MALE-SPECIFIC LETHAL MUTATIONS OF *DROSOPHILA MELANOGASTER*[1]

JOHN M. BELOTE[2] AND JOHN C. LUCCHESI

Curriculum in Genetics and Department of Zoology, University of North Carolina, Chapel Hill, North Carolina 27514

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ABSTRACT

A total of 7,416 ethyl methanesulfonate (EMS)-treated second chromosomes and 6,212 EMS-treated third chromosomes were screened for sex-specific lethals. Four new recessive male-specific lethal mutations were recovered. When in homozygous condition, each of these mutations kills males during the late larval or early pupal stages, but has no detectable effect in females. One mutant, mle^{ts}, is a temperature sensitive allele of maleless, *mle* (FUKUNAGA, TANAKA and OISHI 1975), while the other three mutants identify two new loci: male-specific lethal-1 (*msl-1*) (two alleles) at map position 2–53.3 and male-specific lethal-2 (*msl-2*) at 2–9.0.——The male-specific lethality associated with these mutants is not related to the sex *per se* of the mutant flies, since sex-transforming genes fail to interact with these mutations. Moreover, the presence or absence of a *Y* chromosome in males or females has no influence on the male-specific lethal action of these mutations. Finally, no single region of the *X* chromosome, when present as a duplication, is sufficient to rescue males from the lethal effects of *msl-1* or *msl-2*. These results suggest that the number of complete *X* chromosomes determines whether a fly homozygous for a male-specific lethal mutation lives or dies.

IN Drosophila, sexual differences are manifested at a number of levels, ranging from morphological and behavioral differences between males and females, through different genetic requirements for the meiotic divisions and gametogenesis and to different transcription rates for *X*-linked loci (dosage compensation). Many, if not all, of these processes appear to be under genetic control at two levels. The initial determinant of which mode of development is followed is provided by the *X* chromosome:autosome ratio. Thus, it has been demonstrated that the X:A balance controls both sex determination (BRIDGES 1939) and dosage compensation (LUCCHESI 1977). An alternative level of control is provided by a number of individual loci at which point mutants alter the developmental pathways followed by the two sexes. Existence of such regulatory mutants is particularly clear in the case of sex determination (BAKER and RIDGE 1980).

Over the years, mutants with different phenotypic effects in the two sexes have been discovered and studied. Many of these probably identify individual steps

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[2] Present address: Department of Biology, B-022, University of California, San Diego, La Jolla, California 92093.

in developmental pathways unique to one sex or the other. A particular class of mutants exhibiting sexual dimorphism is that of sex-specific lethals (REDFIELD 1926; BELL 1954; GOWEN 1961; PIERRE 1972; GOLUBOVSKY and IVANOV 1972; FUKUNAGA, TAKANA and OISHI 1975; TANAKA, FUKUNAGA and OISHI 1976; CLINE 1976, 1978, 1979; T. SKRIPSKY, personal communication). These mutants, located throughout the genome, affect either males or females and exhibit lethal phases ranging from the embryo to the pharate adult; several mutants display a maternal effect.

Sex-specific lethality should reflect biochemical or physiological differences that exist between the two sexes and are essential for their development. The study of sex-specific lethal mutants may, therefore, provide a means for understanding the nature of these differences and how they are elicited and maintained during development. To this end, we have induced and recovered new sex-specific lethal mutations on the autosomes. The purpose of this paper is to describe these mutants and to present the results of experiments carried out to define the genetic parameters involved in their sex-specific lethality. The molecular basis of this lethality will be addressed in a separate publication (BELOTE and LUCCHESI 1980).

MATERIALS AND METHODS

Flies were cultured at 25° in half-pint milk bottles on a cornmeal-molasses-yeast-agar medium containing proprionic acid as a mold inhibitor and seeded with active dry yeast.

Males to be mutagenized were collected from two lines that had been made isogenic for chromosomes *X*, *2* and *3*. Cytological examination of salivary gland nuclear spreads revealed these lines to be free of obvious aberrations. The sex ratio of the line marked with *cn* (cinnabar) and *bw* (brown) was 53.3% males:46.7% females ($n = 3{,}498$); that of the line marked with *bw* and *st* (scarlet) 54.6% males:45.4% females ($n = 2{,}761$). Both lines have good viability and fertility. A full description of these and all other genetic markers and special chromosomes used may be found in LINDSLEY and GRELL (1968), unless otherwise noted.

Induction and isolation of mutants: Males were fed a 0.005 M solution of EMS according to the procedure of LEWIS and BACHER (1968). The genetic scheme used to screen for sex-specific lethal mutations on chromosome *2*, derived from a screen by WRIGHT (1970), is outlined in Figure 1. A similar scheme was used for chromosome 3 mutants, except that *TM3, Ser* was the balancer, *DTS-2* (WRIGHT 1974) was the dominant temperature-sensitive lethal used and treated males were from the *bw;st* line. In both screens, generation 4 flies homozygous for the treated autosome are white-eyed and lack the dominant marker of the balancer chromosome. This phenotype allowed the ready assessment of the sex ratio among homozygous individuals by observation through the walls of the culture vials.

To rid the new sex-specific lethal-bearing chromosomes of other deleterious genes that might have been induced during the EMS treatment, each original mutant line was made heterozygous with Canton S wild-type chromosomes and allowed to recombine freely for 3 or 4 generations. The sex-specific lethal mutants were then reextracted, and three of the most viable and fertile lines bearing each mutant were saved and used in subsequent experiments. Some lines, not carrying a sex-specific lethal mutation, were saved from these crosses to be used as control stocks.

Genetic mapping and cytological localization: Initial mapping for all four male-specific lethal mutants was done with a chromosome marked with *Sp* (2–22.0), *Bl* (2–54.8) and *N-2G* (2–72.0).

In the initial mapping cross, *msl-2* was estimated to be 17 map units to the left of *Sp*. For a more precise localization, females heterozygous for *msl-2* over *al dp* (map positions 0.0 and 13.0, respectively), were testcrossed. Male progeny that carried a recombinant chromosome,

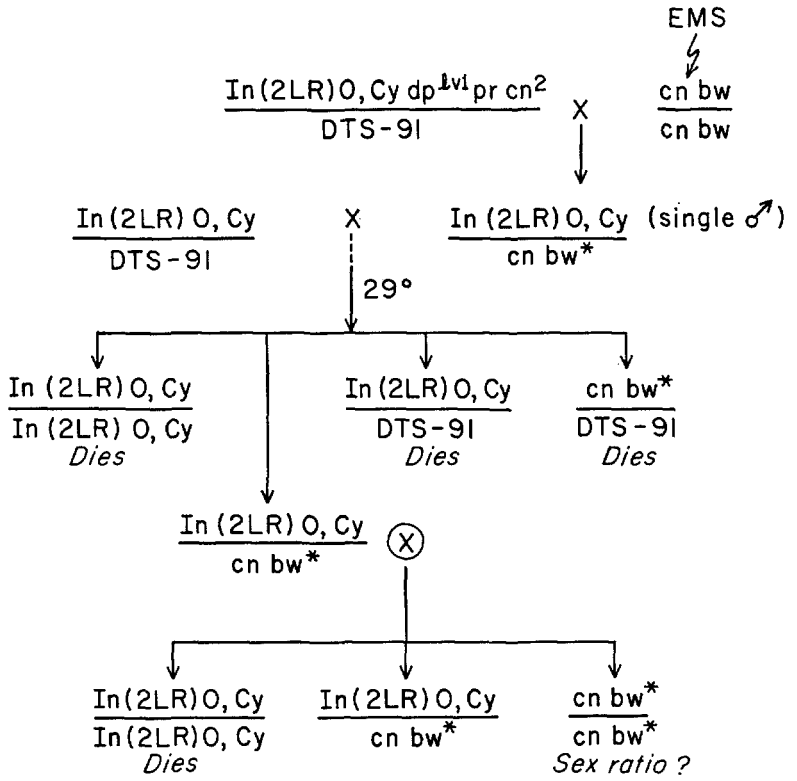

FIGURE 1.—Crossing scheme for the induction and isolation of chromosome *2* sex-specific lethal mutations.

i.e., *al* + or + *dp* in phenotype, were crossed singly to females carrying *In(2LR)CyO*, and *Cy* male progeny were selected. Approximately half of these males, in each line, should carry the recombinant chromosome balanced over *In(2LR)CyO*. For each line, 10 males were crossed singly to homozygous *msl-2* females, and each of the 10 vials per line was scored for evidence of male-specific lethality of non-*Cy* progeny, indicating that the recombinant chromosome carried *msl-2*. Among the 45 lines established, 14 represented recombinants between *msl-2* and *dp*, and 31 between *al* and *msl-2*. The locus of *msl-2* is therefore 9.0 map units from *al*, at *2*–9.0, with a standard error of 0.9 (after STEVENS 1942).

Genetic recombination data place *msl-1* at position *2*–53.3 (95% confidence interval, 51.5 to 54.4 after STEVENS 1942) and *msl-1*^{b} at *2*–54.2. WRIGHT, HODGETTS and SHERALD (1976) have generated a series of overlapping deficiencies that uncover most of the surrounding proximal region of 2L. These deficiencies were used in the deletion mapping of *msl-1* and *msl-1*^{b}. Homozygous *msl-1* or *msl-1b* females were crossed to males carrying a deficiency chromosome balanced over the *In(2LR)CyO* chromosome and the non-*Cy* progeny scored for male-specific lethality. The results indicated that *Df(2L)3*, *Df(2L)E71*, *Df(2L)50* and *Df(2L)137* uncover the region containing the two *msl-1* alleles; whereas, *Df(2L)158*, *Df(2L)2*, *Df (2L)12*, *Df(2L)1* and *Df(2L)161* do not uncover *msl-1*. These results place *msl-1* in salivary region 36F7–37B8.

Preliminary mapping of *mle*^{ts} placed it approximately 1 map unit to the right of *Bl*. A further mapping relative to *cn* localized *mle*^{ts} to the left of this marker at *2*–56.8 (95% confidence interval, 55.8 to 57.3). Deletion mapping of our allele was attempted using chromosomes deficient for proximal regions of 2L or 2R. The results demonstrated that *msl*^{ts} does not lie within the proximal heterochromatin of either 2R or 2L since it complements com-

pletely the following: *Df(2R)MS2⁴*, *Df(2R)MS2⁸*, *Df(2R)MS2¹⁰*, *Df(2L)D'*, *Df(2L)D*, *Df(2L)C* and *Df(2L)C'* (see HILLIKER and HOLM 1975 for a description of these deficiencies). It also is not located in the proximal euchromatin of 2L uncovered by the above deficiencies of WRIGHT, HODGETTS and SHERALD (1976). *Df(2R)42*, which deletes region 42C3–8 to 42D2–3, also failed to uncover the *mle^{ts}* locus. These observations, taken together with the genetic mapping data, suggest that *mle* is most likely located on 2R, just distal to *Df(2R)MS2⁴*. This was confirmed by the demonstration that homozygous *mle* males are rescued by the presence of a *Y* chromosome that carries an insertional duplication of regional 41A to 43A. This *Y* chromosome is from the translocation stock *T(Y;2)L12* of the Seattle-LaJolla Drosophila Laboratories (1971).

Temperature-shift experiments: Half-pint bottles containing 40 pairs of flies each were inverted over 35 × 10 mm plastic Petri dishes containing food medium and active dry yeast. Eggs were collected at 22° or 29° for 4 hr. The food with the deposited eggs was then transferred to culture bottles and placed at 18° or 29°. At various times following egg collection, bottles were shifted from 18° to 29°, or *vice versa*. All resulting live progeny were scored.

Determination of lethal phase: For each cross, eggs were collected as above and transferred with a blunt needle to another Petri dish with fresh medium. The number of hatched eggs was determined at 24 and 36 hr post-collection. Larvae were transferred to food vials where the remainder of their development was monitored. Pupal cases were counted after 14 to 17 days and all emerging adults were scored.

Two types of control crosses were set up to determine the amount of background death due to causes other than the male-specific lethal mutation itself. The first control cross involved lines carrying the wild-type allele of the male-specific lethal and the *In(2LR)CyO* balancer chromosome. These control lines were established along with the various mutant lines during the crossing scheme carried out to replace most of the mutagenized chromosome *2*. Thus, they should contain much of the same genetic background as the mutant line. Another control cross involved either homozygous or heterozygous male-specific lethal female and wild-type males from the control line.

Effect of X-chromosome duplications: In order to generate male zygotes bearing duplications of specific regions of the *X* and homozygous for *msl-1* or *msl-2*, the following crosses were performed: *C(1)RM, y v f/Y*; *msl/CyO* females by *y dor^{l}/Y/Dp(1;f)R, y+*; *msl/CyO* males or by *w^{a}/Y/Dp(1;3)w^{vco}*; *msl/CyO* males or by *y w dor^{l}/Y/Dp(1;3)w^{m49a}*; *msl/CyO* males. In addition, *msl-1/CyO* or *msl-2/CyO* were introduced into a series of *T(X;Y)* translocation stocks (STEWART and MERRIAM 1975). These translocations (B29, B36, J8, J2, B44, B39, B24, B28, B35, B18) were used to make a series of duplications of the *X* chromosome, from 3E to 20 on the cytological map, according to the method illustrated in Figure 2 of STEWART and MERRIAM (1975).

RESULTS

Isolation and preliminary characterization of sex-specific mutants

Recovery: In the screen for chromosome *2* mutations (Figure 1), 7,416 matings in generation 3 were fertile. Of these, approximately 65% produced only *Cy* progeny, indicating that the mutagenized *cn bw* chromosome *2* now carried a recessive lethal mutation. Among the cultures producing homozygous *cn bw* flies, 61 were saved as putative sex-specific lethals. In the screen for chromosome *3* mutants, a total of 6,212 crosses in generation 3 were fertile. Approximately 48% of these lines yielded only *bw*; *Ser* progeny, indicating that a recessive lethal mutation had been induced on the mutagenized chromosome *3*. Of 30 lines saved as putative sex-specific lethals, none were confirmed in subsequent generations. Most of the chromosome *2* lines saved as putative sex-specific lethals gave rise to normal sex ratios in the next generation and were therefore discarded; however, four separate chromosome *2* male-lethal mutant lines were

confirmed. What appeared to be two new female-specific lethal mutant lines, upon further testing, turned out to be two new alleles of the sex-transforming mutant *tra-2* (WATANABE 1975); a characterization of these *tra-2* alleles will be reported elsewhere.

The four male-specific lethals were named male-specific lethal-1 (*msl-1*), male-specific lethal-1^b (*msl-1^b*), male-specific lethal-2 (*msl-2*) and maleless-temperature sensitive (*mle^{ts}*), for reasons outlined below. Retests of cleaned-up stocks of each of these mutants (see MATERIALS AND METHODS) showed that all four new mutants had no detectable effect on female viability or morphology, although they were invariably lethal when homozygous in the male (Table 1).

To determine how many genes are identified by these male-specific lethal mutations, complementation tests were carried out in all possible pair-wise combinations of these mutants plus maleless, *mle* (FUKUNAGA, TANAKA and OISHI 1975). Females carrying one male-lethal mutant balanced over the *In(2LR)CyO* chromosome were crossed to males heterozygous for a second male-lethal mutation and the *In(2LR)CyO* balancer; the reciprocal cross was also carried out. Absence of non-*Cy* sons in these crosses indicated that the two mutants failed to complement. The results clearly showed that these mutants represent three complementation groups. One of our mutants, *mle^{ts}*, is an allele of *mle* and, as shown below, is temperature sensitive. Two of the remaining mutants are allelic to one another and designated *msl-1* and *msl-1^b*, while the final mutant, *msl-2*, identified a third complementation group.

That these three complementation groups identify three distinct loci is shown by the mapping of these mutants (see MATERIALS AND METHODS). The *msl-1* mutants map to 53.3–54.5 on chromosome *2* and are located in salivary region 36F7–37B8; *msl-2* is located at *2*–9.0, and *mle^{ts}* maps to 2–56.8. The latter is in good agreement with the published location of *mle* at *2*–55.2 (FUKUNAGA, TANAKA and OISHI 1975).

Determination of the lethal phases: As a first step in trying to understand the roles of the loci identified by these male-specific lethals, we determined when these mutations exerted their developmental effects. The lethal stage of *msl-1* was determined in crosses of heterozygous *msl-1/In(2LR)CyO* males and

TABLE 1

Results of crosses between heterozygous msl *males and females*

	Cy ♂	*Cy* ♀	non-*Cy* ♂	non-*Cy* ♀
msl-1/CyO ♀ × *msl-1/CyO* ♂	1961	2125	0	950
	(38.9)*	(42.2)	(0)	(18.9)
msl-1^b/CyO ♀ × *msl-1^b/CyO* ♂	959	1003	0	521
	(38.6)	(40.4)	(0)	(21.0)
msl-2/CyO ♀ × *msl-2/CyO* ♂	1519	1605	0	757
	(39.1)	(41.4)	(0)	(19.5)
mle^{ts}/CyO ♀ × *mle^{ts}/CyO* ♂	1117	1312	0	586
	(37.0)	(43.5)	(0)	(19.4)

* Numbers in parentheses represent percentages.

females. To inquire whether *msl-1*$^+$ exerted a maternal effect, the lethal stage was also determined in progeny of homozygous *msl-1* females crossed to *msl-1/In(2LR)CyO* males. In both crosses, eggs were collected and the numbers of progeny that hatched, went through the larval instars, pupated and eclosed were recorded. Five to 12% unhatched eggs were observed in all control crosses, with an overall hatchability of 91.7% ($n = 4{,}480$) (data not shown). This level of hatching failure is not due specifically to either the *msl-1* mutant allele or the *In(2LR)CyO* chromosome (even *CyO/CyO* individuals usually hatch). The level of background post-hatching death not attributable to the effects of the male-specific lethal mutations was calculated from crosses involving wild-type control lines (Table 2, Crosses A and B). The percent post-hatching death of *Cy* males, *Cy* females and non-*Cy* females was 2.1 for cross A and 6.2 for cross B.

Of the eggs produced by homozygous *msl-1* females crossed to *msl-1/CyO* males, 90.0% hatched and 74.2% of the resulting larvae survived to adulthood (Table 2, Cross C). Since the frequency of unhatched eggs in this cross (10.0%) is comparable to that observed in nonmutant control crosses (8.3%), this suggests that *msl-1*-produced male lethality occurs post-embryonically. These data also establish that *msl-1* does not have any significant effect on the viability of females or heterozygous males. The results of this cross demonstrate that, although most of the male lethality occurs during the larval stages, a substantial number of homozygous *msl-1* males must reach the prepupal stage. In this cross, a number of male larvae exhibited delayed larval development, followed by death at the late larval or prepupal stage.

A comparison of the results from crosses involving homozygous *msl-1* (Table 2, Cross C) or heterozygous *msl-1* (Table 2, Cross D) mothers suggests that there may be an effect of the mother's genotype on the stage at which homozygous mutant sons die. Homozygous *msl-1* females produced 336 individuals that died after hatching and are presumed to be *msl-1* males. Of these, 88, or 26%, survive to the prepupal stage. Heterozygous mothers produced 519 individuals that died after hatching; 2/3 of these are *CyO/CyO* and 1/3, or 173, presumed to be *msl-1* males. In this case, 61 (35%) homozygous male larvae survived to the prepupal stage.

A maternal effect is also suggested by an experiment that involved collecting eggs from A- and B-type crosses and separating the larvae into normal and slow developers (presumably the homozygous mutant males) on the basis of size at two days post-hatching. These slow developers, which were judged to be males by gonad size, are smaller, feed less vigorously and at 25° become third-instar larvae three to four days later than normal. They remain at this stage for a long period (up to 12 days post-hatching, in some cases); many reach normal size and some become prepupae. None progress beyond this stage. In crosses involving *msl-1/msl-1* females × *msl-1/CyO* males, 49 slow developers were still alive two days post-hatching out of an expected 81 *msl-1/msl-1* males (25% of 325). This suggests that about 40% of the *msl-1* males died during the first 48 hours post-hatching. On the other hand, when the mothers were heterozygous for *msl-1*, of the approximately 41 homozygous sons expected (12.5% of 326), 42 slow developers were still alive after two days. Thus, few if any of the mutant males had

TABLE 2

Determination of lethal phase

	Cross ♀♀	×	♂♂	Eggs laid	Eggs hatched	Prepupae	Adults	*Cy*♂♂	*Cy*♀♀	non-*Cy*♂♂	non-*Cy*♀♀
A	+/+		+/*CyO*	980	893 (91.1)	839 (93.6)	820 (91.8)	206	185	232	196
B	+/*CyO*		+/*CyO*	1450	1333 (91.9)	979 (73.4)	968 (72.6)	304	342	155	167
C	*msl-1/msl-1*		*msl-1/CyO*	1450	1303 (90.0)	1055 (81.0)	967 (74.2)	310	308	0	349
D	*msl-1/CyO*		*msl-1/CyO*	1300	1197 (92.0)	739 (61.7)	678 (56.7)	253	283	0	142
E	*msl-1^b/msl-1^b*		*msl-1^b/CyO*	400	375 (93.8)	322 (85.9)	273 (72.8)	97	94	0	82
F	*msl-1^b/CyO*		*msl-1^b/CyO*	250	222 (88.8)	168 (75.7)	139 (62.6)	69	50	0	20
G	*msl-2/msl-2*		*msl-2/CyO*	1400	1287 (91.9)	1025 (79.6)	929 (72.2)	318	302	0	309
H	*msl-2/CyO*		*msl-2/CyO*	1250	1108 (88.6)	712 (64.3)	698 (63.0)	291	269	0	138
I	*mle^{ts}/mle^{ts}* (25°)		*mle^{ts}/CyO*	300	263 (87.7)	240 (93.5)	182 (68.8)	61	62	0	59
J	*mle^{ts}/CyO* (25°)		*mle^{ts}/CyO*	300	276 (92.0)	209 (75.7)	165 (59.8)	63	71	0	31
K	*mle^{ts}/mle^{ts}* (29°)		*mle^{ts}/CyO*	450	380 (84.4)	257 (67.6)	245 (64.5)	77	78	0	90
L	*mle^{ts}/CyO* (29°)		*mle^{ts}/CyO*	650	612 (94.2)	411 (67.2)	347 (56.7)	152	133	0	62

All figures in parentheses represent percentages. For prepupae and adults, the percentages were calculated by taking the number of hatched eggs as 100%.

died by this time. Moreover, in crosses involving homozygous mothers, 15 of 81 (19%) of the expected number of *msl-1* males reached the prepupal stage, whereas 15 of 42 (36%) of the expected number of *msl-1* males formed puparia when the mother carried a wild-type allele of *msl-1*. This agrees well with the 26% *vs.* 35% found in the similar comparison using data from Table 2. Thus, there appears to be a weak maternal effect that affects the viability of the mutant male larvae.

It should be noted that the effect of *msl-1* on first-instar, early second-instar male larvae, *i.e.*, their slower development and sluggishness, provides evidence that the wild-type gene is required fairly early for normal male development. Moreover, the delayed larval development demonstrates that *msl-1* affects larval tissue (and not just imaginal discs), since imaginal discs are not required for normal larval development (SHEARN and GAREN 1974).

An examination of the data in Table 2, crosses E and F, shows that males homozygous for $msl\text{-}1^b$ are less severely affected than are *msl-1* males. This shows up as a higher percentage of the $msl\text{-}1^b$ males reaching the prepupal stage (48% if the mother is homozygous for $msl\text{-}1^b$, 100% if the mother is heterozygous for $msl\text{-}1^b$). As with *msl-1*, a slight maternal effect is observed with respect to the lethal phase.

There is no significant amount of embryonic death caused by *msl-2* (Table 2, crosses G and H). Since there is not significantly more mortality in these crosses than is required to account for the death of the homozygous *msl-2* males, these data suggest that there is no significant effect of *msl-2* on the viability of females or heterozygous males. The data in Table 2 also reveal that, although most *msl-2* males are killed in the larval stages, some do reach the prepupal stage. Observations on the larvae from these crosses indicate that *msl-2/msl-2* males develop very slowly, with a reasonable number of them eventually reaching the third-instar stage. Most remain at this stage for several days (up to 14 days post-hatching at 25°) before dying as larvae or forming a puparium and dying soon afterward, without ever differentiating any adult structures. Like *msl-1*, there is a noticeable effect of *msl-2* on early larval development of homozygous males, suggesting early gene function of this locus as well.

Of the expected number of *msl-2/msl-2* male larvae, 27% reach the prepupal stage when the mother is homozygous for *msl-2* and 11% reach the prepupal stage when the mother carries a wild-type allele of *msl-2*. Therefore, there does not appear to be a maternal effect associated with $msl\text{-}2^+$.

Since temperature has been shown to have an effect on mle^{ts} male-specific lethality (see below), determination of the lethal phase was carried out at 25° and 29°, both of which are restrictive temperatures with respect to this mutation (Table 2, crosses I through L). At 25°, when the mothers are homozygous for mle^{ts}, about 90% of the expected mutant males reach the prepupal stage. When the mothers are heterozygous for mle^{ts} virtually all of the expected number of mutant males reach the prepupal stage. At 29°, only about 13% of the mle^{ts} sons from homozygous mothers form puparia, whereas most reach this stage when the mother is heterozygous. Thus, like $msl\text{-}1^+$, $mle^{ts}+$ is associated with

a weak maternal effect. From the amount of overall lethality and the relative viability of adults at both temperatures, it can be concluded that mle^{ts} is completely recessive in males and has no effect in females. The rate of larval development is slowed in homozygous mle^{ts} males, although this is not as pronounced as with the other mutants. Unlike the other mutants, mle^{ts} males frequently undergo differentiation of adult structures within the pupal case, even when raised at 29°. Overall, the observations on mle^{ts} at the restrictive temperatures are consistent with that seen in the case of *mle* (FUKUNAGA, TANAKA and OISHI 1975; TANAKA, FUKUNAGA and OISHI 1976).

Temperature sensitivity: To determine whether any of these male-specific lethal mutants were temperature sensitive, males and females heterozygous for one of these mutants (*i.e., msl cn bw/CyO*) were crossed and the cultures maintained at 18° or 29°. In the case of *msl-1*, $msl\text{-}1^{b}$ or *msl-2*, no mutant males were recovered at either temperature. In contrast, mle^{ts} showed an effect of temperature on the lethal phenotype: no mle^{ts} males were recovered at 29°, but 50 to 60% of the expected number of mutant males reached the adult stage at 18° (data not shown). These escapers eclosed several days later than their heterozygous brothers, exhibited a phenotype similar to that of a slight Minute mutation (*i.e.*, thin bristles, rough eyes) and were invariably sterile. The external and internal reproductive systems of these males appeared normal; the testes contained mature, but nonmotile, sperm. At a fairly low frequency, these males exhibited a small darkened scar in a region between the abdominal sternites or had small nicks along the wing border. None of these mutant males were observed to fly. Finally, their lifespan was significantly reduced. Since the male-lethality of the original *mle* allele is not temperature dependent (our observations), temperature-sensitivity is a property of the mle^{ts} allele and is not a general characteristic of mutants at this locus.

To define the temperature-sensitive period (TSP) of mle^{ts}, temperature shifts experiments were carried out. These consisted of shifting cultures from low temperature (18°) to high temperature (29°), and *vice versa*, at various times after the initiation of development. The earliest shift from the restrictive to the permissive temperature that begins to exhibit the mutant effect marks the beginning of the TSP. Likewise, the first shift from the permissive to the restrictive temperature that lessens the effect of the mutation defines the end of the TSP. The results of such an experiment, shown in Figure 2, demonstrate that the TSP is between the late third larval instar and the middle of the pupal stage. Since we have shown that mle^{ts} delays larval development, it is clear that the TSP is not simply the time that the product of mle^{ts} is synthesized or functions. However, it does define a time at which the male is hypersensitive to the combined effects of mle^{ts} and 29°.

Effect of sexual differentiation on male-specific lethality

If the sex-specific lethality of *msl* mutants were related to the process of sexual differentiation and/or to differences in physiology or anatomy between the two sexes, an alteration in the phenotypic sex of homozygous mutant individuals

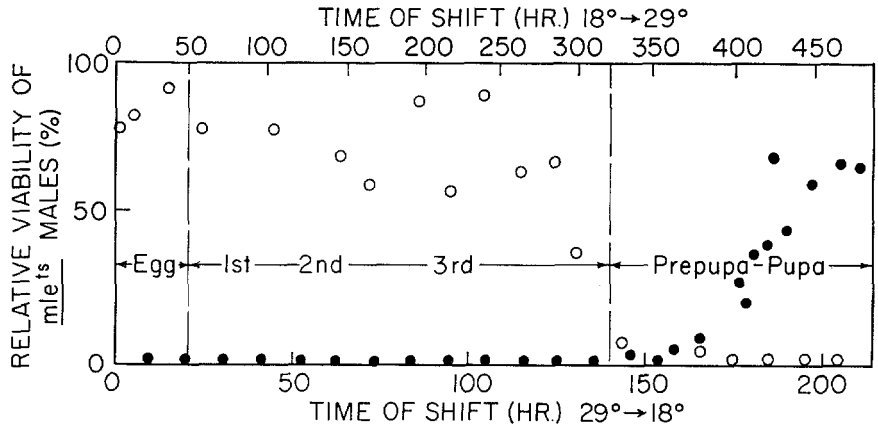

FIGURE 2.—Determination of the temperature-sensitive period (TSP) of *mle*^{ts}. Open circles represent cultures shifted from 29° to 18°; closed circles represent cultures shifted from 18° to 29°.

might be expected to alter their response to the mutation. Specifically, *msl* alleles may be lethal in diplo-*X* individuals transformed into extreme male intersexes by the mutant transformer-2 (*tra-2*) (WATANABE 1975). Conversely, *X/Y* individuals that develop as intersexes because of the mutant *doublesex* (*dsx*) (HILDRETH 1965) might be spared from the male-specific lethal effect.

Results of experiments testing the effects of *tra-2* on the male-killing action of *msl-1* and *msl-2* are shown in Table 3. In these crosses, diplo-*X* individuals carry the attached-*X* chromosome, *C(1)RM*, marked with *y v f*, while single-*X* flies inherit a free *X* with the wild-type alleles of these mutants. Thus, *X·X/Y*; *tra-2/tra-2* "males" can be distinguished from *X/Y*; *tra-2/tra-2* males on the basis of these *X*-linked markers. The relative frequency of transformed diplo-*X* individuals homozygous for *msl-1* (1265 of 2256, or 57%) is slightly higher than that of transformed individuals heterozygous for this male-lethal mutation (345 of 729, or 47%). These results show that shifting the sex of diplo-*X*; *msl-1* individuals towards maleness did not lower their viability. It might be noted that the five y^+ v^+ f^+; *msl-1 tra-2* males in this experiment were in all likelihood diplo-*X* transformed females arising as a result of detachment of the compound-*X* chromosome; all five showed diplo-*X*; *tra-2*-like testes upon dissection (*i.e.*, greatly reduced; no mature sperm) and they appeared in the frequency expected for *C(1)RM* detachment (LINDSLEY and GRELL 1968).

Similar results were obtained for crosses involving *msl-2*. Here, the viability of the *X·X/Y*; *msl-2 tra-2* "males" is equivalent to that control "males," suggesting that this mutation, like *msl-1*, does not interact with the sex-transforming mutant, *tra-2*. The three y^+ v^+ f^+ non-Cy males can be explained as representing *X/X*; *msl-2 tra-2* transformed females resulting from a detachment of the compound-*X* chromosome in the mother. Dissection and examination of these males revealed testes typical of diplo-*X*; *tra-2* flies.

To test the effect of *dsx* on these male-lethal mutations, crosses were made to generate individuals homozygous for a male-specific lethal and *dsx* (Table 4).

TABLE 3

Effect of tra-2 *on* msl-1 *and* msl-2 *lethality*

Cross	*y v f*; *Cy*♀♀	+; *Cy*♂♂	+; *Cy*♀♀	*y v f*; *msl* (or +) *tra-2* "♂♂"	+; *msl* (or +) *tra-2* "♂♂"
1. +/Y; *msl-1 tra-2/In(2LR)CyO,Cy* ♂ ♂ × *C(1)RM,y v f*/Y; *msl-1 tra-2/In(2LR)CyO, Cy* ♀ ♀	2256	4030	5	1265	5
2. +/Y; + *tra-2/In(2LR)CyO,Cy* ♂ ♂ × *C(1)RM,y v f*/Y; *msl-1 tra-2/In(2LR)CyO,Cy* ♀ ♀	729	892	4	345	324
3. +/Y; *msl-2 tra-2/In(2LR)CyO,Cy* ♂ ♂ × *C(1)RM,y v f*/Y; *msl-2 tra-2/In(2LR)CyO,Cy* ♀ ♀	3704	5224	12	1706	3
4. +/Y; + *tra-2/In(2LR)CyO,Cy* ♂ ♂ × *C(1)RM,y v f*/Y; *msl-2 tra-2/In(2LR)CyO,Cy* ♀ ♀	679	880	9	314	341

In the first and third crosses, *tra-2* "♂ ♂" are homozygous for *msl*. In the second and fourth crosses, they carry a wild-type allele of *msl*.

TABLE 4

Effect of dsx *on* msl-1 *and* msl-2 *lethality*

Cross	Diplo-*X*				Single-*X*			
	y²; Cy; Ubx ♀♀	*y²; msl; Ubx* ♀♀	*y²; Cy; dsx* ♂♂	*y²; msl; dsx* ♂♂	*+; Cy; Ubx* ♂♂	*+; msl Ubx* ♂♂	*+; Cy; dsx* ♂♂	*+; msl; dsx* ♂♂
+/Y; *msl-1/SM1,Cy; p^p dsx/TM2,Ubx^130* ♂♂ ×	173	245	72	70	359	0	174	0
C(1)M3,y²/Y; msl-1/msl-1; p^p dsx/TM2,Ubx^130 ♀♀	(2)	(2)	(1)	(1)	(2)	(2)	(1)	(1)
+/Y; *msl-1/SM1,Cy; p^p dsx/TM2,Ubx^130* ♂♂ ×	213	266	67	51	1022	0	477	0
C(1)M3,y²/Y; msl-1/SM1,Cy; p^p dsx/TM2,Ubx^130 ♀♀	(3)	(2)	(2)	(1)	(3)	(2)	(2)	(1)
+/Y; *msl-2/SM1,Cy; p^p dsx/TM2,Ubx^130* ♂♂ ×	179	163	52	25	384	0	191	0
C(1)M3,y²/Y; msl-2/msl-2; p^p dsx/TM2,Ubx^130 ♀♀	(2)	(2)	(1)	(1)	(2)	(2)	(1)	(1)
+/Y; *msl-2/SM1,Cy; p^p dsx/TM2,Ubx^130* ♂♂ ×	105	79	33	9	562	0	250	0
C(1)M3,y²/Y; msl-2/SM1,Cy; p^p dsx/TM2,Ubx^130 ♀♀	(3)	(2)	(2)	(1)	(3)	(2)	(2)	(1)

Numbers in parentheses represent the expected relative proportion of different progeny types in each cross.

In these experiments, diplo-*X* individuals carried the compound-*X* chromosome *C(1)M3*, y^2, while single-*X* flies carried a free *X*, wild type for this marker.

In the first cross, it can be seen that, of the approximately 174 *X/Y*; *msl-1*; *dsx* progeny expected (on the basis of number of *X/Y*; *msl-1/Cy*; *dsx* progeny), none managed to survive to the adult stage, even though the sexuality of these single-*X* flies had been shifted towards femaleness by the *dsx* mutation. Similarly, in the second cross, of the 238 *X/Y*; *msl-1*; *dsx* adults expected, all failed to survive to adulthood. Likewise, examination of the diplo-*X* progeny ratios shows that the *msl-1* mutation in chromosomally female individuals is apparently not lethal, even when these flies are intersexual in phenotype.

In the third cross, involving the *msl-2* mutation, of the 191 single-*X*; *msl-2*; *dsx* progeny expected, none were rescued from the lethal action of *msl-2* by an alteration in their sex. In the last cross, of the 125 *X/Y*; *msl-2*; *dsx* flies expected, again, none survived. The diplo-*X*; *msl-2* progeny in these crosses did show reduced viability, as compared to the expected numbers, when they were homozygous for *dsx*. However, the failure in all of these crosses to obtain all of the classes in the Mendelian ratios suggests that there are factors other than *dsx* and *msl-1* or *msl-2* interacting in the various genotypes produced. Thus, the reduction in viability of diplo-*X*; *msl-2*; *dsx* flies may not indicate a direct interaction between these two genes.

Overall, the results of crosses involving *tra-2* and *dsx* demonstrate that the lethal action of *msl-1* and *msl-2* does not interact with the sex *per se* of the mutant individual, at least at the level affected by these sex-transforming mutants.

The effect of the Y *chromosome*

Another possible basis for the male-specific lethality could be some kind of lethal interaction involving the *Y* chromosome, which is normally present in males but absent in females. Although the *Y* chromosome is not normally required for survival (*XO* males are phenotypically normal, though sterile) and may contain only male fertility genes, pairing regions, satellite DNA and a nucleolus organizer, it is possible that the lethality of male-specific lethals is effected through the *Y* chromosome.

To test for possible interaction between the *Y* chromosome and the male-lethal mutation, *msl-1*, stocks were constructed that carried this mutant balanced over *In(2LR)CyO* and either (1) a compound-*X* chromosome with no free *Y* in the female and an attached-*XY* with no free *Y* in the male, or (2) a compound-*X* chromosome with a free *Y* in the female and a normal free *X* and a normal free *Y* in the male. By making the appropriate crosses (Table 5) with these stocks, homozygous male-specific lethal male and female zygotes can be generated with zero, one, or two *Y* chromosomes. The relative viability of each class can be calculated as the ratio of the number of mutant homozygotes of a particular class divided by half the number of *CyO* heterozygotes of the same class for the experimental cross to the number of wild-type homozygotes of that class divided by half the number of *CyO* heterozygotes of the same class in the control cross.

TABLE 5

Effect of the Y *chromosome on* msl-1

Crosses		XO			XY			XYY			XX			XXY			XXYY		
		Cy	+	*msl-1*	*Cy*	+	*msl-1*	*Cy*	+	*msl-1*	*Cy*	+	*msl-1*	*Cy*	+	*msl-1*	*Cy*	+	*msl-1*
$Y^{S}X{\cdot}Y^{L}$,*In(1)EN,y*/O; *msl-1/CyO* ♂ ♂	×	1893	—	0	1738	—	0	—	—	—	—	—	—	2033	—	907	1492	—	669
$y^{+}/Y^{S}X{\cdot}Y^{L}$,*In(1)EN,y*; *msl-1/CyO* ♀ ♀																			
$Y^{S}X{\cdot}Y^{L}$,*In(1)EN,y*/O; +/*CyO* ♂ ♂	×	1110	497	—	1026	489	—	—	—	—	—	—	—	1217	584	—	850	427	—
$y^{+}/Y^{S}X{\cdot}Y^{L}$,*In(1)EN,y*; *msl-1/CyO* ♀ ♀																			
$Y^{S}X{\cdot}Y^{L}$,*In(1)EN,y*/O; *msl-1/CyO* ♂ ♂	×	—	—	—	—	—	—	2153	—	0	1659	—	1020	—	—	—	—	—	—
C(1)DX,y/Y; *msl-1/CyO* ♀ ♀																			
$Y^{S}X{\cdot}Y^{L}$,*In(1)EN,y*/O; +/*CyO* ♂ ♂	×	—	—	—	—	—	—	1922	940	—	1262	868	—	—	—	—	—	—	—
C(1)DX,y/Y; *msl-1/CyO* ♀ ♀																			
+/Y; *msl-1/CyO* ♂ ♂ × +/+; *msl-1/CyO* ♀ ♀		—	—	—	1961	—	0	—	—	—	2125	—	950	—	—	—	—	—	—
+/Y; +/*CyO* ♂ ♂ × +/+; *msl-1/CyO* ♀ ♀		—	—	—	844	385	—	—	—	—	871	438	—	—	—	—	—	—	—

(—) refers to a progeny class not expected in the cross.

The results of these experiments fail to demonstrate any relationship between the presence or absence of a *Y* chromosome and the lethal effects of *msl-1*. Identical crosses performed with *msl-2* yielded similar results (data not shown). Also tested in these experiments was the viability of homozygous mutant females that carry a normal *X* and an *X* deficient for most of the proximal heterochromatin, *i.e.*, the $In(1)sc^{4L}sc^{8R}$ chromosome. The viability of these females was not significantly different from that of heterozygous females with the same *X*-chromosome constitution (data not shown).

The effect of X-*chromosome duplications*

FUKUNAGA, TANAKA and OISHI (1975) and TANAKA, FUKUNAGA and OISHI (1976) have suggested that the number of doses of a nondosage-compensated *X*-linked locus may be responsible for the sex-specific lethality of *mle*. This predicts that homozygous *msl-1* or *msl-2* males duplicated for the region of the *X* that bears this nondosage compensated gene should be rescued, while females carrying an *X* that is deficient for the region in question should succumb to the lethal effects of the mutations.

Male zygotes duplicated for varying portions of the *X* chromosome and homozygous for *msl-1* or *msl-2* were constructed either by using the *T(X;Y)* chromosomes of STEWART and MERRIAM (1975) or by means of known *X*-duplication stocks (see MATERIALS AND METHODS).

The results of such crosses are listed in Table 6. Heterozygous males, duplicated for all regions of the *X* except for regions 3E2 to 3E7–8 (representing the region between the $Dp(1;3)w^{m49a}$ and *T(X;Y)B29 X* chromosome breakpoints)

TABLE 6

Effect of segmental X-*chromosome hyperploidy on* msl-1 *and* msl-2 *lethality*

X-chromosome region	Hyperploid males			
	msl-1/Cy	*msl-1/msl-1*	*msl-2/Cy*	*msl-2/msl-2*
1A3-4—3A	124	0*	236	0
2B17-C1—3C4-5	110	0	95	0
3A10-B1—3E2-3	59	0	72	0
3E—5C	44	0	107	0
5C—8C	60	0*	37	0
8C—9A	162	0	118	0
9A—11A	246	0	69	0*
11A—11D	148	0	75	0*
11D—12E	0	0†	0	0†
12E—13F	226	0	22	0
13F—16F-17A	26	0	60	0*
15B—16F-17A	217	0	—	—
16F-17A—base	29	0	18	0

* Half of the number of recovered *msl/Cy* males were expected.
† No control males were obtained (see text); therefore, no homozygous *msl* males need be expected.

and 11D to 12E, were produced in substantial numbers; whereas, homozygous males never survived regardless of whether or not they carried a specific *X* duplication. Failure to generate any duplication-bearing males for region 11D-12E using these *T(X;Y)* chromosomes is consistent with the experience of STEWART and MERRIAM (1973, 1975).

Thus, with the possible exception of regions 3E2 to 3E7–8 and 11D–12E, no single region of the *X* chromosome, when present in two doses, is sufficient to rescue males from the lethal effects of *msl-1* or *msl-2*.

The effects of *X* deficiencies in mutant females were not tested for most regions, since most of the deficiencies generated by these particular *T(X;Y)* crosses were inviable in females. However, it was noticed that some homozygous mutant females carrying a deficiency of a particular region of the *X* were always recovered whenever the heterozygous females deficient for that same region were viable.

DISCUSSION

Our relatively extensive search for sex-specific lethals on the major autosomes yielded no female-specific lethals and four male-specific lethals representing two alleles of one new gene (*msl-1* and *msl-1*^{b}), one allele of another new gene (*msl-2*) and one allele of a previously known gene (*mle*^{ts}). These results suggest that male-specific lethality is a phenotype associated with a relatively small number of genes and is not the result of particular types of alterations at a wide variety of loci. It is also striking that when the effects of all known sex-specific lethals in *D. melanogaster* are examined (Table 7), a substantial majority of these mutants are male specific. If this is not due to limited or biased sampling, it would suggest that most essential sex-specific functions act during male development.

In these studies, we have attempted to delimit the cause of male-specific lethality in these mutants, with the aim of deducing the nature of the functions of the wild-type alleles of these loci. The first possibility addressed was that male-specific lethality may be based on the interaction of a mutant with some aspect of male sexual differentiation, physiology or anatomy. We have attempted to test this possibility by introducing sex-transforming genes into the genotypes of individuals homozygous for *msl* alleles. The absence of interaction between *tra-2* or *dsx* and *msl-1* or *msl-2* suggests that the sex-specific lethality of these mutants is not related to sexual differentiation *per se*. These results are similar to those of FUKUNAGA, TANAKA and OISHI (1975), which showed no interaction between *mle* and *dsx* or *tra-3*. Although sex-transforming genes have been shown to be active early in development—by the first instar for *tra-3* (SEIDEL 1963) and by the first 48 hr of development for *dsx* (COLAIANNE and BELL 1972)—it is possible, of course, that *msl-1* and *msl-2* produce their fundamental lethal actions before *dsx* and *tra-2* act to alter the sexual phenotype.

A second difference between males and females that could provide the basis for sex-specific lethality is the presence of a *Y* chromosome in males but not in

TABLE 7

Sex-specific lethal mutations of Drosophila Melanogaster

Mutant	Locus	Comments	Major references
Sex-lethal^{F1} (Sxl^{Fl}); also called Female-lethal (*Fl*)	*1*–19.1 (6E1–7B7)	Viability of *Fl*/+ females varies from 0 to normal depending on maternal genotype; *Fl/Fl* females die as early embryo; *Fl/Y* males have normal viability and phenotype; no interaction with *tra*.	CLINE (1978,1979)
Sex-lethal^{M1} (Sxl^{Ml}); also called Suppressor of daughterless [*Su(da)*]	*1*–19.1 (6E1–7B7)	Male lethal; suppressor of *da* female-killing effect; allelic to *Fl*; no effect in females; appears to cause sex transformation in Sxl^{Ml}/O clones.	CLINE (1978,1979)
sonless (*snl*)	*1*–56.1	*snl*/Y sons from *snl/snl* mothers die in embryo stage (< 48 hr.); exceptional *snl*+/*O* sons from *snl/snl* × +/Y survive; shows interaction with *tra* and *dsx*.	COLAIANNE and BELL (1972)
$T(1;2)X9^{ts}$	Breakpoints at 2D1–2 and 56A1–2.	At 29° translocation is male lethal but female viable; males rescued by w^+Y; male-lethal effect maps to tip of X, between *y* and *cv*; *tra* has no effect.	KAUFMAN and SUZUKI (1974)
Drescher's mutant	*1*–?	Homozygous females die; no effect in males; from natural population; not characterized.	DRESCHER (1964)
Mex. 156	*1*–?	(Same as previous mutant)	GALLO and SALCEDA (1974)
male-specific lethal-2 (*msl-2*)	*2*–9.0	Homozygous males die during larval stages; females not affected; no interaction with *dsx* or *tra-2*; no interaction with *Y* chromosome; EMS induced.	BELOTE and LUCCHESI (this report)

TABLE 7—Continued

Mutant	Locus	Comments	Major references
killer of male(B) [*km(B)*]	2–~20	Homozygous males die in embryo stage; EMS-induced; not well characterized.	PIERRE (1972)
lethal(2) maternal [*l(2)mat*]	2–~25	Maternal-effect mutant; homozygous females produce 5 males for every female.	REDFIELD (1926)
abnormal oocyte (*abo*) hold-up (*hup*) *da-abo*-like (*dal*) wavoid-like (*wal*)	2–~38 (31F–32EF)	Cluster of maternal-effect lethals that show interactions with sex-chromosome heterochromatin; may have sex-differential lethal effects; (also includes *da*).	SANDLER (1977)
daughterless (*da*)	2–41.5 (31CD–F)	At 25° no female survivors among progeny of *da/da* mothers; die as embryo or early larva; no interaction with *tra* or *dsx*; at 18° some daughters are recovered.	BELL (1954) CLINE (1976)
killer of male (A) [*km(A)*]	2–~45	Homozygous males die in embryo stage; EMS-induced; not well characterized.	PIERRE (1972)
male-specific lethal-1 (*msl-1*)	2–53.3 (36F7–37B8)	Homozygous males die during the late larval stages; females not affected; no interaction with *dsx* or *tra-2*; no interaction with *Y* chromosome; EMS induced.	BELOTE and LUCCHESI (this report)
male-specific lethal-1^b (*msl-1^b*)	2–53.3 (36F7–37B8)	Allelic to *msl-1*; similar phenotype; EMS-induced.	BELOTE and LUCCHESI (this report)
male killer (*mak*), an allele of *mle*	2–~54	*mak/mak* males die; *mak/mak* females sterile; not well characterized. *mak/mle* is male-lethal (M. ASHBURNER, personal communication)	GOLUBOVSKY and IVANOV (1972)

TABLE 7—Continued

Mutant	Locus	Comments	Major references
Co 122	2–55	Phenotype similar to *mle*; from natural population.	Loverre and Cicchetti (1977)
maleless (*mle*)	2–55.2	*mle/mle* males die in the late larval stage or pupal stage; females not affected; does not interact with *tra* or *dsx*.	Fukunaga, Tanaka and Oishi (1975) Tanaka, Fukunaga and Oishi (1976)
maleless^{ts} (mle^{ts})	2–56.8 (41A–43A)	Allelic to *mle*; phenotype similar to *mle* at 25° or higher; at 18° some homozygous males survive although they are sterile, small and slow developing. EMS-induced.	Belote and Lucchesi (this report)
son killer (*sok*)	Two loci on chromosome 2; one near *b*, the other on 2R.	Females homozygous for *sok* produce almost no males; no influence of *Y*; no interaction with sex-transforming mutants.	Th. Skripsky (personal communication)
male sex (*ms*)	2–?	Female-lethal or semi-lethal; males are viable and fertile; evidence of maternal effect.	Gowen (1949)
Nelson's mutant (*Ne*)	3–~31	Female progeny from mothers carrying *Ne* die in embryo stage; mutant no longer exists.	Gowen (1961)

females. This possibility was effectively ruled out by constructing *msl* individuals of both sexes with zero, one or two *Y* chromosomes and noting no effect of these permutations on the male-specific lethal phene. FUKUNAGA, TANAKA and OISHI (1975) reported that the presence of a *Y* chromosome in homozygous *mle* females does not affect their viability. Although the effect of the *Y* chromosome on mle^{ts} was not specifically measured in our experiments, we have noticed no effect on viability in mle^{ts} females that carry an attached-*X* chromosome with a free *Y*.

A third possible basis for male-specific lethality could be the difference in the number of *X* chromosomes between the two sexes. This could be due to a difference in dosage of a specific *X*-linked gene or the number of *X* chromosomes as a whole. The former possibility, *i.e.*, interaction with a single gene or gene cluster, has been effectively ruled out by methodically duplicating the *X* chromosome in males, one small segment at a time, and noting that such duplications had no sparing effect on lethality. Mention must be made that the small region defined by the *X* breakpoints of the $Dp(1;3)w^{m49a}$ and *T(X;Y)B29* chromosomes (3E2–3 to 3E7–8) was not duplicated in these experiments. Also, region 11D–12E was not tested since even control males are inviable in the presence of this duplication. Our results do not rule out the possibility that two or a few loci, sufficiently distant from one another that they are not included within any single duplication tested must be present simultaneously in two doses for *msl* males to survive. For operational considerations, we consider this possibility to be equivalent to the statement that male-specific lethality depends upon the relative number of *X* chromosomes present in the genome.

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Corresponding editor: A. CHOVNICK