Studies on the sex-specific lethals of *Drosophila melanogaster*.

IX. Characterization of *fle(3)100*, a female-specific lethal mutation

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ABSTRACT

A female-specific late-lethal mutation, *fle(3)100*, of *Drosophila melanogaster* was examined on its effects in XX-XO mosaics, and its interaction with various alleles of sex-determination genes *tra-2* and *dsx*. Mosaic flies homozygous for *fle(3)100* appeared at a high frequency (more than 80%) compared to the control heterozygous flies. Mosaics, however, rarely appeared when they had relatively large XX tissues. A significant reduction of the frequency of XX genotype was observed in the second and third abdominal sternites, suggesting the presence of a focus or foci of primary gene action in the internal organ or tissue near these structures on the blastoderm fate map. Chromosomal females doubly homozygous for *fle(3)100* and *tra-2* or *dsx* were not rescued. Females homozygous for *fle(3)100* as well as those doubly homozygous for *fle(3)100* and *tra-2* died as pharate adults. Unexpectedly, chromosomal males doubly homozygous for *fle(3)100* and *tra-2* showed variations in viability ranging from about 20% to 100% depending on the alleles of the *tra-2* locus examined. These males also died as pharate adults.

1. INTRODUCTION

Sex-specific lethal mutations in *Drosophila melanogaster* can be divided into two classes. One class includes the male-specific lethals such as malelesses (*mle, mle(3)132*), male-specific lethals (*msl-1, msl-2*), and Sex-lethal (*Sxl^{M*1}, a dominant male-specific lethal allele), and a female-specific lethal, *Sxl^{R*1} (a recessive female-specific lethal allele). All these sex-specific lethals have been shown to play important roles in dosage compensation: for example, homozygous *mle* (late larval or early pupal lethal) male larvae have the X chromosome, in salivary gland chromosome preparations, that is only half as active in transcription as the normal X chromosome (Belote and Lucchesi, 1980; Okuno *et al.*, 1984). A female-specific lethal mutation daughterless (*da*), which is the only one known that is with maternal effect, is also included in this group. For the regulation of dosage

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These sex-specific lethals have been examined for their effects on male (XO) or female (XX) tissues corresponding to the affected sex in XX-XO mosaics (Cline, 1976, 1979; Uenooyama et al., 1982b; Belote, 1983). In all cases, mosaics rarely appeared and those that did appear had smaller and often abnormal patches of affected sex, and no single lethal focus was found.

*Sxl* is a key gene that regulates both dosage compensation and sex determination (Cline, 1978, 1979, 1983, 1984; Lucchesi and Skripsky, 1981). Downstream, these two processes diverge. Male-specific lethals, *mle's* and *msl's*, affect dosage compensation but not sex determination, although these male-specific lethals as well as *da* regulate the expression of *Sxl*, and various combinations of *da, Sxl*, and male-specific lethals produce complex viability interactions, non-sexual morphological abnormality, and sex transformation (Uenooyama et al., 1982a; Skripsky and Lucchesi, 1982; Cline, 1984; Uenooyama, 1984). Downstream sex determination genes such as transformer (*tra*), transformer-2 (*tra-2*), intersex (*ix*), and doublesex (*dsx*), on the other hand, regulate somatic sex determination pathway but not dosage compensation. Sex-specific lethality caused by the first class of genes mentioned above is expressed only on the chromosomal sex and not on phenotypically transformed sex due to the downstream sex determination genes (see Baker and Belote, 1983). For the mechanism of sex determination, see Baker and Belote (1983), Baker et al. (1987), Nöthiger and Steinmann-Zwicky (1987), Nagoshi et al. (1988), and McKeown et al. (1988).

The other class of sex-specific lethals apparently does not affect dosage compensation (Okuno et al., 1984). This class includes only two mutations at the moment, the femaleless on the third chromosome-100 (*fle(3)100*) (Inouchi et al., 1983), and a male-specific lethal *C52* (Uenooyama et al., 1982b). The *fle(3)100* mutation, when homozygous, is lethal specifically for females. The lethal stage varies, but up to 50% of homozygous females survive to the pharate adult stage, and they die just before, or in rare cases in the process of or even just after emergence. The inviable pharate adult females have a slight but distinct morphological abnormality, bending and/or nick, in the third leg tibia, although it has not been rigorously proven that it is due to the *fle(3)100* allele.

In the present study, we attempted to further characterize this non-maternal effect female-specific late-lethal mutation *fle(3)100*. We present here the result of examinations on its effects in XX-XO mosaics, and on its interactions with various alleles of downstream sex-determination genes *tra-2* and *dsx*.

2. MATERIALS AND METHODS

*fle(3)100* stocks

As reported previously (Inouchi et al., 1983), the *fle(3)100*-carrying third
Sex-specific lethal of *Drosophila*

...chromosome had not been freed from semi-lethals. Thus, several recombinant chromosome lines, which bear various other mutations (Table 1), were used in the present study. Originally each of these lines showed, in a cross between heterozygous female and male, about 50% viability in homozygous males. Upon repeated backcrosses to *Sb/TM3, Ser* females, however, certain lines began to show good segregation for homozygous males. (*Sb* is a dominant marker gene on the third chromosome and *TM3, Ser* is a balanced lethal third chromosome marked with a dominant *Ser* gene.) At least in some cases, therefore, nucleo-cytoplasmic interactions are responsible for the lowered viability in homozygous males. Line #219 (Table 1), for example, showed about 80% viability in homozygous males. In the present study the effects of *fle(3)100* were examined in certain inter-line combinations where males showed good segregation. Some examples of viabilities of males homozygous for *fle(3)100* in these inter-line combinations may be found in Table 5.

**Table 1. Third chromosome lines used in the present study**

<table>
<thead>
<tr>
<th>Line #</th>
<th>Mutations carried</th>
</tr>
</thead>
<tbody>
<tr>
<td>39</td>
<td>+ + <em>fle(3)100</em></td>
</tr>
<tr>
<td>79</td>
<td>+ <em>s</em> <em>fle(3)100</em></td>
</tr>
<tr>
<td>119</td>
<td>much + <em>fle(3)100</em></td>
</tr>
<tr>
<td>219</td>
<td>much <em>s</em> <em>fle(3)100</em></td>
</tr>
</tbody>
</table>

* A sterility gene located at 3-41.7 (Inouchi et al., 1983).

**XX–XO mosaic analysis**

XX–XO mosaics were generated using an unstable X chromosome, *In(1)w^C* (Hinton, 1955; see also Hall et al., 1977). External cuticular structures were examined under the dissecting microscope at ~4×20 magnifications: *y* and *w* mutations served as markers for their being XO.

**Sex-determination genes**

Five alleles of the *tra-2* locus were examined with respect to their possible interactions with *fle(3)100*: these were *tra-2* (Watanabe, 1975), *tra-2^{OTF}* (Fujihara et al., 1978), *tra-2^{ts1}* and *tra-2^{ts2}* (Belote and Baker, 1982, 1983), and *tra-2^{B}* (Belote and Baker, 1983). The latter three alleles were kindly provided by Dr. J. Belote in 1983. Mutations in the *tra-2* locus cause XX chromosomal females to differentiate as phenotypic sterile males. Among the alleles used in the present study, *tra-2* and *tra-2^{B}* are null mutations transforming chromosomal females into flies completely male in external sexually dimorphic characters, *tra-2^{OTF}* is a leaky mutation causing incomplete sex transformation, while *tra-2^{ts1}* and *tra-2^{ts2}* are temperature-sensitives. XX chromosomal females homozygous for these *ts's*
develop as phenotypic males when reared at 29°C, while they are normal or nearly normal females (although sterile in *tra-2*TS1) at 16°C. The *tra-2* locus also has an effect on fertility of chromosomal males (Belote and Baker, 1983). Chromosomal males homozygous for *tra-2*OTF or for *tra-2*ts2 at permissive temperature (18°C) are fertile, but those homozygous for other alleles mentioned above become sterile.

Two alleles of the *dsx* locus (Hildreth, 1965; see also Baker and Belote, 1983; Nöthiger et al., 1987) were similarly examined. One is *dsx*m which when homozygous transforms chromosomal males into intersexes, and the other is *dsx*mf which transforms both males and females into intersexes. These mutations were EMS-induced, and kindly provided by Dr. A. Garen in 1982.

For mutantios and balanced lethal chromosomes without direct citation, refer to Lindsley and Grell (1968).

Flies were reared on a standard glucose-dry powdered yeast-agar medium with propionic acid added as a mold inhibitor, and at 24±1°C unless otherwise mentioned.

3. RESULTS

**XX–XO mosaics homozygous for fle(3)100**

Up to 50% of homozygous *fle(3)100* females survive to the pharate adult stage and thus form most, if not all, external cuticular structures (Inouchi et al., 1983). Most probably, then, the lethality is not caused by the impairment in external cuticular tissues, or in presumptive external imaginal cells. If the action of *fle(3)100* is restricted to certain internal tissues or cells, we would be able to locate the primary site of action on the blastoderm fate map upon XX–XO mosaic analysis.

Table 2 shows the result of a cross to generate XX–XO mosaic flies homozygous

<table>
<thead>
<tr>
<th>Genotype*</th>
<th>fle/TM3</th>
<th>fle/fle</th>
</tr>
</thead>
<tbody>
<tr>
<td>y w spl/y w spl</td>
<td>3315</td>
<td>4</td>
</tr>
<tr>
<td>y w spl/B*Y</td>
<td>3027 (100.0)**</td>
<td>1292 (100.0)**</td>
</tr>
<tr>
<td>y w spl/In(1) wcC</td>
<td>581</td>
<td>1</td>
</tr>
<tr>
<td>In (1) wcC/B*Y</td>
<td>19 (0.6)</td>
<td>11 (0.9)</td>
</tr>
<tr>
<td>y w spl/0</td>
<td>666 (22.0)</td>
<td>267 (20.7)</td>
</tr>
<tr>
<td>Gynandromorph</td>
<td>137 (4.5)</td>
<td>49 (3.8)</td>
</tr>
</tbody>
</table>

* TM3 is a balanced lethal third chromosome, and B*Y is a Y chromosome marked with a dominant B* gene.

** The percentage of progeny flies with each genotype relative to y w spl/B*Y is shown in the parentheses.
or heterozygous for fle(3)100. Males, y w spl/BSY, homozygous for fle(3)100 appeared at 85% the expected value (1292 compared to 3027/2). (y, w, and spl are X-lined marker genes, and BSY is a Y chromosome marked with a dominant B\(^S\) gene.) For the sake of comparison, the numbers of males heterozygous or homozygous for fle(3)100 were each expressed as 100% in parentheses, and the number of certain other genotypes were normalized accordingly. As the heterozygous (control) fle(3)100, 137 mosaics (4.5%) were obtained, and 49 (3.8%) as the homozygous (experimental) fle(3)100. Thus, 84% of mosaics homozygous for fle(3)100 developed to adulthood. Most of these mosaics were quite healthy (see below).

Table 3 summarizes frequencies of individual external cuticular landmark structures scored as being XX. These frequencies are somewhat lower than those normally encountered in mosaic analysis (e.g., Bryant and Zornetzer, 1973). The reason for this is not clear, but probably represents the particular genetic

<table>
<thead>
<tr>
<th>Landmark</th>
<th>Frequency of XX genotype (%)</th>
<th>(1) fle/TM3</th>
<th>(2) fle/fle</th>
<th>(2)/(1)×100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head*</td>
<td>18.6–29.2</td>
<td>13.3–21.4</td>
<td>66–86</td>
<td></td>
</tr>
<tr>
<td>Thorax**</td>
<td>16.6–22.1</td>
<td>11.2–17.3</td>
<td>56–86</td>
<td></td>
</tr>
<tr>
<td>(except for haltere)</td>
<td>21.2</td>
<td>9.2</td>
<td>43</td>
<td></td>
</tr>
<tr>
<td>Haltere</td>
<td>28.3</td>
<td>15.3</td>
<td>54</td>
<td></td>
</tr>
<tr>
<td>Tergite</td>
<td>27.8</td>
<td>16.3</td>
<td>59</td>
<td></td>
</tr>
<tr>
<td>2nd</td>
<td>29.2</td>
<td>20.9</td>
<td>72</td>
<td></td>
</tr>
<tr>
<td>3rd</td>
<td>27.7</td>
<td>18.9</td>
<td>68</td>
<td></td>
</tr>
<tr>
<td>4th</td>
<td>32.3</td>
<td>20.4</td>
<td>63</td>
<td></td>
</tr>
<tr>
<td>5th</td>
<td>32.9</td>
<td>7.1</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>6th</td>
<td>27.9</td>
<td>14.3</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>Sternite</td>
<td>27.6</td>
<td>16.3</td>
<td>55</td>
<td></td>
</tr>
<tr>
<td>2nd</td>
<td>29.6</td>
<td>26.0</td>
<td>83</td>
<td></td>
</tr>
<tr>
<td>3rd</td>
<td>31.2</td>
<td>25.0</td>
<td>68</td>
<td></td>
</tr>
</tbody>
</table>

* Head; antenna, anterior orbital bristle (b), posterior orbital b., vibrissae, palpus, postorbital b., ocellar b., postvertical b., innerventral b., outervertical b., occiput, proboscis.

** Thorax; humeral b., anterior notopleural b., posterior notopleural b., presutural b., anterior supraalar b., posterior supraalar b., anterior postalar b., posterior postalar b., anterior dorsocentral b., posterior dorsocentral b., scutellar b., sternopleural b., wing, first leg, second leg, third leg.
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background and/or stability of the $In(1)w^{sc}$ chromosome used. Nevertheless, the values are similar among individual landmarks except for a few cases, but not so between flies heterozygous or homozygous for $fle(3)100$. When these frequencies were compared in each landmark between heterozygous and homozygous flies, however, the ratios were rather uniformly high (54–86%) in all but a few landmarks. The lowest two values were 30% for the second, and 34% for the third abdominal sternites. The difference appears to be real, for the next lowest values were observed in the haltere (43%), and in the fourth abdominal sternite (48%), both of these structures are close to the second and third abdominal sternites in the adults and probably also on the blastoderm fate map.

Relating to the results in Table 3, we examined how much XX external areas individual mosaics had. For this purpose, each of the external landmark structures scored (Table 3) was given one point when it is XX, 0 when XO, and 0.5 when it is itself a mosaic. A maximum total point for a fly (female) is thus 79 (right and left structures counted separately except for the genital structure). Note that this gives only a measure and not the real value for the external area the XX cells occupy in each mosaic fly. Table 4 summarizes the results. It may be noted that sum of the frequencies of mosaics at XX values less than 30.0 are around 80% in mosaics both heterozygous or homozygous for $fle(3)100$. (The percentage of mosaics homozygous for $fle(3)100$ was expressed as that to the estimated total, and not to the surviving animals.) At the XX values higher than that, mosaics heterozygous for $fle(3)100$ still appeared, while those homozygous for $fle(3)100$ no longer appeared except for three individuals at 39, 41, and 78. (The last two mosaics were the only ones which looked unhealthy among all mosaics.) These results are reflected in Table 3 in which the frequencies of XX

Table 4. Distribution of mosaics with varying numbers of XX landmarks

<table>
<thead>
<tr>
<th>Number of XX landmarks</th>
<th>Number of mosaics</th>
<th>$fle/TM3$</th>
<th>$fle/fle$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5–10.0</td>
<td>54 (39.4)*</td>
<td>24 (41.4)**</td>
<td></td>
</tr>
<tr>
<td>10.5–20.0</td>
<td>28 (20.4)</td>
<td>17 (29.3)</td>
<td></td>
</tr>
<tr>
<td>20.5–30.0</td>
<td>22 (16.1)</td>
<td>5 ( 8.6)</td>
<td></td>
</tr>
<tr>
<td>30.5–40.0</td>
<td>15 (10.9)</td>
<td>1 ( 1.7)</td>
<td></td>
</tr>
<tr>
<td>40.5–50.0</td>
<td>9 ( 6.6)</td>
<td>1 ( 1.7)</td>
<td></td>
</tr>
<tr>
<td>50.5–60.0</td>
<td>5 ( 3.6)</td>
<td>0 ( 0.0)</td>
<td></td>
</tr>
<tr>
<td>60.5–70.0</td>
<td>1 ( 0.7)</td>
<td>0 ( 0.0)</td>
<td></td>
</tr>
<tr>
<td>70.5–78.5</td>
<td>3 ( 2.2)</td>
<td>1 ( 1.7)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>137</td>
<td>49</td>
<td></td>
</tr>
</tbody>
</table>

* The percentage of mosaics is shown in the parentheses.

** The percentage of mosaics homozygous for $fle(3)100$ to the estimated total (58), not to the surviving animals (49), is shown in the parentheses.
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The genotype for homozygous flies is lower than those for heterozygous flies in all landmarks.

It was noted previously (Inouchi et al., 1983) that the inviable pharate adult females homozygous for fle(3)100 had a slight but distinct morphological abnormality: bending in or the presence of a nick in legs, particularly in the third leg tibia. This character was also observed in mosaics homozygous for fle(3)100 at high frequency: 15 out of 18 mosaics which had XX legs had these abnormalities. These abnormalities were not restricted to XX tissues. When mosaics homozygous for fle(3)100 had relatively broad XX area, the abnormalities were found even in XO legs (5 cases observed). These results indicate that the abnormality is not autonomous.

Interaction between fle(3)100 and sex-determination genes tra-2 and dsx

No interactions between fle(3)100 and various alleles of the tra-2 locus examined were observed as to the female viability (Tables 5 and 6). Chromosomal females homozygous for fle(3)100 are killed regardless of their phenotypic sex. Unexpectedly, however, viability of the tra-2; fle(3)100 doubly homozygous males decreased to various degrees depending on the alleles examined. Table 5 shows the results for the null tra-2 allele. Here the interaction was examined in three inter-line crosses using four lines of fle(3)100. Chromosomal males homozygous for the second chromosome and heterozygous for the third chromosome, or vice versa, showed good segregations. Those homozygous for both chromosomes, on the other hand, showed very poor viabilities: only 24% of the expected number in

Table 5. Result of the cross X/X; tra-2/SM1; fle(3)100/TM6 × X/B*Y; tra-2/SM1; fle(3)100/TM6, using four fle(3)100 lines

<table>
<thead>
<tr>
<th>Genotype*</th>
<th>Expected ratio</th>
<th>Number of progeny</th>
<th>#79 × #39</th>
<th>#119 × #219</th>
<th>#219 × #119</th>
</tr>
</thead>
<tbody>
<tr>
<td>X/X;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tra-2/SM1; fle/TM6</td>
<td>4</td>
<td>375</td>
<td>558</td>
<td>660</td>
<td></td>
</tr>
<tr>
<td>tra-2/tra-2; fle/TM6</td>
<td>2</td>
<td>163</td>
<td>284</td>
<td>255</td>
<td></td>
</tr>
<tr>
<td>tra-2/SM1; fle/fle</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>tra-2/tra-2; fle/fle</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>X/B*Y:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tra-2/SM1; fle/TM6</td>
<td>4</td>
<td>341</td>
<td>534</td>
<td>555</td>
<td></td>
</tr>
<tr>
<td>tra-2/tra-2; fle/TM6</td>
<td>2</td>
<td>159</td>
<td>238</td>
<td>218</td>
<td></td>
</tr>
<tr>
<td>tra-2/SM1; fle/fle</td>
<td>2</td>
<td>186</td>
<td>246</td>
<td></td>
<td></td>
</tr>
<tr>
<td>tra-2/tra-2; fle/fle</td>
<td>1</td>
<td>21</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% viability of males homozygous for second and third chromosomes</td>
<td>24</td>
<td>18</td>
<td>41</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* SM1 is a balanced lethal second chromosome and TM6 is a balanced lethal third chromosome.
the cross line #79\times line #39 [21/(341/4)\times 0.93\times 1.09], 18% in the cross #119\times #219, and 41% in the cross #219\times #119. It is not clear whether the last two crosses, the reciprocal crosses, indicate the involvement of the same cytoplasmic factor(s) causing lowered viability in males homozygous for fle(3)100 (see MATERIALS AND METHODS), but if so it probably acts as a general, rather than specific, deleterious factor.

In separate experiments we recovered and examined dead pharate adults from two crosses, #119\times #219 and #219\times #119, shown in Table 5. In the cross #119\times #219, 88 females, 24 non-B males (chromosomal females transformed due to tra-2) and 33 B males were obtained. All of the chromosomal females had morphological abnormalities (nick and/or bending) in the third leg tibia. The 33 B males also all had similar abnormalities. Although it is not possible to distinguish the Cy marker in the SM1 balancer in dead pharate adults, we may assume that these B males are tra-2; fle(3)100 double homozygotes. Similarly, in the cross #219\times #119 we obtained 126 females, 42 non-B males and 39 B males, all of which had the morphological abnormalities in the third leg tibia.

Table 6 shows the result of crosses as above but using various alleles of tra-2 locus. Much lowered viabilities than the expected were observed for the tra-2^{ts2} allele at both 24°C and 29°C, and for the tra-2^{ts1} allele at 29°C. No such viability interactions were observed for the tra-2^{ts1} allele, the leaky tra-2^{OTF} allele, and the null tra-2^{B} allele all at 24°C. In addition to the crosses shown in Table 6, the reciprocal crosses for the third chromosome (line #119\times line #219), and the various inter-line crosses, that involved other lines of fle(3)100 (#39, #79) as well

Table 6. Result of the cross X/X; tra-2/SM1; fle(3)100, #219/TM6\times X/B°Y; tra-2/SM1; fle(3)100, #119/TM6, using various tra-2 alleles

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Expected ratio</th>
<th>tra-2 alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>tr-a-2</td>
<td>tr-a-2^{OTF}</td>
</tr>
<tr>
<td>X/X; tra-2/SM1; fle/TM6</td>
<td>4</td>
<td>660</td>
</tr>
<tr>
<td>tra-2/tra-2; fle/TM6</td>
<td>2</td>
<td>255</td>
</tr>
<tr>
<td>tra-2/SM1; fle/fle</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>tra-2/tra-2; fle/fle</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>X/B°Y; tra-2/SM1; fle/TM6</td>
<td>4</td>
<td>555</td>
</tr>
<tr>
<td>tra-2/tra-2; fle/TM6</td>
<td>2</td>
<td>218</td>
</tr>
<tr>
<td>tra-2/SM1; fle/fle</td>
<td>2</td>
<td>246</td>
</tr>
<tr>
<td>tra-2/tra-2; fle/fle</td>
<td>1</td>
<td>40</td>
</tr>
</tbody>
</table>

% viability of males homozygous for second and third chromosomes

| X             | 41 | 108 | 112 | 31 | 22 | 33 | 86 |

* Reared at 29°C.
as #119 and #219, were undertaken for each tra-2 allele. In some inter-line combinations, reciprocal crosses produced different results in viability as the results in Table 5. Even in those combinations, however, the viabilities of doubly homozygous males were always either less than 50% or more than 75%. The property of viability of doubly homozygous males observed in Table 6 was maintained in every case. The viability was less than 50% in all crosses examined involving the tra-2(ts1) allele at 29°C, and tra-2(ts2) allele at both 24°C and 29°C. On the other hand, the viability was no less than 75% in all crosses that involved the tra-2(ts1) allele, the leaky tra-2(OTF) allele, and the null tra-2(2R) allele, all at 24°C.

In spite of a rather extensive effort, we have not been able to produce, by recombination, a third chromosome carrying both fle(3)100 (3–45; Inouchi et al., 1983) and tra (3–45), apparently due to the close linkage. We did obtain, however, the third chromosome carrying fle(3)100 and either dsx^m or dsx^mf (3–48.1: see MATERIALS AND METHODS) as described below. Two hundred males heterozygous for TM3 from the cross, dsxm[fle(3)100, #219] females × Pr/TM3 virgin females, and each extracted chromosome was tested whether it had both fle(3)100 and dsx^m. One chromosome had both mutations, and it was maintained over TM3. Similar series of crosses were made for dsx^mf. Two chromosomes that had both fle(3)100, #219 and dsx^mf were found out of two hundred, and one of them was used for further examinations below. Table 7 shows the results using these third chromosomes. Chromosomal females homozygous for fle(3)100 were killed regardless of their phenotypic sex. Males homozygous for both fle(3)100 and dsx emerged at 114% of the expected number for dsx^m and at 89% for dsx^mf, respectively. These viabilities were higher than those of males homozygous for the original fle(3)100, #219 chromosome (79%) (see MATERIALS AND METHODS). Thus no interactions were detected between fle(3)100 and two dsx alleles, dsx^m and dsx^mf.

Table 7. Result of the crosses involving fle(3)100 and dsx^m or dsx^mf:

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Expected ratio</th>
<th>Cross 1 (fle dsx^m)</th>
<th>Cross 2 (fle dsx^mf)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of progeny</td>
<td>Number of progeny</td>
<td></td>
</tr>
<tr>
<td>X/X; fle dsx^m/TM3</td>
<td>2</td>
<td>311</td>
<td>289</td>
</tr>
<tr>
<td>fle dsx^m/fle dsx</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>X/B^A Y;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>fle dsx^m/TM3</td>
<td>2</td>
<td>299</td>
<td>246</td>
</tr>
<tr>
<td>fle dsx^m/fle dsx</td>
<td>1</td>
<td>170</td>
<td>109</td>
</tr>
</tbody>
</table>
4. DISCUSSION

The lethal stage of females homozygous for fle(3)100 is very late, and up to 50% of them survive to the pharate adult stage. Adult external structures show normal differentiation in these females except for the presence of a nick and/or bending in the third leg tibia (Inouchi et al., 1983). Although rare, even adults emerge (Tables 2, 5, and 6), but these escapers are very weak and die soon. The XX–XO mosaics homozygous for fle(3)100, however, showed good viability: more than 80% of them became adults and most of them were not weak. Although this overall viability was high, detailed examinations revealed that the XX–XO mosaics with larger areas of tissues being XX did not survive. Two out of three mosaics that did appear (Table 4) with large XX tissues were weak. The present results are rather limited for the mosaic analysis, especially since the frequencies of individual landmarks scored as being XX were only around 20–30% even in the control mosaics (Table 3). Still, it can be concluded that the mutant gene, fle(3)100, has a focus or foci of its primary action in the internal organs somewhere near the second and third sternites on the blastoderm fate map. These observations and the conclusion are in contrast to those obtained in examinations on sex-specific lethals that have roles in dosage compensation, where mosaics rarely appeared and no single focus was found (see INTRODUCTION). It remains to be seen what this site of primary action is doing, and how it leads to the female-specific lethality.

The present results have also shown that the fle(3)100 allele has no interaction with the tra-2 and dsx loci as to its lethality upon females. These results are similar to the observations made previously with sex-specific lethals regulating dosage compensation. The lethality was exerted upon chromosomal females irrespective of their sexual phenotypes. No morphological abnormality was observed in sexually dimorphic external structures of the dead pharate adults. Unexpectedly, however, we have observed viability interactions between various alleles of the tra-2 locus and fle(3)100 in doubly homozygous chromosomal males (Tables 5, and 6). Although the present results do not prove that the observed viability interactions are due to these two genes and not to other genes, that these doubly homozygous males died as pharate adults and had the same morphological abnormality as seen in fle(3)100 females and that the tra-2 alleles used came from different background, taken together, might implicate that these two genes are responsible. Two functions have been known for the tra-2 locus: the female-specific somatic sex determination function (see Baker and Belote, 1983), and a function in the male gametogenesis (Belote and Baker, 1983). The present results might indicate a third heretofore unknown function in the male somatic tissues for the tra-2 locus. This third function, if proven by further examinations, does not have a simple relationship with the two functions noted above. Both tra-2 and tra-2B are null with respect to these functions (Belote and Baker,
Sex-specific lethal of Drosophila

1983). When made doubly homozygous with *fle(3)100* in chromosomal males, *tra-2* reduced the viability while *tra-2B* did not.

REFERENCES


Lucchesi, J. C. and Skripsky, T. (1981) The link between dosage compensation and sex differentia-


