Evidence of a Dual Function in fl(2)d, a Gene Needed for Sex-lethal Expression in Drosophila melanogaster

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Manuscript received July 23, 1991
Accepted for publication December 2, 1991

ABSTRACT

In Drosophila melanogaster, the female sexual development of the soma and the germline requires the activity of the gene Sex-lethal (Sxl). The somatic cells need the function of the gene fl(2)d to follow the female developmental pathway, due to its involvement in the female-specific splicing of Sxl RNA. Here we report the analysis of both fl(2)d1 and fl(2)d2 mutations: (1) fl(2)d1 is a temperature-sensitive mutation lethal in females and semilethal in males; (2) fl(2)d2 is lethal in both sexes; (3) the fl(2)d1/fl(2)d2 constitution is temperature-sensitive and lethal in females, while semilethal in males. The temperature-sensitive period of fl(2)d1 in females expands the whole development. SxlM1 partially suppresses the lethality of fl(2)d1 homozygous females and that of fl(2)d1/fl(2)d2 constitution, whereas it does not suppress the lethality of fl(2)d2 homozygous females. The addition of extra Sxl* copies does not increase the suppression effect of SxlM1. The fl(2)d1 mutation in homozygosis and the fl(2)d1/fl(2)d2 constitution, but not the fl(2)d2 in homozygosis, partially suppress the lethality of SxlM1 males. This suppression is not prevented by the addition of extra Sxl* copies. The semilethality of both fl(2)d1 and fl(2)d1/fl(2)d2 males, and the lethality of fl(2)d2 males, is independent of Sxl function. There is no female synergistic lethality between mutations at fl(2)d and neither at sc or da. However, the female synergistic lethality between mutations at Sxl and either sc or da is increased by fl(2)d mutations. We have analyzed the effect of the fl(2)d mutations on the germline development of both females and males. For that purpose, we carried out the clonal analysis of fl(2)d1 in the germline. In addition, pole cells homozygous for fl(2)d2 were transplanted into wild-type host embryos, and we checked whether the mutant pole cells were capable of forming functional gametes. The results indicated that fl(2)d mutant germ cells cannot give rise to functional oocytes, while they can form functional sperm. Moreover, SxlM1 suppresses the sterility of the fl(2)d1 homozygous females developing at the permissive temperature. Thus, with respect to the development of the germline the fl(2)d mutations mimic the behavior of loss-of-function mutations at the gene Sxl. Females double heterozygous for fl(2)d and snf621 are fully viable and fertile. fl(2)d2 in heterozygosis partially suppresses the phenotype of female germ cells homozygous for snf621; however, this is not the case with the fl(2)d1 mutation. The fl(2)d2 mutations partially suppress the phenotype of the female germ cells homozygous for oeg651. We conclude that the gene fl(2)d has a dual function: a female-specific function involved in the splicing of Sxl RNA and a non-sex-specific function. Furthermore, the gene fl(2)d is required in the female germline for Sxl expression.

In Drosophila melanogaster, sex determination, dosage compensation and oogenesis are under the control of the gene Sex-lethal (Sxl). The functional state of Sxl is determined by the ratio of the number of X chromosomes to autosomal sets (X:A). In 2X;2A flies Sxl will be ON, while in X;2A flies Sxl will be OFF (Cline 1978). Activation of Sxl also requires the maternal daughtercless (da) product (Cline 1978). Two X elements of this X:A signal have been identified: sisterless-a (sis-a) (Cline 1986) and a region of the achaete-scute complex that has been named sis-b (Cline 1988) and which corresponds to the gene scute (Torres and Sanchez 1989; Parkhurst, Bopp and Ish-Horowitz 1990; Erickson and Cline 1991). Once the state of activity of Sxl is determined, an event that occurs at blastoderm stage, the X:A signal is no longer used and the activity of Sxl remains fixed (Sanchez and Nöthiger 1983; Bachiller and Sanchez 1991). The capacity of Sxl to function as a stable switch is thought to be due to a positive autoregulatory function of the Sxl product (Cline 1984; Bell et al. 1991).

The gene Sxl controls the expression of two independent sets of regulatory genes: the sex determination and the dosage compensation genes (Lucchesi and Skrisky 1981). The sex determination genes when mutated alter the normal sexual development instructed by the X:A signal through the gene Sxl, while they do not have any effect in the mechanism that brings about dosage compensation (Baker and Belote 1983; Nöthiger and Steinmann-Zwicky 1985). This process, that is, the hypertranscription of the single X chromosome in males requires the activity

Genetics 130: 597–612 (March, 1992)
of the male-specific lethal genes (BELOTE and LUCCHESI 1980; OKUNO, SATOU and OISHI 1984). Mutations in these genes affect dosage compensation but have no effect on somatic sex determination (BELOTE 1983; UENOYAMA, FUKUNAGA and OISHI 1982; BACHILLER and SÁNCHEZ 1989).

The gene Sxl produces two temporally separate sets of transcripts. The early set is composed of three transcripts found only around blastoderm stage. These transcripts would be produced from a female-specific promoter that responds to the X:A signal (SALZ et al. 1989; TORRES and SÁNCHEZ 1991). The late set is formed by three other transcripts, present in both females and males, which appear slightly later in development and in adult life. These transcripts would be produced from a non-sex-specific promoter, giving rise to the primary Sxl RNA that subsequently will be differently spliced in females and males. The three female late transcripts overlap extensively and share most exons, but differ at their 3' ends. The three male late transcripts are similar to their females counterparts, except for the presence of an additional internal exon (exon 3), which contains a translation stop codon; consequently, the male late transcripts give rise to presumably inactive truncated proteins (BELL et al. 1988).

The germline exhibits sexual dimorphism as do the somatic tissues. Cells with the 2X;2A chromosomal constitution will follow the oogenic pathway and X;2A somatic tissues. Cells with the 2X;2A chromosomal constitution will follow the oogenic pathway and X;2A somatic tissues. Cells with the 2X;2A chromosomal combination will follow the oogenic pathway and X;2A somatic tissues. Cells with the 2X;2A chromosomal combination will follow the oogenic pathway and X;2A somatic tissues. Cells with the 2X;2A chromosomal combination will follow the oogenic pathway and X;2A somatic tissues. Cells with the 2X;2A chromosomal combination will follow the oogenic pathway and X;2A somatic tissues. Cells with the 2X;2A chromosomal combination will follow the oogenic pathway and X;2A somatic tissues.

The germ line is based on the use of Sxl, whose activity is required for normal female germline development and in adults. Flies were macerated with 10% KOH at 50° for 15 min, washed in H2O and mounted in Faure's solution. For a description of the sexual dimorphic regions see BRYANT (1978).

Induction, isolation and genetic mapping of f(2)d' mutants: Both f(2)d' and f(2)d' have been induced by treatment of males with EMS according to the procedure of LEWIS and BACHER (1968). f(2)d' was isolated from the analysis of a group of putative female-specific lethals that arose during a mutagenesis carried out by T. SCHÜPBACH, to whom we are grateful for allowing us to examine these lethals. f(2)d' was isolated from a mutagenesis designed to collect more f(2)d' mutant alleles. For that purpose, we mutagenized the cn bw isogenic strain and the mutagenized chromosomes were tested over f(2)d'. The selection criteria was the lethal phenotype of one or both sexes. To rid the lethal-bearing chromosomes of other deleterious mutations that might have been induced during mutagenesis, each original mutant line of both f(2)d' and f(2)d' was allowed to recombine with chromosomes carrying different marker mutations to control the recombinational events, and thus providing an initial map position of the new mutations. This initial mapping located both mutations in the cn-bw interval. For a more precise location we mapped both mutations by analyzing the recombinant chromosomes from females of genotype Bl'cn f(2)d' bw.

X-ray-induced mitotic recombination in the germline: We irradiated at 24-48 hr after oviposition if flies were grown at 29°, and at 72-96 hr if grown at 18°. The irradiation dose was 1000 rad, applied by a Philips X-ray machine at a rate of 300 rad/min. The clonal analysis in the germ line is based on the use of Fs(2)D. This is a dominant female-sterile mutation (YARGER and KING 1971), which autonomously affects the development of the germline, while having no effect on the somatic tissues involved in oogenesis (WIESCHAUS 1980). Figure 1 shows the scheme that we followed for the clonal analysis. The genotype of the experimental and control females was cn f(2)d' bw/Fs(2)D and cn f(2)d' bw/Fs(2)D, respectively. Since f(2)d' is a temperature-sensitive mutation, the clonal analysis was performed at both 18° and 29°.

Transplantation of pole cells: Pole cells were transplanted according to SANTAMARIA's (1986) procedure. We followed the experimental scheme of STEINMANN-ZWICKY, SCHMID and NÖTHIGER (1989), which is based on the use of
host embryos lacking their own pole cells, because they come from mothers homozygous for the oskar (osk) mutation (Lehmann and Nüsslein-Volhard 1986). To generate the host embryos we crossed \( \varphi ^{osk^{lo}} \) homozygous females with Oregon-R males. To generate the donor embryos we crossed \( cn^{fl(2)d^{lo}} bw/SM5 \) females and males. The transplantation of pole cells was carried out at 18°C, and the injected embryos were kept at this temperature until the larvae hatched. These were then transferred to vials with Drosophila food and cultured at 25°C for the rest of their development. The adult hosts were individually testcrossed. Host females and males were crossed with \( cn bw \) males and females. The expected donor-derived progeny is as follows: (a) females and males with white phenotype when the donor pole cells were homozygous for \( cn^{fl(2)d^{lo}} bw \), (b) females and males with either white or Curly-cinnabar phenotype when the donor pole cells were \( cn^{fl(2)d^{lo}} bw/SM5 \), and (c) females and males with Curly-cinnabar phenotype when the donor pole cells were homozygous for SM5. The sterile adult hosts were dissected in Ringer's solution and their gonads were examined with phase contrast under a compound microscope.

The sex of the germ cells was identified by morphological criteria. Germ cells following the oogenic pathway will form egg chambers that consist of 15 polyplid nurse cells and one diploid oocyte. This has a clear nucleus with a dark nucleolus, while the nuclei of the nurse cells are larger with multiple nucleoli (King 1970). In testes, germ cells were classified as oogenic only when clusters of 15 nurse cells and an oocyte were present. Germ cells that follow the spermatogenic pathway are easily identifiable when they form spermatids or sperm. Spermatocytes are identified by their spherical nucleus with a light center in the nucleolus and the assembly of mitochondria around the nucleus (Meyer, Hess and Beer mann 1961; Seidel 1963; Cooper 1965). In the absence of a Y chromosome, spermatocytes form so-called crystals (Meyer, Hess and Beer mann 1961) that serve as a reliable criterion for spermatogenesis.

**RESULTS**

The lethal phenotype of \( fl(2)d \) mutations: The effect of \( fl(2)d^{lo} \) and \( fl(2)d^{lo} \) on the viability of females and males is shown in Table 1. \( fl(2)d^{lo} \) is a recessive
temperature-sensitive mutation, the permissive temperature being 18°, and the restrictive one 29°. The effect on females is stronger than that on males. No \( f_{l(2)d}^1 \) homozygous females survive at 29°. The females that survive at 18° are sterile. On the contrary, the \( f_{l(2)d}^1 \) homozygous males are fertile, whether raised at 18 or 29°. \( f_{l(2)d}^2 \) is a recessive non-temperature-sensitive mutation, which is lethal in both sexes. The \( f_{l(2)d}^1/f_{l(2)d}^2 \) females are lethal at 29°, while having a reduced viability at 18°. The males are more viable at 18° than at 29°. The females that survive are sterile, while the males are fertile at both temperatures. \( f_{l(2)d}^2 \) animals developing at 29° die as larvae, although they can eventually reach the third larval instar. \( f_{l(2)d}^2 \) animals die very early in larval development.

The \( f_{l(2)d} \) gene is located in the right arm of the second chromosome, at position 70.08 ± 0.43 map units (m.u.). For the cytogenetic mapping, we tested the \( Df(2R)^{wg^3} \) (49B1-C1;49F15-50A1), the \( Df(2R)^{wg^8} \) (49D3-4; 50A2-5), the \( Df(2R)^L^{++} \) (50F-51A1; 51B), and the \( Df(2R)^{trix} \) (51A1-2; 51B6). Both females and males double homozygous for these deficiencies and the \( f_{l(2)d} \) mutations are fully viable. Since \( f_{l(2)d} \) maps at position 70.08 m.u., while \( vestigial \) (49C1-49E6) maps at position 67.0 m.u. and \( Lobe \) (51A1) maps at position 72.0 m.u., we conclude that \( f_{l(2)d} \) is placed within the chromosomal bands 50A2-5; 50F-51A1. The \( f_{l(2)d} \) mutations are not associated with any chromosomal aberrations.

The \( Sxl^{M1} \) mutation suppresses the lethality of \( f_{l(2)d} \) mutant females, while the \( f_{l(2)d} \) mutations suppress the lethality of \( Sxl^{M1} \) males: The viability of the \( f_{l(2)d}^1 \) homozygous females increases by the presence of \( Sxl^{M1} \) (Table 2, cross A; for comparison see Table 1), a mutation that constitutively expresses the \( Sxl \) functions characteristic of females (CLINE 1978). The suppressor effect of \( Sxl^{M1} \) is temperature-sensitive, being higher at 18° than at 29°. Since \( Sxl^{M1} \), itself, is not a temperature-sensitive mutation (our unpublished data), the thermosensitivity of the suppressor effect should be due to the temperature-sensitive phenotype of \( f_{l(2)d}^1 \). In fact, a positive correlation can be established between the degree of \( f_{l(2)d} \) activity and the suppressor effect by \( Sxl^{M1} \).

The elimination of the \( Sxl^+ \) copy from the \( Sxl^{M1}/ Sxl^+ \) females homozygous for \( f_{l(2)d}^1 \) decreases their viability, and those that survive at 18° exhibit an intersexual phenotype (Table 2, cross B). This indicates that the single \( Sxl^+ \) copy does not provide, in mutant conditions for \( f_{l(2)d} \), sufficient \( Sxl^+ \) function for normal female development, whereas it does in a \( f_{l(2)d}^+ \) background. This further indicates that the \( Sxl^+ \) copy present in \( Sxl^{M1}/Sxl^+; f_{l(2)d}^1/f_{l(2)d}^1 \) females is functional, even at the most restrictive conditions for \( f_{l(2)d} \) (29°). The \( Sxl^{M1}/Sxl^+; f_{l(2)d}^1/f_{l(2)d}^1 \) females are lethal at 29° and only 1% of them survive at 18° (Table 2, cross C). \( Sxl^{M1}/f_{l(2)d}^1 \) is a mutation induced on the \( Sxl^{M1} \) allele that retains its constitutivity but has affected the \( Sxl \) functions (CLINE 1984). The different viabilities of these females relative to the \( Sxl^+/ Sxl^+; f_{l(2)d}^1/f_{l(2)d}^1 \) females at 18° (see Table 1) is explained by the reduced level of \( Sxl^+ \) activity provided by the \( Sxl^{M1}/f_{l(2)d}^1 \) allele. In fact, \( Sxl^{M1}/Sxl^+; f_{l(2)d}^1/f_{l(2)d}^1 \) females have a similar viability to that of \( Sxl^+/ Df(Sxl); f_{l(2)d}^1/f_{l(2)d}^1 \) females (Table 2, cross D).

The viability of the \( Sxl^{M1}/Sxl^+; f_{l(2)d}^1/f_{l(2)d}^1 \) females is not increased by the presence of an extra \( Sxl^+ \) copy, either at 18° or 29° (Table 2, cross E). Moreover, the intersexual phenotype of these females, developing at 29°, is the same, whether they contain an extra \( Sxl^+ \) copy or not. These results demonstrate a limitation in the \( Sxl^+ \) function due to the \( f_{l(2)d}^1 \) mutation, which is more evident in conditions where the amount of \( f_{l(2)d}^+ \) activity is more reduced (29°). Moreover, these results indicate that the degree of constitutivity of \( Sxl^{M1} \); i.e., the amount of \( Sxl^+ \) function supplied by this allele, depends on the level of \( f_{l(2)d}^+ \) activity.

The \( Sxl^{M1} \) mutation suppresses the lethality of the \( f_{l(2)d}^1/f_{l(2)d}^1 \) females (Table 2, cross G), however this is not the case with the \( f_{l(2)d}^2 \) homozygous females (Table 2, cross F). This suppressor effect is higher at 18° than at 29°, due to a higher level of \( Sxl^+ \) function. At 29°, these flies are intersexual, while at 18° are sterile females. The replacement of \( Sxl^{M1} \) by \( Sxl^{M1}/f_{l(2)d}^1 \) eliminates the suppressor effect (Table 2, cross H). These results are compatible with the proposal that the level of \( Sxl^+ \) function depends on \( f_{l(2)d} \), and that \( f_{l(2)d}^2 \) is stronger than \( f_{l(2)d}^1 \) (see below).

As mentioned above, the \( Sxl^{M1} \) mutation constitutively expresses the \( Sxl \) functions characteristic of fe-

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**Table 1**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Temperature (°C)</th>
<th>Relative viability, %</th>
<th>(No. of control flies)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( f_{l(2)d}^1 )</td>
<td>18</td>
<td>16</td>
<td>75</td>
</tr>
<tr>
<td>( f_{l(2)d}^1 )</td>
<td>29</td>
<td>0</td>
<td>51</td>
</tr>
<tr>
<td>( f_{l(2)d}^2 )</td>
<td>18</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>( f_{l(2)d}^2 )</td>
<td>29</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>( f_{l(2)d}^1 )</td>
<td>18</td>
<td>4</td>
<td>85</td>
</tr>
<tr>
<td>( f_{l(2)d}^2 )</td>
<td>29</td>
<td>0</td>
<td>52</td>
</tr>
</tbody>
</table>

Crosses: \( f_{l(2)d}^1 \) bw/SM5, females and males; \( f_{l(2)d}^1 \) bw/SM5, females and males; \( f_{l(2)d}^2 \) bw/SM5, females and \( f_{l(2)d}^1 \) bw homozygous males. Control flies were heterozygous for the \( f_{l(2)d} \) mutations.
males, and this determines the male-specific lethal phenotype of this mutation (CLINE 1978). The lethality of the Sxl+ males is partially suppressed by fl(2)d+; the suppressor effect being higher at 18° than at 29° (Table 3, cross A; for comparison see Table 1). While at 18°, 25% of these males are fertile, all of them are sterile at 29° and a few of them present intersexual traits. These results agree with the idea of the Sxl+ function being dependent on fl(2)d+ function. Due to the hypomorphic character of fl(2)d+ (unpublished data), in some of the Sxl+/Sxl+/fl(2)d+ males there is still enough Sxl+ function to cause their lethality. This is confirmed by the viability increase of these males when the Sxl+ allele is replaced by Sxl-3 (Table 3, cross B). The addition of an extra Sxl+ copy to the Sxl+ males homozygous for fl(2)d+ does not reduce their viability (Table 3, cross C). Moreover, both Sxl+ and Sxl+/Dp(Sxl+) males homozygous for fl(2)d+, developing at 29°, exhibit identical intersexual traits: some sexcombs are composed of pure male bristles and intersexual ones. Thus, as it happens in females (Table 2, cross E), in the Sxl+ males there is also a limitation in the amount of Sxl+ function supplied by the Sxl+ allele due to the fl(2)d mutation.

The viability of the fl(2)d+ homozygous males is the same whether they carry the Sxl+ allele (see Table 1) or a deficiency of it (Table 3, cross D). This would indicate that the fl(2)d+ homozygous males present a certain lethality, higher at 29° than at 18°, which is independent of Sxl. Also the fl(2)d+ homozygous males are lethal, even if they carry a deficiency of Sxl. Moreover, fl(2)d does not suppress the lethality of the Sxl+ males (data not shown). The fl(2)d+/fl(2)d combination, in turn, partially suppresses the lethality of the Sxl+ males; this suppression being higher at 18° than at 29° (Table 3, cross E). The viability of these males is increased if the Sxl+ allele is replaced by Sxlfirst (Table 3, cross F).

**TPS of fl(2)d+:** To determine the developmental time when the fl(2)d+ function is required, we carried
TABLE 3

Interaction between Sxl and fl(2)d in X;2A flies

<table>
<thead>
<tr>
<th>Cross</th>
<th>Genotype</th>
<th>Development at 18°</th>
<th>Development at 29°</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Relative viability, % (No. of control flies)</td>
<td>Sexual phenotype</td>
</tr>
<tr>
<td>A</td>
<td>Sxl&lt;sup&gt;M1&lt;/sup&gt;, fl(2)d&lt;sup&gt;1&lt;/sup&gt; fl(2)d&lt;sup&gt;1&lt;/sup&gt;</td>
<td>43 (247)</td>
<td>Male</td>
</tr>
<tr>
<td>B</td>
<td>Sxl&lt;sup&gt;M1&lt;/sup&gt;, fl(2)d&lt;sup&gt;1&lt;/sup&gt; fl(2)d&lt;sup&gt;1&lt;/sup&gt;</td>
<td>79 (100)</td>
<td>Male</td>
</tr>
<tr>
<td>C</td>
<td>Sxl&lt;sup&gt;M1&lt;/sup&gt;, fl(2)d&lt;sup&gt;1&lt;/sup&gt; fl(2)d&lt;sup&gt;1&lt;/sup&gt; Df(Sxl) &lt;sup&gt;+&lt;/sup&gt;</td>
<td>52 (172)</td>
<td>Male</td>
</tr>
<tr>
<td>D</td>
<td>Df(Sxl), fl(2)d&lt;sup&gt;1&lt;/sup&gt; fl(2)d&lt;sup&gt;1&lt;/sup&gt;</td>
<td>74 (248)</td>
<td>Male</td>
</tr>
<tr>
<td>E</td>
<td>Sxl&lt;sup&gt;M1&lt;/sup&gt;, fl(2)d&lt;sup&gt;1&lt;/sup&gt; fl(2)d&lt;sup&gt;2&lt;/sup&gt;</td>
<td>59 (104)</td>
<td>Male</td>
</tr>
<tr>
<td>F</td>
<td>Sxl&lt;sup&gt;M1&lt;/sup&gt;, fl(2)d&lt;sup&gt;1&lt;/sup&gt; fl(2)d&lt;sup&gt;2&lt;/sup&gt;</td>
<td>77 (124)</td>
<td>Male</td>
</tr>
</tbody>
</table>

Crosses: A, cross A of Table 2; B, cross C of Table 2; C, cross E of Table 2; D, cross D of Table 2; E, cross G of Table 2; F, cross H of Table 2. Control flies were the same as in Table 2, since the FM6 balancer chromosome has detrimental effects on male viability. In the case of cross D, the control flies were Df(Sxl) males heterozygous for fl(2)d<sup>1</sup>.

out temperature shifts between the restrictive and the permissive temperature, in both directions. The first shift from the restrictive to the permissive temperature displaying the mutant phenotype marks the beginning of the TPS, while the end of that period is defined by the first shift from the permissive to the restrictive temperature displaying the wild-type phenotype.

Figure 2a shows the TPS of the females homozygous for fl(2)d<sup>1</sup>. It was surprising the two peaks of viability obtained for the temperature shifts from 29 to 18° during the first 36 hr of development; however, the fact that the first and second points represent the shift at 0–2 and 2–4 hr of development, respectively, is an indication of the very early requirement for fl(2)d<sup>+</sup> function. With respect to the shifts from 18 to 29°, we did not recover any female homozygous for fl(2)d<sup>1</sup>, independently of the developmental time when the shift was performed. We conclude that the female TPS of fl(2)d<sup>1</sup> spans the whole development, which further indicates that the fl(2)d<sup>+</sup> function is required throughout the development of female flies. This agrees with the idea that the gene fl(2)d is needed for Sxl<sup>+</sup> function, since this gene is continuously required for the development of female flies (Cline 1979; Sánchez and Nöthiger 1982).

Figure 2b shows the TPS of the fl(2)d<sup>1</sup> homozygous males. There are oscillations in the viability values for the temperature shifts at the first stages of development, although they are not as strong as in the case of the fl(2)d<sup>1</sup> females. The viability of the fl(2)d<sup>1</sup> males decreases gradually: the longer the males stay at 29°, the lower is their viability. This contrasts with the abrupt viability decrease of the fl(2)d<sup>1</sup> females. These different temperature-sensitive profiles of mutant females and males may reflect the different causes of the lethality of both sexes.

**Interaction between fl(2)d and genes involved in Sxl activation:** As mentioned in the Introduction, the state of activity of Sxl, which occurs around blastoderm stage, is specified by the X:A signal and the da maternal product. Since fl(2)d activity is required for Sxl<sup>+</sup> function from the beginning of development (see TPS of fl(2)d<sup>1</sup>), as a further test for the role of fl(2)d we have analyzed the interaction between this gene and sc, an X element of the X:A ratio signal, as well as the interaction between fl(2)d and the maternal da product. Loss-of-function mutations at da and Sxl display female-specific dominant synergism, each enhancing the other's sex-specific lethal effect (Cline 1980). A similar synergistic interaction has been observed between loss-of-function mutations at either da and sc, or sc and Sxl (Cline 1986, 1988; Torres and Sánchez 1989). No female-specific lethal synergism exists between mutations at fl(2)d and Sxl; as females double heterozygous for fl(2)d<sup>1</sup> or fl(2)d<sup>2</sup>, and a deficiency for Sxl are fully viable (data not shown).

We have measured the viability of females heterozygous for fl(2)d and deriving from mothers heterozygous for da<sup>2</sup> (Table 4, cross A). The experiment was performed at 29° because of the temperature-dependence for the lethal effects involving the mater-
and are as viable as the control flies when they are derived from da+ homozygous mothers (Table 7).

We crossed \( \text{cnjZ}(2)d' \) females double heterozygous for \( J(2)d' \) and \( da' \) in males (b). We analyzed the interaction between \( J(2)d' \) and the maternal \( da \) product in more restrictive conditions for this product: we measured the viability of females and males.

The numbers in parentheses refer to the flies used as viability reference: \( \text{cnjZ}(2)d' \) heterozygous females (a) and males (b). The egg-laying period was for 2 hr.

In Table 5 we present the data on the viability of females double heterozygous for \( fl(2)d' \), or \( fl(2)d^2 \), and \( sc \) mutations. The viability of these females (crosses A and B) is similar to that of the control females (\( P > 0.1 \) for cross A and \( P > 0.5 \) for cross B). As reported before, \( sc^{51}/Sxl^{MM} \) females have a reduced viability (Cline 1988; Torres and Sanchez 1989), which significantly (\( P < 0.001 \)) drops to zero in heterozygous conditions for \( fl(2)d' \) (Table 5, cross C).

**The sterile phenotype of \( fl(2)d' \) mutant females:** Females homozygous for \( fl(2)d' \) developing at 18° are sterile. They never lay eggs. We have analyzed the ovaries of these females at different times after eclosion. Some females were kept at 18° and others were shifted to 29° immediately after eclosion (the eclosed flies were collected every 4 hr). No differences were observed between the ovaries developed at 18° and those developed at 29°. In both cases, the first oogenic stages seemed to occur normally, at least at the microscopic level. In the great majority of the cases, oogenesis was arrested at the 8th and 9th previtellogenic stages. Occasionally, some eggs chambers continued to develop, but they gave rise to abnormal oocytes, in which the yolk was disorganized (Figure 3A). In other cases, we observed multicellular cysts (Figure 3B), resembling those formed by loss-of-function mutations at the gene Sxl (Schupbach 1985; Steinmann-Zwicky, Schmid and Notthiger 1989; Nothiger et al. 1989). These types of abnormalities were observed in young \( fl(2)d' \) mutant females, which had no time to develop later oogenic stages. This eliminated the possibility that the alterations observed in the ovaries of mutant females were the consequence of a reabsorption process due to a failure in oviposition.

\( Sxl^{MM} \) suppresses the sterility of the \( fl(2)d' \) homozygous females developing at 18° and kept at this temperature after eclosion. Both the \( Sxl^{MM}/Sxl^+;fl(2)d'/fl(2)d' \) females developing at 18° and shifted to 29° after eclosion, and the \( Sxl^{MM}+/;fl(2)d'/fl(2)d' \) females developing at 18°, contained well developed ovarioles in which all normal oogenic stages were observed; these females, however, remained sterile due to a failure in oviposition. Thus, \( Sxl^{MM} \) overcomes the arrest that the mutation \( fl(2)d' \) in homozygosis, or the \( fl(2)d'/fl(2)d^2 \) combination, imposed on the germ cells. This further suggests that the ovarian phenotype showed by the \( fl(2)d' \) mutant females is caused by alteration of \( Sxl \) expression.

We can reject the possibility that the sterile phenotype of the \( fl(2)d' \) mutant females was caused by a second female-sterile mutation coinduced with \( fl(2)d' \). First, each original mutant line went through several recombination events (see MATERIALS AND METHODS). Second, if the oogenesis arrest was independent of the \( fl(2)d' \) mutations and then caused by a second muta-
interaction, it is not expected that this arrest was suppressed by Sxl'. Third, fl(2)d1 is a temperature-sensitive mutation and the suppression of the oogenesis arrest by Sxl' is also temperature-sensitive. And fourth, Sxl''/+; fl(2)d1/" females developing at 18° have normal ovaries, in which all oogenic stages were observed.

Clonal analysis of fl(2)d1 in the germline: To determine whether fl(2)d1 homozygous germ cells can develop into normal eggs within a wild-type female ovary, we have induced mitotic recombination in the germline of fl(2)d1 mutant females. For that purpose we followed the experimental scheme of Figure 1 (see MATERIALS AND METHODS). The results are shown in Table 6. At 29°, we have not found any fertile experimental female, while we have found eight fertile control females. At 18°, we have found both experimental and control females that were fertile, although the frequency of the latter was higher than that of the former. Among the six fertile experimental females, four of them gave rise to a few progeny, which died as larvae or pupae. The other two females gave rise to adult progeny, which in both cases was homozygous for bw and heterozygous for cn. This indicates that these two females contained a clone of germ cells that could be either homozygous or heterozygous for fl(2)d1, depending on whether mitotic recombination took place proximal or distal to fl(2)d1, respectively (see Figure 1). To answer this question, we testcrossed the bw male offspring of the two females with cn fl(2)d1 bw/CyO females, at 29°. The progeny revealed that one of these two females contained a clone of germ cells homozygous for fl(2)d1, which were able to develop into functional eggs. The remaining four fertile experimental females that only produced a few larvae and pupae, at 18°, might presumably contain fl(2)d1 homozygous germ cells, which had enough fl(2)d

### Table 4

<table>
<thead>
<tr>
<th>Cross</th>
<th>T*</th>
<th>Maternal genotype</th>
<th>Paternal genotype</th>
<th>Relative viability of da2/fl(2)d females, % (No. of control flies)</th>
<th>Relative viability of da2/fl(2)d females, % (No. of control flies)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>29°C</td>
<td>da2/fl(2)d1</td>
<td>da2/fl(2)d2</td>
<td>96 (282)</td>
<td>96 (282)</td>
</tr>
<tr>
<td>B</td>
<td>18°C</td>
<td>da2/fl(2)d1</td>
<td>da2/fl(2)d2</td>
<td>98 (281)</td>
<td>98 (281)</td>
</tr>
<tr>
<td>C</td>
<td>29°C</td>
<td>da2/fl(2)d1</td>
<td>da2/fl(2)d2</td>
<td>96 (119)</td>
<td>96 (119)</td>
</tr>
<tr>
<td>D</td>
<td>29°C</td>
<td>da2/fl(2)d1</td>
<td>da2/fl(2)d2</td>
<td>98 (337)</td>
<td>98 (337)</td>
</tr>
</tbody>
</table>

Crosses:
A. y/y; da2/CyO females and cn fl(2)d1 bw/SM5. Control flies were males double heterozygous for da2 and fl(2)d2.
B. da2/da females and cn fl(2)d1 bw/CyO males. Control flies were males heterozygous for da2.
C and D. cn fl(2)d1 bw/CyO females and da2/da1 males. Control flies were males heterozygous for da2.

### Table 5

<table>
<thead>
<tr>
<th>Cross</th>
<th>T*</th>
<th>Maternal genotype</th>
<th>Paternal genotype</th>
<th>Relative viability of sc*/+; fl(2)d1/+ females, % (No. of control flies)</th>
<th>Relative viability of sc*/Sxl; fl(2)d1/+ females, % (No. of control flies)</th>
<th>Relative viability of sc*/Sxl; fl(2)d1/+ females, % (No. of control flies)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td></td>
<td>sc*/+; fl(2)d1/+</td>
<td></td>
<td>83 (235)</td>
<td>83 (235)</td>
<td>83 (235)</td>
</tr>
<tr>
<td>B</td>
<td></td>
<td>sc*/+; fl(2)d1/+</td>
<td></td>
<td>92 (150)</td>
<td>92 (150)</td>
<td>92 (150)</td>
</tr>
<tr>
<td>C</td>
<td></td>
<td>sc*/+ Sxl; fl(2)d1/+</td>
<td></td>
<td>18 (144)</td>
<td>18 (144)</td>
<td>18 (144)</td>
</tr>
</tbody>
</table>

Crosses: Df(1)Rt343, sc*/FM7 females were crossed with either y/Y; Bt fl(2)d1/SM5 males (A), or cn fl(2)d1 bw/SM5 males (B), and sc*/w ftsu/FM7 females were crossed with cm Sxl0ct/Y; cn fl(2)d1 bw/CyO males (C). Control flies: (A) and (B) females heterozygous for sc; (C) females heterozygous for Sxl0.
product to form some oocytes, although insufficient for these to become normal, as they show an abortive development, producing only larvae and pupae but not adults. Taken together the results at 29 and 18 °C, we conclude that the f1(2)d’ mutation autonomously affects the development of the germline. This effect is higher at 29 °C than at 18 °C, as expected due to the temperature-sensitive character of this mutation.

Transplantation of pole cells mutant for f1(2)d’2:  
The f1(2)d’2 mutation is lethal to both females and males. By means of pole cell transplantation, we have explored the capacity of f1(2)d’2 germ cells to develop into functional gametes. The experiment has two aims. First, we wanted to know if 2X germ cells homozygous for f1(2)d’2 mimic the behavior of 2X germ cells homozygous for loss-of-function mutations at the gene Sxl, as is the case for the somatic cells (GRANADINO, CAMPUZANO and SÁNCHEZ 1990). If so, we assumed that these cells follow an abortive spermatogenic pathway, as 2X germ cells lacking Sxl’ activity do (SCHÜPBACH 1985; STEINMANN-ZWICKY, SCHMID and NÖTHIGER 1989; NÖTHIGER et al. 1989). Second, we asked if XY germ cells homozygous for f1(2)d’2 are capable of giving rise to functional sperm. We injected 2487 embryos, from which only 205 reached the adult stage: 113 were females and 92 were males. From these adults, 33 females and 22 males contained donor pole cells. Among the 33 females, 9 of them were fertile. None of these females contained donor pole cells homozygous for f1(2)d’2, nor for SM5, but all of them contained f1(2)d’2/SM5 pole cells.

Five other females contained well developed ovarioles, in which we observed all oogenic stages, that seemed normal at the microscopical level. These females, however, laid few eggs from which no adult offspring emerged. Presumably, they represent females that received SM5 homozygous pole cells. The possibility remains that these females received f1(2)d’2/SM5 germ cells, and that they were unfertile due to a mechanical block induced by the transplantation procedure. We reject the possibility that these females received f1(2)d’2 homozygous germ cells, since germ cells homozygous for f1(2)d’, a weaker mutation than f1(2)d’2, are mainly arrested at previtellogenic stages and the few that continue development form abnormal egg chambers, and in some cases form multicellular cysts (see above).

Three females had small ovaries, containing multicellular cysts with undifferentiated cells (Figure 4A). An abnormal oogenic stage was also present in these three females. This indicates that these females received 2X,2A donor pole cells. We believe that these females received 2X germ cells homozygous for f1(2)d’2, which followed an abortive pathway (formation of multicellular cysts), similar to that of germ cells homozygous for loss-of-function mutations at the gene Sxl (SCHÜPBACH 1985; STEINMANN-ZWICKY, SCHMID and NÖTHIGER 1989; NÖTHIGER et al. 1989).

Eleven females contained multicellular cysts with undifferentiated and/or degenerating cells and cysts with well developed spermatocytes (Figure 4B). The absence of crystals in the cytoplasm of these spermatocytes indicates that these females received XY germ cells (STEINMANN-ZWICKY, SCHMID and NÖTHIGER 1989).

Finally, five females are characterized by having small ovaries with no detectable oogenic, nor spermatogenic, stages. They exclusively contained cysts with a high number of undifferentiated and/or degenerating cells, whose karyotype could not be determined. This class most likely represents females that received XY germ cells, which did not reach the spermatocyte stage (STEINMANN-ZWICKY, SCHMID and NÖTHIGER 1989).

These data confirm the results of clonal analysis; i.e., the f1(2)d’2 homozygous germ cells are not able to develop into functional oocytes, and in addition suggest that these cells presumably enter an abortive pathway similar to that of 2X germ cells lacking Sxl activity.

With respect to the adult males with donor pole cells, we found six fertile males: four of them received f1(2)d’2/SM5 pole cells and the other two received pole cells homozygous for f1(2)d’2. We found four males containing spermatocytes without crystals and spermatids, but never sperm; presumably they represent males that received XY pole cells homozygous for SM5. In six males we found well developed spermatocytes with crystals (Figure 4C), indicating that they received 2X germ cells (STEINMANN-ZWICKY, SCHMID and NÖTHIGER 1989). Finally, we found six males with non-gametogenic testes, which contained cysts with undifferentiated and/or degenerating cells, whose karyo-

![Figure 3](image-url)  
**Figure 3.** Ovaries of f1(2)d’ homozygous flies developed at 18 °C. (A) Abnormal advanced oogenic stage; (B) multicellular cyst (arrow).
TABLE 6
Clonal analysis of fl(2)d' in the female germline

<table>
<thead>
<tr>
<th>Genotype of tested females</th>
<th>No. of tested females</th>
<th>No. of fertile females</th>
<th>No. of females with progeny heterozygous for cn, homozygous for bw</th>
<th>No. of females with progeny homozygous for cn bw</th>
<th>No. of females with germ cells homozygous for fl(2)d'</th>
</tr>
</thead>
<tbody>
<tr>
<td>29°  cn fl(2)d'bw</td>
<td>931</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F(2)D</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>29°  cn fl(2)d'bw</td>
<td>921</td>
<td>8</td>
<td>2</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>F(2)D</td>
<td></td>
<td></td>
<td>(5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18°  cn fl(2)d'bw</td>
<td>1015</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>F(2)D</td>
<td></td>
<td></td>
<td>(4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18°  cn fl(2)d'bw</td>
<td>1145</td>
<td>9</td>
<td>4</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>F(2)D</td>
<td></td>
<td></td>
<td>(5)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Nonirradiated experimental (503 at 29° and 467 at 18°) and control (536 at 29° and 485 at 18°) females were fully sterile. In parentheses is given the number of fertile females which did not produce enough progeny.

FIGURE 4.—Transplantation of pole cells involving the fl(2)d' mutation. (A) Multicellular cysts (arrow) and germarium stages (asterisk) formed in ovary by pole cells homozygous for fl(2)d'. (B) Spermatocytes without crystals formed in ovary by XY pole cells. (C) Spermatocytes with needle-like crystals (arrow) in testis formed by XX pole cells.

otype could not be determined, and that presumably correspond to males that received 2X pole cells whose development was arrested (STEINMANN-ZWICKY, SCHMID and NÖTHGER 1989). We conclude that germ cells homozygous for fl(2)d' can give rise to functional sperm.

Interaction between fl(2)d and snf: Females trans-heterozygous for snf (liz in Steinmann-Zwicky’s terminology) and Sxl mutations show sexual transformations, ovarian tumors and greatly reduced viability. These results led to the proposition that snf is needed to activate or to maintain Sxl expression (OLIVER, PERRIMON and MAHOWALD 1988; STEINMANN-ZWICKY 1988). Since fl(2)d is also needed for Sxl expression, we have analyzed the interaction between snf and fl(2)d mutations. Females double heterozygous for snf^{1621}, or a deficiency of this gene (Df{1}F{70}), and fl(2)d {at 29°}, or fl(2)d' {at 25°}, are fully viable and fertile, and they do not show any sign of sexual transformation. Their ovaries look normal and all oogenetic stages are observed in all ovarioles. Furthermore, both the viability and sexual phenotype of females homozygous for snf^{1621} are not affected if they are heterozygous for fl(2)d' {at 29°}, or fl(2)d' {at 25°}.
Functional Relationship between Sxl and fl(2)d

The ovaries of the snf621/snfl621:fl(2)d1/+ females developing at 29° do not differ from that of their snf621/snfl621 sisters: they are small and contain few ovarioles with multicellular cysts (Figure 5A). The same phenotype is observed in females homozygous for snf621 developing at 25°; however, their snf621/snfl621:fl(2)d2/+ sisters have better developed ovaries, in which it is possible to observe, together with ovarioles containing multicellular cysts, other ovarioles with egg chambers that reach the oogenic stage 6 to 11 (Figure 5B). Thus, heterozygosity for fl(2)d1, but not for fl(2)d2, partially suppresses the germline phenotype of snf621.

Interaction between fl(2)d and ovo: Mutations at ovo result in a defective female germline, while the male germline is not affected (Oliver, Perrimon and Mahowald 1987; Oliver, Pauli and Mahowald 1990). Since fl(2)d is required for female, but not male, germline development, we have analyzed the interaction between this gene and ovo. Three dominant ovoD1 mutations have been characterized. Oogenesis of ovoD1/+ females is mainly arrested prior to stage 4, while that of ovoD2/+ females prior to stage 6. ovoD1/+ females have a near normal oogenesis, difficult to distinguish from wild-type in dissected ovaries (Oliver, Perrimon and Mahowald 1987). Oogenesis in females double heterozygous for any of these three ovoD mutations and fl(2)d1 (at 29°), or fl(2)d2 (at 25°), do not differ from that of ovoD/+ sisters mentioned above (data not shown).

Females double heterozygous for the strong loss-of-function ovoD1S1 mutation, or a deficiency of this gene (Df(l)J6C70), and fl(2)d1 (at 29°), or fl(2)d2 (at 25°), are fully viable and fertile: their ovaries are normal in size and all of the ovarioles contain egg chambers in all oogenic stages (data not shown). Adult females homozygous for ovoD1S1 do not have ovaries, because their germ cells die (Oliver, Perrimon and Mahowald 1987; our personal observations). However, their sisters homozygous for ovoD1S1 and heterozygous for fl(2)d1 (at 29°) (Figure 6A), or fl(2)d2 (at 25°) (Figure 6B), have small ovaries with a few ovarioles containing mainly multicellular cysts and cysts with 16 cells resembling young egg chambers. No great differences were observed between these females heterozygous for fl(2)d1 or fl(2)d2. Thus, the fl(2)d mutations par-
tially suppress the specific-lethal female germline phenotype of the loss-of-function vgo mutations.

DISCUSSION

The gene fl(2)d seems to have a dual function: Loss-of-function mutations at either fl(2)d or Sxl are equivalent with regard to the processes controlled by Sxl: sex determination and dosage compensation (GRANADINO, CAMPUZANO and SÁNCHEZ 1990). Thus, fl(2)d function is required for Sxl function. This agrees with the TSP of fl(2)d', that revealed the necessity of fl(2)d activity throughout the development of females. Moreover, fl(2)d is also required throughout the adult life of females for the processes that require Sxl activity, but not for males (GRANADINO, SAN JUÁN and SÁNCHEZ 1991). The gene fl(2)d is needed for the female-specific splicing of Sxl RNA, showing the involvement of fl(2)d in the maintenance autoregulatory pathway of Sxl (GRANADINO, CAMPUZANO and SÁNCHEZ 1990).

Nevertheless, fl(2)d mutations also affect male viability. This cannot be attributed to lack of Sxl activity, since this function is unnecessary for male development (SALZ, CLINE and SCHEDL 1987). Moreover, we have found that the semilethality, or lethality, of males homozygous for fl(2)d1, or fl(2)d2, respectively, is the same, whether they contain a Sxl copy or a deficiency of this gene. On the other side, it is known that SxIM is a mutation that constitutively expresses the Sxl functions characteristics of females, which determines its dominant male-specific lethal phenotype (CLINE 1978). The fl(2)d mutations, however, can partially suppress the lethality of SxIM males, this suppression being higher at 18° than at 29°. Thus, there seems to exist a contradiction. On one hand, SxIM/Y;fl(2)d/+ fl(2)d+ males are lethal, while SxIM males mutant for fl(2)d are viable; i.e., the viability of SxIM males is negatively correlated with the degree of fl(2)d activity. On the other hand, SxIM males mutant for fl(2)d are more viable at 18° than at 29°; i.e., the viability of SxIM males is positively correlated with the degree of fl(2)d activity. Furthermore, the TSP profile of fl(2)d1 is clearly different in females and males. All these results are explained if the gene fl(2)d has a dual function: it is needed for sex determination and dosage compensation through its involvement in the female-specific splicing of Sxl RNA (female-specific function), and it is needed for a vital process which is independent of Sxl (non-sex-specific function). This process seems not to be related to basic metabolic cell functions, because first, the fl(2)d mutations are not cell lethals (GRANADINO, CAMPUZANO and SÁNCHEZ 1990; GRANADINO, SAN JUÁN and SÁNCHEZ 1991), and second, transplanted male germ cells homozygous for fl(2)d2 can develop into functional sperm (this report). This result further shows that this non-sex specific function is exclusively needed in a process of somatic nature. Since the fl(2)d mutations do not affect the adult life of males, and since the effect of these mutations in the adult life of females is suppressed by SxIM' (GRANADINO, SAN JUÁN and SÁNCHEZ 1991), we further conclude that the non-sex-specific function of fl(2)d has to do with a specific process in the development of both females and males.

We discard the possibility that the dual function attributed to fl(2)d would really represent two functions corresponding to two different genes. First, fl(2)d1 and fl(2)d2 arose in different mutagenesis, so it is practically impossible that both mutations could be coinduced with a common second mutation. Second, to rid both fl(2)d1 and fl(2)d2 lethal-bearing chromosomes of other deleterious mutations that might have been induced during the EMS treatment, each original mutant line was allowed to recombine with chromosomes carrying different marker mutations to control the recombinational events. Third, the fl(2)d1-bearing chromosome in homozygosis shows a temperature-sensitive phenotype for both the Sxl-splicing and the non-sex-specific function. Then, it is rather unlikely that if both functions correspond to two different genes, the coinduced mutations were temperature-sensitive at the same time.

Following the proposal on the dual function of fl(2)d, the female lethality and the male semilethal phenotype of fl(2)d1 would be due to this mutation affecting the Sxl splicing function more than the non-sex-specific function. In contrast, the fl(2)d2 mutation would strongly affect both functions, thus explaining the lethality of both females and males. In the case of the fl(2)d1/fl(2)d2 combination, the Sxl splicing function would be more affected than the non-sex-specific function. This would explain why the fl(2)d1/fl(2)d2 combination is lethal in females while viable in males. That fl(2)d2 is affecting the Sxl-splicing function is deduced from the fact that all of the clones homozygous for fl(2)d2, the female-specific splicing pattern, but the male one. This results in the formation of the nonfunctional Sxl products typical of males, and then these would be
viable. This is confirmed by the viability increase when Sxl^{M1} is replaced by Sxl^{M1,P}, a mutation induced on the Sxl^{M1} allele that retains its constitutivity but has affected the Sxl functions (CLINE 1984). However, the viability increase is not complete. This is explained by the alteration of the fl(2)d non-sex-specific function, which is independent of Sxl. The higher viability of the Sxl^{M1} males mutant for fl(2)d at 18° relative to 29° is explained by a better improvement at 18° of the non-sex-specific function of fl(2)d respect to the improvement of its Sxl-splicing function. On the other hand, the Sxl^{M1} males homozygous for fl(2)d^{2} are lethal. Although in these males the Sxl RNA would follow the male-specific splicing, fl(2)d^{2} is also affecting the non-sex-specific function.

The suppression of the lethality of females homozygous for fl(2)d^{1} and those of genotype fl(2)d^{1}/fl(2)d^{2} by Sxl^{M1} is attributed to the constitutive character of this mutation (see below). This suppression, however, depends on the amount of fl(2)d activity, since the suppression is higher at 18° than at 29°, and at the latter temperature these females show sexual transformation. The addition of an extra Sxl^{+} copy does not increase the viability of the Sxl^{M1}/Sxl^{+} females mutant for fl(2)d. This agrees with the idea that the amount of fl(2)d activity is a limiting factor for the amount of Sxl activity. Sxl^{M1} does not completely suppress the lethality of the fl(2)d^{1} homozygous females, nor that of the fl(2)d^{1}/fl(2)d^{2} constitution. This is explained because both females are also affected by the non-sex-specific function of fl(2)d. On the other hand, Sxl^{M1} does not suppress the lethality of the females homozygous for fl(2)d^{2}. The different behavior of Sxl^{M1} in fl(2)d^{1} relative to fl(2)d^{2} females would be due to the stronger character of this latter mutation.

The gene fl(2)d seems necessary for Sxl expression in the female germline: Our results show that fl(2)d mutations autonomously affect the female germline, while having no effect on the male germline. Thus, loss-of-function mutations at either the gene fl(2)d or the gene Sxl are equivalent with regard to germline development. We propose that fl(2)d is required in the female germline for the expression of Sxl. This would explain that Sxl^{M1} suppresses the sterility of the fl(2)d^{1} homozygous females that survive at 18°. Sxl^{M1}/+;fl(2)d^{1}/fl(2)d^{1} females developing at 18° and shifted to 29° after eclosion, and also Sxl^{M1}/+;fl(2)d^{1}/fl(2)d^{2} females developing at 18°, do not lay eggs. This cannot be attributed to a failure of Sxl^{M1} to suppress the oogenesis arrest of these females, but to a failure in oviposition, since these females show normal ovaries with all ovarioles containing egg chambers in all oogenic stages.

The gene fl(2)d is needed in the somatic tissues for the female-specific splicing pattern of Sxl RNA (GRANADINO, CAMPUZANO and SÁNCHEZ 1990). On the other hand, two Sxl transcripts have been identified in the female germline (SALZ et al. 1989). We suggest that the requirement of fl(2)d for the female germline development is related to its involvement in Sxl splicing, so that in fl(2)d homozygous germ cells the male-specific exon cannot be eliminated from the primary Sxl RNA, and consequently nonfunctional truncated Sxl proteins will be produced, thus explaining the similar behavior of mutations at both fl(2)d and Sxl genes.

The fl(2)d^{1} females developing at 18° are sterile. In these females, both the somatic tissues involved in oogenesis and the germline are mutant. We have found, however, that at 18° fl(2)d^{1} germ cells in a wild-type soma are capable of developing into functional oocytes. We conclude, then, that the fl(2)d^{1} females have also affected the somatic component of oogenesis. Since the fat body of these females has still some capacity to synthesize yolk proteins (GRANADINO, SAN JUÁN and SÁNCHEZ 1991), the somatic component affected may be the gonadal soma. This result leads to the further conclusion that not only the germline but also the soma supporting its development require fl(2)d function. This is in agreement with the necessity of fl(2)d function for the development of female somatic cells.

Cell autonomous and somatic inductive signals determine the sex of the germline by regulating the gene Sxl (STEINMANN-ZWICKY, SCHMID and NÖTHIGER 1989; NÖTHIGER et al. 1989). The nature of the somatic signal remains unknown. A possibility could be that this signal is made up by the Sxl and the fl(2)d products from the female gonadal soma. These would be required in the germline for initiating the first female-specific splicing of the Sxl RNA, which is produced as a response to the cell autonomous X:A signal.

A working hypothesis on the functional relationship between Sxl and fl(2)d: Our results indicate that the gene fl(2)d seems to have a dual function: a female-specific and a non-sex-specific function. This raises the question of how the female-specific function of fl(2)d is controlled. Our results discard the possibility that the X:A signal would directly control this function: Sxl^{M1}/Y;fl(2)d^{+}/fl(2)d^{+} males, in which this function should not be activated (X:A = 0.5), are lethal. Moreover, there exists no female-specific lethal synergistic interaction between mutations at fl(2)d and either sc, or a reduced amount of maternal da product, as expected if the female-specific function depends on the X:A signal. On the other hand, mutations at Sxl and sc, or a reduced amount of maternal da product, display female-specific dominant synergism, each enhancing the other's sex-specific lethal effect (CLINE 1980, 1986, 1988; TORRES and SÁNCHEZ 1989). In all these cases, the lethality is the consequence of a failure for stabilizing the activation of Sxl, since there
is a reduction of the initial expression of this gene. This synergistic lethality is increased by mutations at \( f(2)d \). We suggest that this is a consequence of the female-specific function of \( f(2)d \) being under the control of Sxl: the reduced amount of initial Sxl products causes a reduced amount of this initial \( f(2)d \) function, since only one \( f(2)d^+ \) allele is available and, consequently, a stable positive cycle between Sxl and \( f(2)d \) is not established. Thus, we propose that the female-specific function of \( f(2)d \) is under the control of Sxl and that the female-specific splicing of Sxl RNA is under the control of the female-specific function of \( f(2)d \), therefore a stable positive feedback might be established between Sxl and \( f(2)d \).

A possibility is that \( f(2)d \) would code for a single product. In this case, the two functions of \( f(2)d \) could reflect the different sensitivity of the two functions to \( f(2)d \) levels. The postulated stable positive cycle between Sxl and the female-specific function of \( f(2)d \) would determine that a higher level of \( f(2)d \) product was attained in females relative to males. Alternatively, the two functions of \( f(2)d \) could be carried out by two different products coded by this gene, one of which was under the control of Sxl and then female-specific.

Sxl\(^{M1} \) suppresses the lethality of \( f(2)d \) females: Since \( f(2)d \) is needed for the female-specific splicing of Sxl RNA, it would be expected that Sxl\(^{M1} \) did not suppress the lethality of females mutant for \( f(2)d \), contrary to observation. One possibility could be that Sxl\(^{M1} \) produces a transcript that is driven into the female mode of splicing, independently of both Sxl and \( f(2)d \) products. If this was the case, the \( f(2)d \) mutations would not suppress the lethality of Sxl\(^{M1} \) males, because in these the Sxl\(^{M1} \) transcript would follow the female mode of splicing. We believe that the suppression effect of Sxl\(^{M1} \) is a consequence of the postulated positive feedback cycle between Sxl and \( f(2)d \).

The molecular analysis of Sxl\(^{M1} \) has revealed the existence of the B104 transposable element inserted downstream of the male-specific exon and upstream of the coding sequences for the early Sxl transcripts (MAINE et al. 1985). One possibility is that Sxl\(^{M1} \) represents the \( \alpha \)-activation of Sxl by promoter and/or enhancer sequences contained in the B104 element (MAINE et al. 1985). In this respect, it is worth mentioning that B104 is specifically transcribed during the early stages of development (SCHERER et al. 1982). Thus, the transcripts from Sxl\(^{M1} \) early in development might not contain the male-specific exon. The Sxl\(^{M1} \)/Sxl\(^* \)/\( f(2)d^+ \)/\( f(2)d^1 \) females would contain two types of Sxl primary RNAs: those that do not contain (from Sxl\(^* \)) and those that do contain (from Sxl\(^* \)) the male-specific exon. In these females the amount of Sxl proteins at early stages of development would be higher than in the Sxl\(^+ \)/Sxl\(^* \)/\( f(2)d^+ \)/\( f(2)d^1 \) females, and then a higher amount of zygotic \( f(2)d \) product will be produced in Sxl\(^{M1} \)/+;\( f(2)d^+ \)/\( f(2)d^1 \) females. Consequently, a stable positive feedback cycle between Sxl and \( f(2)d \) could be established. The suppression effect is higher at 18° than at 29° because at the former temperature there is more functional \( f(2)d \) product, so that a higher amount of Sxl products will also be present, and then the probability of reaching a stable feedback cycle between Sxl and \( f(2)d \) is increased.

This would also explain why the Sxl\(^{M1/P} \)/Sxl\(^* \)/\( f(2)d^+ \)/\( f(2)d^1 \) and Sxl\(^{M1/P} \)/Sxl\(^* \)/\( f(2)d^+ \)/\( f(2)d^2 \) females are lethal. Recalled that Sxl\(^{M1/P} \) is a mutation induced on the Sxl\(^{M1} \) allele that retains its constitutivity but has affected the Sxl functions (CLINE 1984). Then, in these females the amount of early Sxl proteins is reduced and consequently no stable positive feedback cycle can be established between Sxl and \( f(2)d \).

The ideas that we have discussed are subject to being tested at the molecular level, when the gene \( f(2)d \) is cloned, and its pattern of expression being analyzed. Recently, we have generated a P-induced \( f(2)d \) mutation (L. VICENTE, B. GRANADINO and L. SANCHEZ, experiments in progress), so that the cloning of this gene is opened.

Interaction of \( f(2)d \) with snf and ovo: snf seems necessary for Sxl expression (OLIVER, PERRIMON and MAHOWALD 1988; STEINMANN-ZWICKY 1988). Moreover, snf\(^{621} \) suppresses the lethality of Sxl\(^{M1} \) males (STEINMANN-ZWICKY 1988). Despite these similarities between snf and \( f(2)d \), there is a fundamental difference between both genes; namely, that snf seems only necessary for early Sxl expression (OLIVER, PERRIMON and MAHOWALD 1988; our unpublished observations), while \( f(2)d \) is needed throughout development of females to maintain the activity state of Sxl (GRANADINO, CAMPUZANO and SANCHEZ 1990; this report). Furthermore, no synergistic interaction occurs between snf\(^{621} \), or a deficiency of this gene, and \( f(2)d^+ \) or \( f(2)d^1 \), and between these and Sxl mutations; while there is a lethal synergistic interaction between snf and Sxl mutations (OLIVER, PERRIMON and MAHOWALD 1988; STEINMANN-ZWICKY 1988). On the other hand, \( f(2)d^2 \), but not \( f(2)d^1 \), partially suppresses the effect of snf\(^{621} \) on the development of the female germline: while females homozygous for snf\(^{621} \) exclusively contain ovarioles with multicellular cysts, some ovarioles of females homozygous for snf\(^{621} \) and heterozygous for \( f(2)d^1 \) contain egg chambers corresponding to oogenic stages 6 to 11. Thus, an interaction between snf and \( f(2)d \) seems to exist in the germline, but not in the soma. The role of \( f(2)d \) in Sxl expression is due to its involvement in the female-specific splicing of Sxl RNA (GRANADINO, CAMPUZANO and SANCHEZ 1990), while that of snf remains unknown. It is possible that snf is also required for Sxl
splicing, and that the interaction between \( snf \) and \( fl(2)d \) in the germline, but not in the soma, might reflect the different requirement of both tissues to \( snf \) and \( fl(2)d \) levels.

The \( fl(2)d \) mutations also partially suppress the lethal phenotype of female germ cells homozygous for the loss-of-function \( ovo^{2131} \) mutation. The surviving germ cells give rise to multicellular cysts similar to those formed by \( Sxl^- \) germ cells, and occasionally to cysts with 16 cells resembling young egg chambers. A similar interaction has been reported for \( ovo^{2131} \) and \( Sxl^- \) mutations (Oliver, Pauli and Mahowald 1990). These authors suggested that this suppression might be the consequence of compensatory changes in both somatic and germline sexual identity: reducing the dose of somatic \( Sxl \) might allow the greater survival of \( ovo^- \) germ cells. Because \( fl(2)d \) is needed for somatic \( Sxl \) function, the interaction between \( fl(2)d \) and \( ovo \) mutations may resemble that of \( Sxl \) and \( ovo \).

Such an explanation, however, is not completely satisfactory, since all of these experiments (with \( ovo \) and \( snf \)) have a major caveat; namely, that both soma and germline have the same genotype. It is known that somatic inductive and autonomous germline signals are required for the development of the germline into the female pathway (Steinmann-Zwicky, Schmid and Nöthiger 1989; Nöthiger et al. 1989). Then, it is not so straightforward to clearly determine the role of \( snf \) and \( ovo \) with \( Sxl \) is occurring. The same caveat exists in the analysis of the interaction between \( snf \) and \( ovo \) with \( fl(2)d \). The transplantation of mutant and wild-type germ cells in both directions will help to understand the functional relationships between these genes in the female germline.

We are grateful to R. de Andrade and D. Mateos for their technical assistance. We are also grateful to M. Torres, L. Vicente and M. de la Escalera for their comments on the manuscript, and to R. Nöthiger and P. Ripoll for their comments on the effect of \( fl(2)d \) mutations in the germline. This work was supported by grant PB87-0239 from Comisión Interministerial de Ciencia y Tecnología (CICYT). B.G. and A.B.S.J. are recipients of a predoctoral fellowship during part of this work.

**LITERATURE CITED**


Cline, T. W., 1978 Two closely-linked mutations in Drosophila melanogaster that are lethal to opposite sexes and interact with dauleerless. Genetics 90: 685-698.


Cline, T. W., 1983 Functioning of the genes daughterless (da) and Sex lethal (Sxl) in Drosophila germ cells. Genetics 104 (Suppl.): s16-17.


Cline, T. W., 1988 Evidence that "sisterless-a" and "sisterless-b" are two of several discrete "numerator elements" of the X:A sex determination signal in Drosophila that switch Sex-lethal between two alternative stable expression states. Genetics 119: 829-862.


