MALELESS, A RECESSIVE AUTOSOMAL MUTANT OF *DROSOPHILA MELANOGASTER* THAT SPECIFICALLY KILLS MALE ZYGOTES[1]

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ABSTRACT

A second chromosome male-specific lethal gene, maleless (*mle*), in *D. melanogaster* is described. It kills males but not females in homozygous condition, regardless of whether female parents are heterozygous or homozygous for *mle*. Many, if not most, homozygous males survive up to the third instar larval stage, but cannot pupate and die eventually as larvae. No interactions with sex-transforming genes, *tra* and *dsx*, were observed. It is proposed that *mle* interacts with a gene(s) on the *X* chromosome, which is not dosage compensated.

ABNORMAL sex-ratio conditions are not very rare phenomena in *Drosophila*. These conditions can result either from aberrant meiotic and/or associated processes (e.g., ZIMMERING, SANDLER and NICOLETTI 1970) or from sex-differential zygotic mortality (e.g., POULSON 1963, and those mentioned below). Sex-differential lethality should be the reflection of the biochemical differences between male and female sexes, although they may or may not be related to sex differentiation, at the time of the action of lethal genes. Studies of such mutants should be of interest in the developmental genetics of Drosophila, especially if mutations lethal at different developmental stages can be recovered.

Sex-differential lethals that have been described in *Drosophila melanogaster* and studied in detail are all maternal effect mutants (sonless, *snl*, COLAIANNE and BELL 1970, 1972; daughterless, *da*, BELL 1954; COLAIANNE and BELL 1968; SANDLER 1972; MANGE and SANDLER 1973). Sex-specific lethals without maternal effects have also been reported but detailed studies appear to be lacking (killer of male, *km*, PIERRE 1972; male killer, *mak*, GOLUBOVSKY and IVANOV 1972). We describe here one such mutation, maleless (*mle*), which is located on the second chromosome of *D. melanogaster* and is lethal specifically for males when present in homozygous condition.

ORIGIN AND ELEMENTARY GENETICS OF *mle*

One second chromosome extracted in 1971 from a natural population of *D. melanogaster* in Kofu, Japan was found to be lethal for male zygotes. This finding was made by DR. C. OSHIMA and his colleagues at the National Institute of Genetics, Japan, in the course of studying second chromosome deleterious genes

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in natural populations. The strain balanced with *SM1(Cy)* (for description of mutants mentioned without direct citations, see LINDSLEY and GRELL 1968) was kindly given to one of the authors (K. O.) for further examination. The original strain supplied carried a cytoplasmic factor which caused the homozygous *mle* females to be sterile. From a cross between *SM1(Cy)/Pm* females and *SM1 (Cy)/mle* males,*SM1(Cy)/mle* females and males were recovered and crossed. Homozygous *mle* females thus obtained in the next generation proved to be fertile, and the strain was maintained by crossing *mle/mle* females with *SM1 (Cy)/mle* males. Strain maintenance and experiments were carried out at 24 ± 0.5° on a sucrose-yeast-agar medium (per liter of water: sucrose 55 g, dry powdered yeast 35 g, agar 19 g, with 4 ml of propionic acid added).

It was repeatedly found during these experiments that the fecundity of *mle/mle* females decreases gradually when the strain was maintained as above. Thus whenever necessary *SM1(Cy)/mle* females and males were crossed or *SM1(Cy)/Pm* females were crossed with *SM1(Cy)/mle* males to restore normal fecundity. A cytoplasmic sterility factor which may have a chromosomal basis such as that in the case of *delta* (MINAMORI 1972) appears to be interacting with the *mle*-carrying second chromosome. This cytoplasmic factor will be dealt with elsewhere.

The gene, *mle*, is completely recessive and produces no morphological abnormalities in homozygous females. The effects of *mle* so far noted are that it kills homozygous males and that it possibly slows down the developmental rate of homozygous females. The second chromosome carrying *mle* was freed from deleterious genes prior to these experiments by crossing *SM1(Cy)/mle* males and wild-type (Ore-R) females and using *SM1(Cy)/Pm* strain subsequently in the F_4 generation, recovering *mle/mle* females and *SM1(Cy)/mle* males with second chromosomes derived from a single origin. Table 1 shows that *mle* kills all the homozygous males but no females, regardless of whether parental females are homozygous or heterozygous for *mle*. Examination of the salivary gland chromosome preparations revealed that the *mle*-bearing chromosome is not associated with any detectable aberrations.

The *mle* locus was mapped following the procedures of WATANABE and OSHIMA (1966). *SM1(Cy)/Sp Bl L* females were crossed with *SM1(Cy)/mle*

TABLE 1

Frequency of heterozygous and homozygous mle *females and males in F_1 progenies from two kinds of crosses*

Cross	Phenotype of F_1 progeny			
	Cy ♀	Cy ♂	+ ♀	+ ♂
mle/mle females × *SM1(Cy)/mle* males	1116	1031	1063	0
SM1(Cy)/mle females × *SM1(Cy)/mle* males	568	557	286	0

TABLE 2

Results of cross between Sp Bl L/mle *females and* SM1(Cy)/mle *males*

Phenotype	Frequency of flies			
	Cy		non-Cy	
	♀	♂	♀	♂
Sp Bl L	259	303	329	291
+ + +	425	390	378	0
Sp + +	109	110	109	0
+ Bl L	96	95	91	95
Sp Bl +	26	33	40	28
+ + L	39	36	24	1
Sp + L	11	15	10	0
+ Bl +	6	10	7	12

males and the *Sp Bl L/mle* F_1 females were crossed with *SM1(Cy)/mle* males. F_2 progeny were first separated into *Cy* flies and non-*Cy* flies, then scored according to the dominant characters and to the sex (Table 2). Directing attention to the non-*Cy* male progeny, results show that the locus of *mle* is between *Bl* and *L* and is very close to *Bl*. Thus the genotype of *Sp Bl L/mle* was actually *Sp Bl mle*$^+$ *L*/+ + *mle* +. The distances, *Bl–mle* and *mle–L*, are a function of crossovers in these regions, respectively. F_2 survivors with a crossover in *Bl–mle* are + + *mle*$^+$ *L*, + + *mle*$^+$ +, *Sp* + *mle*$^+$ +, and *Sp* + *mle*$^+$ *L* (total one F_2 individual). Similarly, F_2 survivors with a crossover in the region *mle–L* are *Sp Bl mle*$^+$ +, + + *mle*$^+$ +, + *Bl mle*$^+$ +, and *Sp* + *mle*$^+$ + (total 40 F_2 individuals). Since the distance between *Bl* (2-54.8) and *L*(2-72.0) is 17.2 according to the standard map, the locus of *mle* was estimated to be 55.2 without correction and is probably closer to *Bl*.

TIME OF LETHAL ACTION OF *mle*

Table 3 shows the viability at various developmental stages in progeny from a cross between *mle/mle* females and *SM1(Cy)/mle* males. Parental flies were transferred into new culture bottles every 24 hours and eggs were allowed to

TABLE 3

Viability at various developmental stages in the progeny of cross between mle/mle *females and* SM1(Cy)/mle *males*

Egg	First instar larva	Pupa	Adult	Segregation in adults*			
				Cy ♀	Cy ♂	+ ♀	+ ♂
458	393	314	293				
(1.00)	(0.86)	(0.69)	(0.64)	111	91	85	0

* Out of 293 adults emerged, 6 individuals died immediately and thus their phenotypes could not be determined.

develop. Out of 458 eggs collected and examined, 393 (86%) hatched, 314 (69%) pupated and 293 (64%) eclosed.

In these particular experiments, it is probable that some *SM1(Cy)/mle* eggs died during development as well as some *mle/mle* females, apparently because the *mle*-carrying second chromosome and other chromosomes had accumulated some deleterious genes. It is clear, however, that once pupated, practically no individuals die. It is also clear that not all the *mle/mle* males die as embryos. It was noted that many individuals remained as larvae for several days after others pupated. Salivary gland chromosome preparations from the larvae that eventually died were made and examined in a separate experiment. Eighty-seven larvae were collected and examined, and 82 determined to be the male sex by gonad size inspection. Salivary gland chromosome preparations, however, proved to be quite difficult. Many of the larvae had salivary glands reduced in size and their chromosomes appeared in the process of degeneration with the loss of normal banding structure and integrity. Only 15 larvae produced reasonably good chromosome preparations, all of which had normal second chromosomes (namely *mle*-carrying chromosomes) instead of an *SM1(Cy)* and a normal chromosome.

Since only a slightly improved egg hatchability was observed when *mle/mle* females were crossed with *SM1(Cy)/+* males rather than *SM1(Cy)/mle*, it may be concluded that much of the egg mortality (14%) observed in Table 3 cannot be assigned to *mle/mle* males and that most, if not all, *mle/mle* males hatched. Of these, most survived to the third instar larval period but died before pupation.

EFFECT OF *mle* ON SEX-TRANSFORMING GENES

The male-specific lethal action of *mle* was examined in relation to sex differentiation by using the two third chromosomal imaginal sex-transforming genes, transformer (*tra*) and doublesex (*dsx*). With *SM1(Cy)* chromosome and *TM2(Ubx)* or *TM6(Ubx)* chromosome as balancers of the second and third chromosome, respectively, and with an attached *XX* chromosome, *C(1)M3*, or a *Y* chromosome, $B^s \cdot Y$, as a marker of genotypic females or males, respectively, strains were constructed and crossed.

Table 4 shows the results with *tra*. In combination with *C(1)M3*, homozygous *tra* chromosome had a lowered viability of about 60% that of the expected value, and homozygous *mle* about 80%. Using four genotypes having *XX/Y* chromosomes, a test for the independence was performed. The χ^2 value was 1.04 (d.f.=1) and the probability, P, was 0.5–0.25. Thus, the frequency of *XX/Y*; *mle/mle*; *tra/tra* individuals (21) was not significantly different from the expected (17.88). Similarly in combination with *X/X*, homozygous *tra* chromosome had a reduced viability of about 75% that of the expected value and homozygous *mle* about 75%. The χ^2 value, calculated as above, was 4.68 (P=0.05–0.025). Although this value shows a significant difference at the 5% level, observed frequency of the double homozygote (127) was not less than the expected (110.8). It was concluded that *XX*; *tra/tra* transformed "males" are not affected

TABLE 4

Frequency of various genotypes in F_1 progenies of crosses (1) C(1)M3, y^2/Y; SM1(Cy)/mle; TM2(Ubx) *or* TM6(Ubx)/tra × X/Y; SM1(Cy)/mle; TM2(Ubx) *or* TM6(Ubx)/tra, *and* (2) X/X; SM1(Cy)/mle; TM2(Ubx) *or* TM6(Ubx)/tra × X/B^s•Y; SM1(Cy)/mle; TM2(Ubx) *or* TM6(Ubx)/tra

Genotype	Sex	Genotypic segregation*	Observed frequency
Cross (1)			
XX/Y; Cy/mle; Ubx/tra	♀	4	129
tra/tra	♀ → ♂	2	37
mle/mle; Ubx/tra	♀	2	53
tra/tra	♀ → ♂	1	21
X/Y; Cy/mle; Ubx/tra	♂	4	125
tra/tra	♂	2	41
mle/mle; Ubx/tra	♂	2	0
tra/tra	♂	1	0
Cross (2)			
X/X; Cy/mle; Ubx/tra	♀	4	697
tra/tra	♀ → ♂	2	258
mle/mle; Ubx/tra	♀	2	259
tra/tra	♀ → ♂	1	127
X/B^s•Y; Cy/mle; Ubx/tra	♂	4	570
tra/tra	♂	2	265
mle/mle; Ubx/tra	♂	2	0
tra/tra	♂	1	0

*Disregarding the male-killing effect of *mle*.

by *mle*, and that the presence of a *Y* chromosome in genotypic females does not alter the results.

Table 5 shows the results with *dsx*. The χ^2 values are 0.048 for cross (1) and 2.68 for cross (2), and P for the latter is 0.25–0.1. Conclusions were that *XX; dsx/dsx* intersexes are not killed by the lethal action of *mle*, that *X/Y; dsx/dsx* intersexes are not rescued, and that the presence of a *Y* chromosome in genotypic females does not affect the results.

DISCUSSION

The lethal action of *mle* is specifically limited to the male sex, or rather to individuals with a single *X* chromosome, regardless of whether female parents are heterozygous or homozygous for *mle*. These characteristics and its location on the second chromosome make this mutant look very similar to *mak*, which is located near *Bl* but to the left of it (GOLUBOVSKY and IVANOV 1972). It remains to be examined, however, whether *mak* as well as *km* (PIERRE 1972) can be influenced by imaginal sex-transforming genes. Sex differential lethal genes, *snl* on the *X* and *da* on the second chromosome, are both maternal effect mutants. The action of *snl* is related to the process of imaginal sex differentiation in some way yet to be determined (COLAIANNE and BELL 1972), while *da* is not affected

TABLE 5

Frequency of various genotypes in F_1 progenies of crosses (1) C(1)M3, y^2/Y; SM1 (Cy)/mle; TM2(Ubx) *or* TM6(Ubx)/dsx × X/Y; SM1(Cy)/mle; TM2(Ubx) *or* TM6(Ubx)/dsx, *and* (2) X/X; SM1(Cy)/mle; TM2(Ubx) *or* TM6(Ubx)/dsx × X/B^s•Y; SM1(Cy)/mle; TM2(Ubx)*or* TM6(Ubx)/dsx

Genotype	Sex	Genotypic segregation*	Observed frequency
Cross (1)			
XX/Y;Cy/mle; Ubx/dsx	♀	4	119
dsx/dsx	♀ → ♂	2	43
mle/mle; Ubx/dsx	♀	2	49
dsx/dsx	♀ → ♂	1	19
X/Y; Cy/mle; Ubx/dsx	♂	4	127
dsx/dsx	♂ → ♂	2	55
mle/mle; Ubx/dsx	♂	2	0
dsx/dsx	♂ → ♂	1	0
Cross (2)			
X/X; Cy/mle; Ubx/dsx	♀	4	718
dsx/dsx	♀ → ♂	2	307
mle/mle; Ubx/dsx	♀	2	275
dsx/dsx	♀ → ♂	1	94
X/B^s•Y; Cy/mle; Ubx/dsx	♂	4	857
dsx/dsx	♂ → ♂	2	332
mle/mle; Ubx/dsx	♂	2	0
dsx/dsx	♂ → ♂	1	0

*Disregarding the male-killing effect of *mle*.

(COLAIANNE and BELL 1968). However, the differential lethal effect of *da* is known to be influenced by the amount of sex chromosome as well as autosomal heterochromatin (SANDLER 1972; MANGE and SANDLER 1973). It should also be noted that at least many, if not all, *mle/mle* males can survive up to the third instar larval period while *km*, *snl*, and *da* are all embryonic or early larval lethals.

Sex-transforming genes, *tra* and *dsx*, are apparently already acting during the first 48-hour period of larval development or earlier, since the lethality of *snl*, which interacts with these sex-transforming genes, is exerted in these periods. Since many, if not most, *mle/mle* males survive into the third larval instar, and since no interactions with imaginal sex-transforming genes have been observed, it is most probable that the action of *mle* is in no way related to the process of imaginal sex differentiation. Then the same explanation presented for the male-killing effect of SR-spirochetes infecting Drosophila (MIYAMOTO and OISHI 1975) can be applied for the mechanism of *mle* lethality. Thus we may assume that the *X* chromosome contains a gene(s) whose action is not related to the imaginal sex differentiation, but this gene is not dosage-compensated. Interactions between this gene and *mle* may result in the selective death of individuals with a single dose of the assumed gene. It would be of interest to see whether the same

explanation can be applied for sex-specific lethals other than *mle* and, if so, whether the same gene(s) on the *X* chromosome is responsible for all the cases.

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