

Table VI. Absence of maternal liz product

<table>
<thead>
<tr>
<th>mother</th>
<th>liz/Y</th>
<th>liz SxM/Y</th>
<th>liz Sx6°/Y</th>
</tr>
</thead>
<tbody>
<tr>
<td>Df 3E8</td>
<td>446</td>
<td>0.95</td>
<td>0.91</td>
</tr>
<tr>
<td>liz</td>
<td>250</td>
<td>0.53</td>
<td>0.98</td>
</tr>
<tr>
<td>liz/</td>
<td>120 (4)</td>
<td>0.27</td>
<td></td>
</tr>
</tbody>
</table>

Chromosomes were marked as follows: Df 3E8 to 4F11 cm SxM, liz v, liz cm Sx6°, y w cm Sx6° f.

1b and c). Some of the males were fertile and, when crossed with females homozygous for the mutation a, rescued females which shows that they in fact carry the SxM allele. Viable males carrying the mutation SxM 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 SxM to males of three different genotypes: males mutant for liz, males doubly mutant for both liz and SxM, and males carrying Sx6. 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 oocytes, which represents the typical liz phenotype (Figure 1d). Presence of only one SxM allele was more deleterious to females than having no liz product. In the absence of maternal liz activity, genotype liz/Sx6 is lethal. The few surviving females of genotype Df 3E8 to 4F11 SxM/Sx6 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 SxM allele was very efficient in compensating for the missing liz activity. Females of genotype liz/liz SxM 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 SxM/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 SxM/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 SxM/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 SxM/Y; Dp/+ should now be able to
survive although they have a wild-type \textit{liz} gene. These males can be recognized because they carry the mutation \textit{cm}, which is very closely linked to \textit{Sxl\textsuperscript{M1}} and because they display the marker \textit{Co}, which identifies the duplication. A total of 38 \textit{Co cm} males were found. These males, whose genotype corresponds to \textit{liz}\textsuperscript{+}/\textit{Sxl\textsuperscript{M1}}/\textit{Y}, show all the characteristics of the \textit{liz} \textit{Sxl\textsuperscript{M1}}/\textit{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 \textit{cm} are expected to occur as frequently as \textit{+/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 \textit{Sxl\textsuperscript{M1}} can survive in the absence of maternal or zygotic \textit{liz} product, and that maternal \textit{liz} product is involved in the activation of zygotic \textit{liz}\textsuperscript{+} genes.

**Males carrying a duplication for \textit{liz} are vital.** Males of genotype \textit{Df 3E8 to 4F11/DP 1A to 7D} can survive or die as pharate adults, whereas genotype \textit{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 \textit{liz}\textsuperscript{+} gene, the latter two. The question arises whether duplicating \textit{liz}\textsuperscript{+} in \textsf{X}Y animals can produce a female signal. If this were the case, males carrying two \textit{liz}\textsuperscript{+} genes should be subvital. If \textit{liz} acts through \textit{Sxl}, males carrying two \textit{liz}\textsuperscript{+} genes and two \textit{Sxl}\textsuperscript{+} genes might be expected not to survive at all. To test this, I crossed females of genotype \textit{D(f1)cho 23, y w \textit{liz}\textsuperscript{+} /D(f1)HC 244, \textit{liz}\textsuperscript{+}; Dp 3C to 5A, \textit{liz}\textsuperscript{+}/+} to males carrying a \textit{Dp Sxl\textsuperscript{+}} 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 \textit{liz}\textsuperscript{+} and 1 \textit{Sxl}\textsuperscript{+}; (ii) 114 with 2 \textit{liz}\textsuperscript{+} and 2 \textit{Sxl}\textsuperscript{+}; (iii) 176 control males with 1 \textit{liz}\textsuperscript{+} and 1 \textit{Sxl}\textsuperscript{+}; (iv) 159 with 1 \textit{liz}\textsuperscript{+} and 2 \textit{Sxl}\textsuperscript{+}. The males with 2 \textit{liz}\textsuperscript{+} and 1 \textit{Sxl}\textsuperscript{+} were vital, those with 2 \textit{liz}\textsuperscript{+} and 2 \textit{Sxl}\textsuperscript{+} were the least frequent class, but still present in substantial numbers. I conclude that duplicating \textit{liz}\textsuperscript{+} alone does not produce a strong female signal in males. It appears that \textit{Dp 1A to 7D} contains several genes that, when duplicated, cause cells to choose the female pathway. If, however, region \textit{3E8 to 4F11}, that contains \textit{liz}, is not duplicated as well, the female pathway is not chosen.

**Discussion**

**Product of \textit{liz} is required in females for \textit{Sxl\textsuperscript{+}} activity**

Maternal and zygotic products of \textit{liz}\textsuperscript{+} act in females to ensure proper sex determination, dosage compensation and oogenesis. The mutation \textit{liz} is defective for these functions: this becomes apparent in females of genotype \textit{liz}/\textit{Sxl}\textsuperscript{0} some of which are lethal while the others are sterile and show male characters; in females that carry only one \textit{Sxl}\textsuperscript{+} gene and whose mother was heterozygous for \textit{liz} which are poorly viable; in females homozygous for \textit{liz} which are sterile; and in animals of genotype \textit{liz Sxl\textsuperscript{M1}}/\textit{Y} which now survive as males, whereas genotype \textit{Sxl\textsuperscript{M1}}/\textit{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 \textit{liz} alleles and \textit{Sxl} mutations, a gene known to regulate this process.

The mutation \textit{Sxl\textsuperscript{M1}} can rescue all female-specific defects caused by absence of \textit{liz} product in the germ line and in the soma. This means that \textit{liz} is required for the expression of \textit{Sxl\textsuperscript{+}}. But even though \textit{Sxl} product is required in all tissues for female differentiation, females of genotype \textit{liz}/\textit{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 \textit{liz} activity (Table VI, cross \textit{Df 3E8 to 4F11 Sxl\textsuperscript{M1}/liz \times liz}). Although the phenotype of \textit{liz}/\textit{liz} was not significantly different from that of \textit{Df 3E8 to 4F11 liz} (they both had ovaries filled with undifferentiated germ cells and an occasional oocyst), the mutation \textit{liz} is probably hypomorphic. Females of genotype \textit{Df 3E8 to 4F11/Sxl\textsuperscript{+}} were definitely more affected than females of genotype \textit{liz}/\textit{Sxl\textsuperscript{0}} 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 \textit{liz} probably still provides soma and germ line with some residual function. Occasional activation of \textit{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 \textit{liz}/\textit{liz} daughters derived from \textit{Df 3E8 to 4F11 Sxl\textsuperscript{M1}/liz} mothers alive as females. This rest of \textit{liz} activity, however, is neither sufficient to kill males of genotype \textit{liz Sxl\textsuperscript{M1}}/\textit{Y} nor to transform them into females, except for a few cells. Nevertheless, the possibility that \textit{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 \textit{liz} activity can survive will be presented later.

Crossing females of genotype \textit{Df 3E8 to 4F11 Sxl\textsuperscript{M1}/liz} to \textit{liz Sxl\textsuperscript{M1}}/\textit{Y} males (Table VI) showed that \textit{Sxl\textsuperscript{M1}} can rescue all daughters (genotype \textit{liz}/\textit{liz} \textit{Sxl\textsuperscript{M1}} survived best). Two phenotypes resulting from lack of \textit{liz} activity, however, are only rescued to a small extent. In both cases, females of genotype \textit{Df 3E8 to 4F11 Sxl\textsuperscript{M1}/Sxl\textsuperscript{0}} are affected. If they receive only half the amount of maternal \textit{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, \textit{Sxl\textsuperscript{M1}} does not express a somatic function that can be activated by maternal \textit{liz} product.

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

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

If \textit{Sxl\textsuperscript{M1}} is active in males of genotype \textit{liz Sxl\textsuperscript{M1}}/\textit{Y}, only two different models can provide an explanation for the survival of these males. In the wild-type female, the gene products of \textit{liz}\textsuperscript{+} and \textit{Sxl}\textsuperscript{+} act in parallel to bring about female
differ. or liz+ is activated by Stxl+ and transmits the female signal. Both models, however, cannot explain why males carrying StxlM1 and a liz- allele can survive if there is no maternal liz product, nor why StxlM1 can rescue all female-specific phenotypes caused by lack of liz product. If, however, StxlM1 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 Stxl.

Is there published evidence that StxlM1 might not always be active? Initially, StxlM1, that carries a transposable element within the Stxl gene (Maine et al., 1985), was believed to express constitutively all female-specific Stxl functions because of its ability to rescue daughters of females mutant for da (Cline, 1978). Newer results, however, indicate that StxlM1 does not produce female-specific Stxl activity throughout development. Using a hypomorphic mutation of the gene runt to monitor dosage compensation, Gergen (1987) could show that StxlM1 remains without effect in male or female embryos at the blastoderm stage, a time at which Stxl is already differentially regulated in the two sexes (Sanchez and Nöthiger, 1983; Gergen, 1987). Although it was shown that tissue of genotype StxlM1/O can differentiate female-specific structures, 2 of 7 legs with StxlM1/O tissue in the sex comb region differentiated male sex comb teeth (Cline, 1979). It is possible that StxlM1 was not active in these cells. Alternatively, the products of StxlM1 might not always be sufficient for dictating the female pathway.

In the germ line, Stxl+ activity is required for normal oogenesis (Schüpbach, 1985; Salz et al., 1987). My results showing that StxlM1 can rescue a germ line mutant for liz confirm such a requirement and indicate that, in the female, StxlM1 expresses this function. Germ cells of genotype StxlM1/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 StxlM1 is not active in germ cells with only one X chromosome or that StxlM1 activity alone does not dictate the female pathway.

A unifying hypothesis that explains all the results assumes that StxlM1 is controlled by both the X:A ratio and by liz product. With an X:A ratio of 0.5, StxlM1 may not become active in every cell, or StxlM1 may not become stably activated. Maternal and zygotic product of liz- could help to initiate or maintain expression of StxlM1. This assumption is supported by the observation that tissue of genotype StxlM1/O is mostly female with some male cells, whereas genotype liz StxlM1/Y, or StxlM1/Y but lacking maternal liz product, is male with some female cells. Such a mosaic pattern suggests that StxlM1 needs liz product and an X:A ratio of 1 for stable activation.

Thus, StxlM1 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, StxlM1 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 StxlM1. An interesting situation is the few surviving females of genotype Df 3E8 to 4F11 StxlM1/StxlO (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 StxlM1 allele.

**liz and Sxl in the wild-type female**

In the germ line of the wild-type female, Stxl+ genes require zygotic liz+ activity to be functional. But liz is also needed in the soma. Females that carry only one Stxl+ gene, or one StxlM1 allele, and StxlO 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 StxlM1/StxlO lacking maternal liz product are poorly viable, I have argued that StxlM1 does not express a function that can be activated by maternal liz. This function can also be provided by Stxl+. In fact, in some cases, deleting one Stxl+ had a more drastic 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 Stxl+ genes or with one Stxl- 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 Stxl- and/or help to keep the state of activity stable. Two Stxl- genes seem to be more active or more stably active than one. Cline (1984) has suggested that StxlM1 can transactivate an Stxl+ gene present in the same zygote. In my experiments, mutual transactivation between one Stxl+ allele and an Stxl+ gene or between two Stxl+ genes could ensure that the functional Stxl+ genes are active and are kept stably active. If only one Stxl+ and one zygotic liz+ gene are present (genotype Df 3E8 to 4F11/StxlO), 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 oocyte, one or more cells out of the 16 present had chosen the male pathway and if this were detrimental to the oocyte. It is also possible that all cells display a low overall activity of Stxl, 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 Stxl activity is at a threshold, sufficient for some cells, but not for others, to become female. Intermediate levels of Stxl 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 StxlM1 allele present in the maternal germ line was able to transactivate the Stxl+ gene present on its homologue. In the daughters, this Stxl+ allele was able to transactivate the paternal Stxl+ gene, and mutual transactivation could keep the Stxl+ genes active in most cells. Thus, two Stxl+ 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 Stxl+ 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 must be expressed. 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 \( sis-a^- \) 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 \( sis-a/Sxl^{P} \) is poorly viable but fertile, whereas genotype \( liz/Sxl^{P} \) 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 \( Sxl^{P} \) tested, one, \( Sxl^{M1} \), was able to rescue females from the maternal lethal effect of \( da \), whereas the other, \( Sxl^{M1} \), was without effect. Both revertants have lost some of the gene’s functions and are now constitutive for an altered, mostly dysfunctional product. I have tested both revertants and my results obtained with \( liz \) show the opposite of what Cline describes for \( da \): \( Sxl^{M3} \) was able to substitute \( liz \) function whereas \( Sxl^{M1} \) had no such effect (genotype \( Sxl^{M3} \)/\( df \) 3E8 to 4F11 is fertile, genotype \( Sxl^{M1}/df \) 3E8 to 4F11 is sterile; M. Steinnmann-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 for \( Sxl^{P} \), 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), \( sis-a^- \), \( liz^+ \) 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 Lindsay and Grell (1968) and Lindsay 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 \( cho/cho \) were crossed to males of genotype \( X: \text{sex-transformed} \) females.
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+, 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 ovo38 (previously called Fs(1)K1237, Busson et al., 1983). Females of genotype y Sm(1)FM6 were crossed to treated males of genotype ovo80/FM7. Groups of 30 females of genotype ovo80/FM6 were then kept together with their FM6/F male siblings. Vials with progeny showed that the ovo80/Male was lost. The females 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 males on 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 rq were described before. To test for ovo, I used the allele ec89 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)Ms640. 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(1;2)Ms657, a translocation with a breakpoint in 4C (Steinmann-Zwicky and Nöthiger, 1985). Surviving males would show the diplo-lethal phenotype (mdl) deleted.

The interaction with Sxl was tested by crossing females with a deficiency to males carrying Sxl8. 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 small 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 that had been mutagenized with EMS (Lewis and Bacher, 1968), were crossed to males of genotype Df(1)Hec244/Y; T(1;2)Ms640. 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 (eccl) 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)Ms640, the second chromosome with the X-chromosomal segment 3C2 to 5A1, contains both a liz+ gene and a mdl+ gene. Therefore, males with this 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. Dy Sxl8 is Dp(1;3)m640. Males of the four 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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References

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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 Df3E 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.