A genetic analysis was made of the Female-lethal (Fl) locus of Drosophila melanogaster. This is an X-linked mutation which causes lethality only in females. Other alleles do not complement Fl and are either lethal or sterile when homozygous in females. Complementation studies on Fl alleles demonstrate that there is no simple ranking of these alleles in terms of severity of phenotypic effect. Dosage manipulation of Fl alleles indicates that the sex-specificity is not a consequence of gene dosage effects. Viability studies on males carrying Fl alleles show that Fl alleles have no effect on viability regardless of the presence or absence of a Y chromosome. The Fl locus is therefore sex-specific. The hypothesis that Fl+ is involved in the establishment of imaginal phenotypic sex cannot be substantiated on the basis of experiments utilizing sex-change mutations.
was investigated that the sex-limited effects of Fl mutations were due to gene dosage rather than a manifestation of sex difference.

2. MATERIALS AND METHODS

Stocks

Fl alleles were maintained as balanced stocks. X-linked mutations (for description of mutations see Lindsley & Grell, 1968) were used to distinguish different X chromosome genotypes. For the male viability studies stocks with Fl alleles were constructed from common original stocks to minimize other genotypic differences. The Fl2393 allele was kindly supplied by Dr E. Vyse of Montana State University, U.S.A. The translocation stock involving the Fl locus; X\textsuperscript{XX}, ymf/Y females and Df(1)J6/Y; Dp(1;3)sn\textsuperscript{13A1} males; which was used to manipulate the number of copies of Fl alleles was provided by Professor G. Lefevre of California State University, U.S.A. The Df(1)J6 X chromosome consists of a short deficiency induced by irradiation in an In(1)dl-49 chromosome marked with forked (Lefevre & Johnson, 1973). This chromosome is deficient for bands 6E1-7C1 including the

Table 1. Dosage manipulation of Fl

(Mating scheme used to test the viability and fertility of females carrying a varying number of copies of Fl and Fl\textsuperscript{+}.)

(a) Fl ct\textsuperscript{b}/FM6 x Df(1)J6/Y; Dp(1;3)sn\textsuperscript{13A1}/+  

(b1) Fl ct\textsuperscript{b}/Df(1)J6; Dp(1;3)sn\textsuperscript{13A1} x cm Fl f\textsuperscript{6A6}  
Fertility and progeny testing of Fl/Fl\textsuperscript{+}; DpFl\textsuperscript{+}

(b2) Fl ct\textsuperscript{b}/Df(1)J6 x cm Fl f\textsuperscript{6A6}/Y  
Fertility and progeny testing of Fl/Fl\textsuperscript{+}

(c) Fl ct\textsuperscript{b}/cm Fl f\textsuperscript{6A6}; Dp(1;3)sn\textsuperscript{13A1} x cm ct\textsuperscript{b}/Y  
Fertility and progeny testing of Fl/Fl; DpFl\textsuperscript{+}

(d) Fl oc ptg v/FM3 x Fl ct\textsuperscript{b}/Y  
Verification of presence of Fl in Fl/Y males by failure of complementation

(b1) Single-pair testcross of females carrying a third chromosome duplication of Fl\textsuperscript{+} and an X chromosome deficient for Fl\textsuperscript{+}. Phenotypically distinguishable as Bar\textsuperscript{+}, cut\textsuperscript{+} females.

(b2) Single-pair testcross of females deficient for Fl\textsuperscript{+} but without third chromosome duplication. Phenotypically distinguishable as Bar\textsuperscript{+}, cut females.

(c) Fertility and progeny testing of females homozygous for Fl but carrying third chromosome duplication for Fl\textsuperscript{+}. Phenotypically forked\textsuperscript{+}, carmine\textsuperscript{+}.

(d) Complementation test to verify the presence of Fl in males, which are phenotypically cut.
Genetics of Female-lethal 105

carmine, Fl and cut loci and is mutant for forked. The third chromosome
Dp(1;3)sn1381 has bands 6C12-7C9 inserted at band 79E of 3L. It is duplicated for
the carmine+, Fl+ and cut+ loci and is mutant for singed (Lindsley & Grell, 1968).

Crosses

Flies were raised at 25 °C on a standard yeast, maize, sugar medium supple-
mented by live yeast. The crosses used to investigate male viability were of the
general form \( XX, yf/Yf \) females \( \times Fl^D/Y \) males or \( XX, yv^B \) females \( \times Fl^B/Y \) males, where \( Fl^D \) represents the alleles \( Fl, Fl B \)
or \( Fl+ \). In each experimental group, ten to fifteen females were placed in a 10 ml food vial with an equal number of
males and were transferred every 2 or 3 days. The full progenies of five or six
broods were scored. Similar conditions were used in the complementation studies.

Combinations of Fl and the sex-change mutations transformer-dominant (traD),
Masculinizer (Mas), doublesex (dsx) and intersex (ix) were synthesized by balancer
chromosome substitution, and linked markers were used to identify these geno-
types. The mating scheme used to vary the dosage of the Fl allele is shown in
Table 1. Similar schemes were used for the other alleles.

3. RESULTS

(i) Complementation tests

Table 2 shows that, although each mutant allele is either lethal or sterile when
homozygous, the phenotypes produced by heterozygous combinations are more
complex. The lethality observed in crosses two, three and four indicates that \( Fl^b, Fl^{b1} \) and \( Fl^{2593} \) are allelic to \( Fl \). Homozygosity for \( Fl^{2593} \) also results in lethality
(cross eight) in contrast to homozygous (crosses six and seven) and heterozygous
(crosses nine and ten) combinations of \( Fl^b \) and \( Fl^{b1} \) which produce sterile females.
The low viability of these \( Fl^b \) and \( Fl^{b1} \) combinations indicates a semi-lethal effect of
these alleles. However, complementation does occur when \( Fl^{2593} \) is heterozygous
with either \( Fl^a \) (cross eleven) or \( Fl^{s1} \) (cross twelve). The viability of these hetero-
zygous females is high and they are fertile. Thus it appears that these alleles
represent one complementation group but that intra-allelic complementation may
occur.

The \( Fl^b sn^3 \) chromosome appears to be completely lethal when homozygous
(cross five) although homozygous \( y v^b Fl^b sn^3 \) females may survive (cross six).
Such a result was unexpected as the latter chromosome was synthesized from the
\( Fl^b sn^3 \) stock. From these results there is no simple way to rank these alleles in
order of severity of phenotypic effect.

(ii) Viability effects of Fl alleles in males

It was important to distinguish a sex-specific from a sex-differential action of Fl
alleles. A sex-specific action of Fl+ implies that a normal Fl+ gene product is required
specifically in females and not in males. A sex-differential action of Fl would
indicate that Fl+ is required by both males and females, but that males either

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Table 2. Complementation of Fl alleles. Frequencies of genotypes produced in combinations of various Fl alleles

<table>
<thead>
<tr>
<th>Maternal genotype</th>
<th>Paternal genotype</th>
<th>Fl combination</th>
<th>% Regular progeny*</th>
<th>Total progeny size</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Fl oc ptg v/FM6</td>
<td>Fl oc ptg v/Y</td>
<td>Fl/Fl</td>
<td>0</td>
<td>37/38/25/25</td>
</tr>
<tr>
<td>2. Fl oc ptg v/FM6</td>
<td>Fl sn3/Y</td>
<td>Fl/Fl</td>
<td>0</td>
<td>47/33/20</td>
</tr>
<tr>
<td>3. Fl oc ptg v/FM6</td>
<td>Fl sn3/Y</td>
<td>Fl/Fl</td>
<td>0</td>
<td>48/35/16</td>
</tr>
<tr>
<td>4. Fl oc ptg v/FM3</td>
<td>Fl sn3/Y</td>
<td>Fl/Fl</td>
<td>0</td>
<td>48/52/0†</td>
</tr>
<tr>
<td>5. Fl sn3/y CIBv</td>
<td>Fl sn3/Y</td>
<td>Fl/Fl</td>
<td>0</td>
<td>56/44/0†</td>
</tr>
<tr>
<td>6. y w Fl sn3/FM6</td>
<td>Fl sn3/Y</td>
<td>Fl/Fl</td>
<td>6</td>
<td>52/40/3</td>
</tr>
<tr>
<td>7. Fl sn3/y CIBv</td>
<td>Fl sn3/Y</td>
<td>Fl/Fl</td>
<td>0.4</td>
<td>52/47/0</td>
</tr>
<tr>
<td>8. y Fl sn3/Y</td>
<td>Fl sn3/Y</td>
<td>Fl/Fl</td>
<td>0</td>
<td>37/42/20</td>
</tr>
<tr>
<td>9. y Fl sn3/Y</td>
<td>Fl sn3/Y</td>
<td>Fl/Fl</td>
<td>8</td>
<td>45/35/4</td>
</tr>
<tr>
<td>10. Fl sn3/y CIBv</td>
<td>Fl sn3/Y</td>
<td>Fl/Fl</td>
<td>1</td>
<td>48/51/0†</td>
</tr>
<tr>
<td>11. y Fl sn3/Y</td>
<td>Fl sn3/Y</td>
<td>Fl/Fl</td>
<td>39</td>
<td>35/19/6</td>
</tr>
<tr>
<td>12. Fl sn3/y CIBv</td>
<td>Fl sn3/Y</td>
<td>Fl/Fl</td>
<td>26</td>
<td>34/40/0†</td>
</tr>
</tbody>
</table>

* Superscript 1 refers to the maternally inherited allele, superscript 2 to paternally inherited allele.
† Fl+ chromosome carries a recessive lethal mutation.
‡ Exceptional progeny produced by these crosses. Produced because female parents carried a Y chromosome.
tolerate an imperfect gene product better than females or possess other mechanisms to achieve the same developmental goal. Table 3 records the results of experiments designed to test the viability of males carrying Fl alleles. The use of attached-X female parents ensured that the male progeny inherited the Fl0 chromosome from their father. A decrease in the survival of males carrying Fl and/or Fl0 can be detected by comparing the relative segregations of male and female progeny in the experimental groups to the Fl+ control. $\chi^2$ tests performed on the data in Table 3(a) showed no significant differences in Fl/Y or Fl0/Y male viability compared to Fl+/Y male viability.

Table 3. Viability of Fl and Fl0 males

**Table 3. Viability of Fl and Fl0 males**

<table>
<thead>
<tr>
<th>Genotype of progeny</th>
<th>Fl0 experiment</th>
<th>Fl experiment</th>
<th>Fl+ experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td>XX/Y females</td>
<td>475</td>
<td>530</td>
<td>608</td>
</tr>
<tr>
<td>Fl0/Y males</td>
<td>472</td>
<td>612</td>
<td>587</td>
</tr>
</tbody>
</table>

**Table 3(b) X/O male viability**

<table>
<thead>
<tr>
<th>Genotype of progeny</th>
<th>Fl0 experiment</th>
<th>Fl experiment</th>
<th>Fl+ experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td>XX/Y females</td>
<td>620</td>
<td>359</td>
<td>528</td>
</tr>
<tr>
<td>Fl0/O males</td>
<td>725</td>
<td>473</td>
<td>583</td>
</tr>
</tbody>
</table>

* Fl0 represents the alleles Fl+, Fl or Fl0.

The possibility was also examined that males carrying Fl alleles survive because of the presence of the Y chromosome. Partial suppression of X-linked lethal mutations by the Y chromosome is one of many examples of position-effect variegation (Lindsley, Edington & Von Halle, 1960). On the basis of their interaction with heterochromatin the mutations abnormal oocyte (Sandler, 1970) and daughterless (Sandler, 1972) have been implicated in the regulation or synthesis of rDNA. Table 3(b) indicates that the removal of the Y chromosome does not affect the survival of males carrying Fl alleles. The attached-X females used were obtained from a stock of XX, y2 wB females and XY, v cv/O males and possess no free Y chromosome. The X/O constitution of male progeny was verified by crossing 50 males from each experimental group en masse to approximately 100 virgin females. In no case were progenies produced by such crosses. Comparison of the relative segregations of XO males to their female sibs in each group by $\chi^2$ tests in no case yielded a significant value. Therefore the presence of a Y chromosome has no effect on the survival of males carrying these Fl alleles.
(iii) Interactions between Fl alleles and sex-change mutations

The normal function of the Fl locus may relate to the differentiation of female phenotypic sex. This hypothesis was investigated by altering the phenotypic sex in Fl/Fl females using various sex-transforming mutations and determining whether lethality was suppressed. The dominant mutations transformer-dominant (tra°), Masculinizer (Mas) and the recessive mutation intersex (ix) exclusively affect chromosomal (X/X) females, causing them to develop as sterile intersexes. Double-sex (dsx) causes both genetic females and males to develop as intersexes. Analyses of large progenies in which Fl and these mutations were segregating are given in Table 4. In every cross transformed Fl/Fl individuals, should they be produced, would be easily distinguished by virtue of the X-linked markers present. The interaction of traP and Fl/Fl was also investigated as the lethal phase of Fl/Fl is later than Fl/Fl (Marshall, 1977 and in preparation), and might overlap a later period of activity of the sex-transforming mutations. None of these sex-transforming mutations suppressed the lethal phenotype of Fl/Fl or Fl/Fb, although in each cross other progeny classes showed the expected transformed phenotype. Large progenies were examined to detect any poorly viable classes.

The mutation dsx also transforms males to intersexes. Cross (e) indicates that transformed Fl/Y genotypes are viable. Comparison of the relative segregations of Fl/Y; dsx/dsx+ and Fl/Y; dsx/dsx progeny to Fl+/Y; dsx/dsx+ and Fl+/Y; dsx/dsx male progeny, or to the appropriate Fl/Fl+ male progeny, or to the appropriate Fl/FI+ progeny, did not give significant χ² values. Therefore transformation of Fl males by dsx does not reduce their viability.

The presence of a free Y chromosome in the FM6 maternal genome led to a high proportion of exceptional progeny in cross (e). Were they viable, Fl/Fl/Y genotypes would have been found in these crosses but such females were never observed, further indicating that the Y chromosome has no influence on the expression of Fl.

(iv) Dosage manipulation of the Fl locus

The resultant phenotype of a mutation will depend upon both the normal function of that gene and the type of mutation (Muller, 1932). The lethality and/or sterility of Fl alleles exclusively in homozygous females is possibly a consequence of gene dosage rather than physiological sex. One dose of Fl may be tolerated in Fl/Y individuals but two doses (assuming no dosage compensation at this locus) may result in an increased amount of an abnormal gene product with lethal consequences. Fl could even be a dose-dependent neomorph or a hypermorph. This was examined by dosage manipulation of Fl alleles. Should the phenotype of Fl be related to gene dose then Fl/Fl should have the same phenotype as Fl/Fl; DpFl+ as lethality depends solely on the number of copies of Fl.

The translocation stock available, ymf- × Df(1)J6/Y; Dp(1;3)sm1241/+ (see Methods and Table 1), carries a deficiency for the Fl+ locus in males, which can only survive if they also carry the duplication for this region of the X in their third chromosome. By crossing females heterozygous for an Fl allele to Df(1)J6/Y;
Dp(1;3)sn13a1 males it is possible to construct females with heterozygous deficiencies (Fl−) and/or an additional copy of Fl+ (DpFl+) in chromosome 3L. Table 1 shows the series of crosses used to investigate the viability and fertility of Fl/Fl−, Fl/Fl−; DpFl+ and Fl/Fl; DpFl+ female genotypes. A similar procedure was used to investigate the dosage of Fl+, Flb and Flb1; in these cases cm ct6/cm ct6, Flb sn3/FM6 or Flb1 ct6/y CLB v females were initially crossed to Df(Y)J6/Y; Dp(1;3)sn13a1 males. In each programme, several of the genotypes could only be identified by progeny tests for recessive linked markers on the X chromosome. At least 31 females of each necessary genotype were progeny tested. The results

Table 4. Frequencies of distinguishable genotypes in crosses combining Fl alleles with tra°, Mas, ix and dsx

(a) Fl oc ptg v/FM3 × Fl f3oa/Y; tra° Sbe/TM3

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Fl/Fl; Fl/Fl; Fl/Fl+; Fl/Fl+; Fl/Y; Fl/Y; tra°/+</th>
<th>+/+ tra°/+ +/+ tra°/+ tra°/+ +/+ tra°/+ tra°/+</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. observed</td>
<td>0 0 244 211 176 222</td>
<td></td>
</tr>
<tr>
<td>%</td>
<td>0 0 29 25 21 26</td>
<td></td>
</tr>
</tbody>
</table>

* FM3 males are lethal.

(b) Fl sn3/FM6 × Fl f3oa/Y; tra° Sbe/TM3

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Fl/Fl; Fl/Fl; Fl/Fl+; Fl/Fl+; Flb/Y; Flb/Y; Fl+/Y; Fl+/Y; tra°/+</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. observed</td>
<td>0 0 113 151 128 51 78</td>
</tr>
<tr>
<td>%</td>
<td>0 0 18 24 18 8 12</td>
</tr>
</tbody>
</table>

(c) Fl oc ptg v/FM3 × Fl ct6/Y; Mas/+ 

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Fl/Fl; Fl/Fl; Fl/Fl+; Fl/Fl+; Fl/Y; Fl/Y; Mas/+</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. observed</td>
<td>0 0 241 227 413</td>
</tr>
<tr>
<td>%</td>
<td>0 0 27 26 46</td>
</tr>
</tbody>
</table>

(d) Fl f3oa/FM3; ix pr cn/SM5 × Fl oc ptg v/Y; ix pr cn/SM5

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Fl/Fl; Fl/Fl; Fl/Fl+; Fl/Fl+; Fl/Y; Fl/Y; ix/ix</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. observed</td>
<td>0 0 584 137 659 230</td>
</tr>
<tr>
<td>%</td>
<td>0 0 36 8 41 14</td>
</tr>
</tbody>
</table>

* Eight exceptional progeny produced by this cross.

(e) Fl sn3/FM6; Fl f3oa/FM6; p° dsx/TM6 × Fl oc ptg v/Y; p° dsx/TM6

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Fl/Fl; Fl/Fl; Fl/Fl+; Fl/Fl+; Fl/Y; Fl/Y; Fl+/Y; Fl+/Y;</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. observed</td>
<td>0 0 398 193 334 154 180 79</td>
</tr>
<tr>
<td>%</td>
<td>0 0 25 12 21 10 11 5</td>
</tr>
</tbody>
</table>

* 15% exceptional progeny genotypes produced by this cross.

To investigate the dosage of Fl+, Flb and Flb1; in these cases cm ct6/cm ct6, Flb sn3/FM6 or Flb1 ct6/y CLB v females were initially crossed to Df(Y)J6/Y; Dp(1;3)sn13a1 males. In each programme, several of the genotypes could only be identified by progeny tests for recessive linked markers on the X chromosome. At least 31 females of each necessary genotype were progeny tested. The results
showed that the genotypes \(Fl^+Fl^-\) and \(Fl^+/Fl^-\); \(DpFl^+\) were viable and fully fertile in contrast to \(Fl/Fl^-\) and \(Fl^+/Fl^-\) which were completely lethal. Female genotypes \(Fl/Fl^-\); \(DpFl^+, Fl/Fl\); \(DpFl^+, Fl^+/Fl^-\); \(DpFl^+, Fl^+/Fl\); \(DpFl^+\) and \(Fl^+/Fl\); \(DpFl^+\) were all viable and fertile. As the duplication of \(Fl^+\) \((Dp(1;3)sn^{13a1})\) was mutant for \textit{singed}, problems arose in identifying \(Fl B\) females. \textit{Singed} females which could only have been \(Fl B/Fl a; DpFl^+\) or \(Fl s/Fl B\) were fertile. It was concluded that the fertility was due to the presence of \(Fl^+\).

These results show that females homozygous for \(Fl\) are lethal, but even two copies of \(Fl\) produce neither lethality nor sterility when \(Fl^+\) is also present. Thus these alleles are unlikely to be dose-dependent neomorphs or hypermorphs.

4. DISCUSSION

The range of phenotypes shown by the various \(Fl\) alleles confirms that this gene has an essential role in females. The most extreme allele in this series is \(Fl\) but the other alleles do not form a clear linear series in the extent and severity of their effects. The later lethal phase of \(Fl/Fl^{'\text{2593}}\) and \(Fl/Fl^{'\text{s}}\) compared to \(Fl/Fl\) zygotes also indicates that \(Fl\) is more severe in its effects than the other alleles (Marshall, 1977 and in preparation). No confirming evidence exists to suggest that \(Fl\) is neomorphic so \(Fl\) could be either an amorph or a hypomorph whilst the other less severe alleles may be hypomorphs.

The evidence from the viability studies shows that no effect of \(Fl\) alleles can be detected in males. This conclusion is reinforced by experiments to determine the effective lethal phase of \(Fl\) (Marshall, in preparation). Only 25\% of zygotes, accounting only for homozygous \(Fl\) embryos, die when \(Flf36'/+ +\) females are crossed to \(Fl\) males, when compared to control series. Thus \(Fl\) is the second example of a sex-specific mutation affecting only one sex, the other being \textit{maleless} (Fukunaga, Tanaka & Oishi, 1975). All other sex-limited lethal mutations are sex-differential in action. The viability studies on \(X/O\) males, reinforced by the failure to recover viable \(Fl/Fl/Y\) genotypes in the sex-change experiments, indicate that male survival is not influenced by the presence of a \(Y\) chromosome.

Alteration of imaginal phenotypic sex by the action of sex-transforming mutations neither suppresses the lethality of \(Fl/Fl\) and \(Fl/Fl^{'\text{s}}\) in females nor induces lethality in \(Fl\) males, indicating that \(Fl^+\) is not involved in the establishment of the adult sexual phenotype. In this respect \(Fl\) behaves similarly to \textit{daughterless} (Colainne & Bell, 1968), \textit{maleless} (Fukunaga et al. 1975) and the maternal sex-ratio condition (Sakaguchi & Poulson, 1963; Miyamoto & Oishi, 1975). The only sex-limited lethal known to respond to the action of sex-change mutations is \textit{sonless} (Colainne & Bell, 1972) which has a lethal phase extending to 48 h after hatching (Colainne & Bell, 1970). The lethal phase of \(Fl\) occurs during embryogenesis and may precede the action of the sex-transforming mutations. The mutation \textit{transformer} is the most extreme of the sex-change mutations, \(XX^{tra/tra}\) individuals being almost indistinguishable from normal males, yet these pseudomales still possess some female characteristics (Brown & King, 1961); \(Fl^+\)
may function in some female-specific process which persists in these intersexes. The expression of sex-transforming mutations involves imaginal disk derivatives but the embryonic lethality of \( Fl \) may reflect a quite separate developmental response to chromosomal sex from the differentiation of adult morphological sex. Alternatively the selective action of \( Fl \) might reflect differences between the \( XX \) and \( XY \) chromosomal constitution in terms of the amount or type of chromatin and associated proteins. Survival of homozygous \( Fl \) clones induced by mitotic recombination (Marshall, 1977 and in preparation) makes it unlikely that \( Fl^+ \) is involved in any cell-autonomous function.

It is concluded that \( Fl^+ \) codes for a product required specifically in \( X/X \) females. The evidence is consistent with \( Fl \) being an amorph or hypomorph, other alleles being hypomorphs. A role for \( Fl^+ \) in the establishment of female phenotypic sex has not been substantiated. \( Fl^+ \) may be required for a female specific process not directly related to the external sexual dimorphism of the adult.

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REFERENCES


