SHORT PAPER

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

VI. An autosomal, recessive, non-maternal effect female-specific lethal mutant

By Yoshie INOUCHI*, Tadanobu OKUNO*, Kugao OISHI*¹⁾ and
Takao K. WATANABE**

*Department of Biology, Faculty of Science, Kobe University, Nada, Kobe 657
and

**National Institute of Genetics, Mishima 411

(Received June 9, 1983)

ABSTRACT

A recessive, non-maternal effect, female-specific lethal mutant of *Drosophila melanogaster* was recovered from a natural population. The mutant, *fle(3)100*, is located at 3-45±. About one-half of homozygous females show normal development up to the pharate adult stage. Salivary gland chromosomes of third instar homozygous female larvae have normal staining properties in the lactic-aceto-orcein staining, suggesting normal dosage compensation. The results indicate that the *fle(3)100* gene forms a new class of sex-specific lethals.

Studies on the sex-specific lethals of *Drosophila melanogaster* in recent years have provided evidence that they are involved in the primary step regulating sex determination and dosage compensation (Cline 1978, 1979a, b, 1980, 1983; Belote and Lucchesi 1980; Lucchesi and Skripsky 1981; Uenoyama, Fukunaga and Oishi 1982; Skripsky and Lucchesi 1982). It is now clear that the central role is played by the *Sxl* (sex-lethal, 1-19.2) locus: the *Sxl*⁺ product, which is normally present only in 2X2A flies, is required for flies to differentiate as females and to express X chromosome transcription at the female rate. Actions of other sex-specific lethals such as *da* (daughterless, 2-41.5), *mle* (maleless, 2-55.2) and *mle(3)132* (3-25.8) are apparently related to the regulation of the *Sxl* locus in one way or another (references cited above and Uenoyama, in preparation).

Sex-specific lethals so far recovered and studied fall into several groups: male-specific or female-specific is one division, sex-linked or autosomal is another, and maternal-effect or non-maternal effect is yet another. Still another division should now be considered: whether the action is in any way related to the expression of *Sxl* locus. All the sex-specific lethals so far exa-

1) To whom correspondence should be addressed.

Table 1. Results of crosses for mapping the "sex-differential" lethal. Two hundred and three females heterozygous for *TM 3* from the cross, *mwh red e*/#100 $\times$ *Pr/TM 3*, were individually crossed to *Pr/TM 3* males, and each extracted chromosome was made homozygous in F_3 by crossing F_2 females and males both heterozygous for *TM 3*

	Number of chromosomes*	
	"sex-differential" lethal	+
<i>mwh red e</i>	3	45
+ + +	40	2
<i>mwh</i> + +	30	6
+ <i>red e</i>	4	39
<i>mwh red</i> +	0	10
+ + <i>e</i>	12	0
<i>mwh</i> + <i>e</i>	5	1
+ <i>red</i> +	0	6

* Expected segregation ratios in the F_3 are 2 *TM 3* females: 2 *TM 3* males: 1 non-*TM 3* females: 1 non-*TM 3* males. "Sex-differential" chromosomes are those which produced no homozygous (non-*TM 3*) females but some homozygous (non-*TM 3*) males. "+" chromosomes are those that produced both homozygous (non-*TM 3*) females and males.

mined show no interactions with sex transformation mutants, but this criterion should also be kept in mind. Interestingly female-specific lethals are very rare. In fact, except for the female-specific lethal alleles of *Sxl*, there has been only one mutant known: the autosomal, recessive, maternal-effect temperature sensitive mutant *da* which as mentioned above regulates the expression of the *Sxl* locus. We report here on an autosomal, recessive, non-maternal effect female-specific lethal mutant, of which action is probably not related *Sxl*.

A third chromosome (named #100) extracted from a natural population of *Drosophila melanogaster* at Katsunuma, Yamanashi Prefecture, was found to be completely lethal to females when homozygous. Homozygous males were also affected and only about 50% of them were viable. Further, these homozygous males were completely sterile. We mapped this "sex-differential" lethal as follows. Sex-differential here means that the mutant is completely lethal for one sex and semi-lethal for the other. It may be that the mutant is simply a leaky one.

Heterozygous females, *mwh red e*/#100, were crossed to *Pr/TM 3* (*Sb Ser*) males: *TM 3* is a balanced lethal chromosome marked with two dominant genes, *Sb* and *Ser*. For marker genes refer to Lindsley and Grell (1968). Crosses were made at 25°C and on a standard *Drosophila* medium in all the

Table 2. *Examples of inter-line crosses. Number of extracted chromosomes each showing homozygous "sex-differential" lethality were maintained following the crosses shown in Table 1. Some inter-line crosses showed "female-specific" lethality and some others still "sex-differential" lethality, and some combinations showed different results in reciprocal crosses (A). In separate experiments, dead pharate adults were examined and these are shown in parentheses (B)*

Crosses heterozygous <i>TM3</i> females (line No.) × heterozygous <i>TM3</i> males (line No.)	Number of progeny			
	<i>TM3</i>		non- <i>TM3</i>	
	female	male	female	male
A				
4 × 4	121	135	0	32
4 × 79	125	110	0	55
79 × 4	118	106	0	54
79 × 79	118	123	0	23
31 × 31	119	114	0	31
31 × 119	116	125	0	58
119 × 31	133	126	0	32
119 × 119	164	158	0	40
119 × 206	107	119	0	29
206 × 119	115	121	0	29
206 × 206	128	121	0	27
B				
4 × 39	182 (0)	170 (0)	0 (39)	85 (0)
79 × 31	187 (2)	184 (0)	0 (41)	93 (1)
65 × 119	151 (0)	158 (0)	0 (42)	78 (0)
206 × 79	167 (2)	170 (0)	1 (31)	68 (0)

experiments described in the present report. Two hundred and forty F_1 females heterozygous for *TM3* were individually crossed with several *Pr/TM3* males. From each cross in the F_2 , females and males both heterozygous for *TM3* were taken and crossed. The F_3 progeny was examined as to whether homozygous non-*TM3* females appeared. Two hundred and three crosses were successfully examined (seven P females were sterile and thirty F_2 crosses produced no homozygous non-*TM3* flies). Results are shown in Table 1. The female lethality was always associated with the male semi-lethality. These results locate this "sex-differential" lethal between *mwh* (3-0.0) and *red* (3-53.6) to 45.1. From each of these 203 crosses, homozygous non-*TM3* males were taken and crossed to wild type (Oregon-R) females in order to locate the sterility gene noted above. The results (not shown) showed that the sterility gene is located at 41.7.

Number of lines from the F_3 above were maintained, with or without a marker gene(s) and with or without the sterility gene. When these lines were examined several generations later they still showed the sex-differential lethality. Inter-line crosses, however, showed that in some combinations they resulted in complete lethality for females and complete viability for males. Examples are shown in Table 2. Moreover, in some combinations reciprocal crosses produced different results: in one cross males were completely viable and in the reciprocal cross only one-half of the males were viable. These results would suggest that the #100 chromosome carried a female-specific lethal gene, here named femaleless (3) 100 (*fle(3)100*, 3-45), a sterility gene (3-42, male-specific?), and a semi-lethal gene(s) (semi-lethal perhaps for both sexes?) of which action involves a nucleo-cytoplasmic interaction.

Table 2 also shows the number of dead pharate adults for each genotype in parentheses. Up to 50% of homozygous females apparently survived to the pharate adult stage. It was observed that some females even started to eclose and died in the process with the head popped out of the pupal case. Though quite rare, one or a very few females even succeeded in eclosion but they died very soon (Table 2). These pharate adult females, when cooked in 10% KOH, mounted and examined under the compound microscope, were quite normal in external cuticular morphology except for tibia and femur of the legs. In these segments, especially in the third leg tibia, various abnormalities such as bending and the presence of a nick were observed. These abnormalities were distinct but slight, and it appears unlikely that they were the cause for the inability of flies to eclose. Also it is not at all clear as yet whether they have anything to do with *fle(3)100*.

The location of this female-specific lethal, 3-45±, might have suggested that an allele of *tra* (transformer, 3-45, which transforms chromosomal females into phenotypic but sterile males), with semi-lethal effect, or an allele of *tra* linked with a separate semi-lethal gene, is responsible. Results in Table 2 clearly negate this possibility. Also, in stocks established using an *Y* chromosome with a dominant marker gene, *B⁺Y*, males homozygous for the third chromosome carrying *fle(3)100* were all with the *B⁺* phenotype.

As mentioned earlier, the maternal-effect female-specific lethal gene, *da*, is known to regulate the expression of the *Sxl* gene. Daughters from *da/da* mothers die because no *Sxl⁺* product is produced (Cline 1978). This lethal effect can be detected in daughters heterozygous for *Sxl^{f⁴¹}* (a hypomorph) from *da/+* mothers (Cline 1980). Thus, in a cross, *X/X; da/SM1(Cy) × Sxl^{f⁴¹}/Y; +/+*, progeny females appear only at about 20-30% of the expected number (*SM1(Cy)* is a balanced lethal second chromosome marked with the dominant marker gene *Cy*) (Uenoyama, in preparation). The effect of *fle(3)100* is recessive, female-specific, non-maternal effect lethal. It might however have a hidden maternal effect which might be revealed in a combination with

Sxl^{f^h}. A cross was made between *X/X; fle(3)100/TM3* and *Sxl*^{f^h}; +/+ . Progeny flies were 487 *fle*/+ females, 492 *TM3*/+ females, 502 *fle*/+ males and 492 *TM3*/+ males. Thus, *fle(3)100* does not have the kind of viability interaction with *Sxl* as *da* does.

Whether the mutant affects dosage compensation as *Sxl* (Lucchesi and Skripsky 1981) or various autosomal male-specific lethals (Belote and Lucchesi 1980) do was examined in a preliminary way by staining the salivary gland chromosomes of late third instar homozygous *fle(3)100* female larvae with lactic-aceto-orcin. Preparations by a similar procedure of salivary gland chromosomes of *Sxl*^{f^h}/*Sxl*^{f^h}, for example, reveal a pronounced enlargement of X chromosomes (Lucchesi and Skripsky 1981). No abnormalities were observed in preparations of *fle/fle* female larvae.

All these results, taken together, would allow the conclusion that the *fle(3)100* mutant forms a new class of sex-specific lethals of *Drosophila melanogaster*. Mechanism of the lethality remains to be elucidated. The late lethal phase by itself should not be taken to indicate that the action is not basic, because the *mle/mle* males from *mle*/+ mothers can survive to pupal stage, can differentiate most of the head and thoracic adult structures and some abdominal structures, and yet *mle* interacts with *Sxl*^{f^h} (Tanaka, Fukunaga and Oishi 1976; Uenoyama, Fukunaga and Oishi 1982; Skripsky and Lucchesi 1982; Uenoyama, in preparation).

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