LETHALS, STERILES AND DEFICIENCIES IN A REGION OF THE *X* CHROMOSOME OF *CAENORHABDITIS ELEGANS*

PHILIP M. MENEELY AND ROBERT K. HERMAN

Department of Genetics and Cell Biology, University of Minnesota, St. Paul, Minnesota 55108

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ABSTRACT

Twenty-one *X*-linked recessive lethal and sterile mutations balanced by an unlinked *X*-chromosome duplication have been identified following EMS treatment of the small nematode, *Caenorhabditis elegans*. The mutations have been assigned by complementation analysis to 14 genes, four of which have more than one mutant allele. Four mutants, all alleles, are temperature-sensitive embryonic lethals. Twelve mutants, in ten genes, are early larval lethals. Two mutants are late larval lethals, and the expression of one of these is influenced by the number of *X* chromosomes in the genotype. Two mutants are maternal-effect lethals; for both, oocytes made by mutant hermaphrodites are rescuable by wild-type sperm. One of the maternal-effect lethals and two larval lethals are allelic. One mutant makes defective sperm. The lethals and steriles have been mapped by recombination and by complementation testing against 19 deficiencies identified after X-ray treatment. The deficiencies divide the region, about 15% of the *X*-chromosome linkage map, into at least nine segments. The deficiencies have also been used to check the phenotypes of hemizygous lethal and sterile hermaphrodites.

LETHAL and sterile mutations have long been recognized as potentially important tools in trying to understand the genetic basis of eukaryotic development (HADORN 1961). The obvious strategy is to try to infer the role of a gene essential for normal development from an analysis of the differences between mutant and wild-type organisms. Lethals and steriles have been identified and characterized in a wide variety of animals, including vertebrates like chickens and mice, but their most fruitful use has been in Drosophila (for a recent review, see GEHRING 1976). The nematode *Caenorhabditis elegans* may also prove to be particularly suitable for the genetic analysis of development. Its self-fertilizing, hermaphroditic mode of reproduction is clearly useful in the identification of recessive mutations. This and other virtues for genetic manipulation have been cited and demonstrated by BRENNER (1974), who also estimated that the organism has only 2,000 genes with indispensable functions. Furthermore, development is precise, giving rise to a countable set of cells of strictly specified fates: about 810 nongonadal and 2,500 gonadal nuclei in the mature hermaphrodite (SULSTON and HORVITZ 1977; HIRSH, OPPENHEIM and KLASS 1976). Many of the embryonic (DEPPE *et al.* 1978) and virtually all of the postembryonic somatic

cell lineages (SULSTON and HORVITZ 1977; J. KIMBLE, personal communication) that generate these cells have been followed in living individuals.

Most of the lethals and steriles studied so far in *C. elegans* have been temperature sensitive (HIRSH and VANDERSLICE 1976; HIRSH *et al.* 1977; WARD and MIWA 1978). Temperature-sensitive mutations have the advantage of allowing one to assay, by temperature-shift experiments, the times of action of the mutant product in the developmental sequence, assuming temperature sensitivity is due to a temperature-sensitive product (SUZUKI 1970; HIRSH and VANDERSLICE 1976). They are also easily maintained in homozygotes at permissive temperature. An advantage of unconditional lethals and steriles, apart from their relative ease of induction, is that they are more likely to provide null alleles, which should be useful in the detection of missing gene products in defective embryos or animals. Chromosome rearrangements are available to facilitate the maintenance of unconditional lethal and sterile mutations in heterozygous stocks (HERMAN, ALBERTSON and BRENNER 1976; HERMAN 1978). We have used a duplication of part of the *X* chromosome as a balancer in the handling of 21 EMS-induced *X*-linked lethals and steriles that define 14 new genes. In addition, 19 stocks deficient for parts of the duplicated region have been identified following X irradiation and have been used in the mapping and characterization of the lethals and steriles. The deficiencies should expedite characterization of lethals induced in this region in the future.

MATERIALS AND METHODS

Strains and general procedures: *C. elegans* var. Bristol (wild type designated N2) and many of the mutants were obtained from S. BRENNER or were derived from his stocks. MN-H1 has the genotype *mnDp1*/+; *unc-3(e151) X*. Its derivation and properties were described previously (HERMAN, ALBERTSON and BRENNER 1976). The designation *mnDp1* (or *mnDp1(X;V)*) for the duplication is a change from the previous name *Dp(X;V)1*, in keeping with a nomenclature system recently agreed upon by many *C. elegans* workers (HORVITZ, personal communication). The symbols Dpy, Unc, and Unc-3 are examples of phenotypic abbreviations. The duplication carries *unc-3*+ *X* and the closely linked *unc-7*+ and is attached to linkage group V (LGV). It greatly suppresses crossing over in the left half of LGV, and no recombination between the duplication and the *X* chromosome has been observed. When MN-H1 is self-fertilized, three kinds of progeny are produced: worms lacking the duplication and hence uncoordinated; worms homozygous for the duplication, normally coordinated, but slow-growing and with an undeveloped gonad, hence sterile; and wild-type fertile worms, which have the same genotype as the parent and are therefore used to maintain the stock.

Other genes and alleles used were: *dpy-5(e61) I*, *dpy-11(e224) V*, *dpy-7(e88) X*, *unc-7 (e139) X* (all described by BRENNER 1974), *tra-2(e1094) II*, *dpy-10(e128) II*, *tra-1(e1099) III*, and *dpy-18(e1096)* III (HODGKIN and BRENNER 1977). J. KIMBLE and A. FODOR supplied us with strains bearing mutations *b246*ts (HIRSH and VANDERSLICE 1976) and *e1470*, respectively.

Media and culture techniques were as described by BRENNER (1974). Mating and mapping procedures were as described by BRENNER (1974) and HERMAN (1978). Incubations were done at 20° except where noted.

Generation, identification, and complementation of X-linked recessive lethal and sterile mutations balanced by mnDp1: The aim of this procedure was to produce mutations in the essential genes in the region of the *X* chromosome covered by *mnDp1*. Specifically, we were looking for mutants with the following genotype: *mnDp1*/+; *unc-3 let*, where *mnDp1* carries *unc-3*+ *let*+, and *let* symbolizes a recessive lethal or sterile mutation. The selection scheme is shown

EMS treat: *mnDp1* V/+; *unc-3* X

Among wild-type F_1: *mnDp1*/+; *unc-3 let-1*/*unc-3 let-1*+

Among wild-type F_2: *mnDp1*/+; *unc-3 let-1*

F_3	*mnDp1*/*mnDp1*; *unc-3 let-1*	Sterile, non-Unc
	mnDp1/+; *unc-3 let-1*	Wild-type
	+/+; *unc-3 let-1*	Inviable or sterile Unc

FIGURE 1.—Selection scheme for isolating EMS-induced mutants carrying recessive lethals or steriles balanced by *mnDp1(X;V)*. Reproduction is by self-fertilization.

in Figure 1. Young adult MN-H1 hermaphrodites were treated with ethyl methanesulfonate (EMS) by a procedure previously described (HERMAN 1978) and then placed individually on small *E. coli*-seeded petri plates and allowed to self-fertilize. Eight to ten wild-type progeny of each mutagenized animal were separately cultured, and two or three F_2 animals from each F_1 were picked. Each F_2 animal was allowed to self-fertilize, and six to nine Unc progeny of each, if present, were picked and cultured together. If the Unc progeny of the F_2 were absent, inviable, or sterile, a wild-type sibling was chosen to maintain the stock.

Not every F_2 worm that segregated inviable or sterile Unc progeny carried an *X*-linked lethal. Two classes of autosomal mutants were also isolated by this scheme. One class included lethals and steriles on LGV balanced by the *mnDp1*-bearing chromosome. These have the following genotype: *mnDp1/let*; *unc-3*. The other class consisted of mutations lethal only in an *unc-3*, and not in an *unc-3*+, background. The procedure for identifying the *X*-linked lethals and steriles is given in Figure 2. A wild-type stock carrying *mnDp1* and a *let* was crossed to N2 males. The absence of viable Unc male offspring from this cross indicated the presence of an *X*-linked *let* in the hermaphrodite parent. On the other hand, the presence of Unc males did not necessarily disprove the existence of an *X*-linked *let* since the mutation could act late in development or it could act only on hermaphrodites; therefore, a second cross was performed. Wild-type males issuing from the first cross were backcrossed to the wild-type stock carrying *mnDp1* and the *let*. The production of wild-type male progeny signaled a successful mating.

(A) *mnDp1*/+; *unc-3 let-1* ♀ × +; +/O ♂

♂ progeny { +/+; *unc-3 let-1*/O — Unc, inviable?
mnDp1/+; *unc-3 let-1*/O — Wild-type

(B) *mnDp1*/+; *unc-3 let-1* ♀ × *mnDp1*/+; *unc-3 let-1*/O ♂

Unc ♀ progeny: +/+; *unc-3 let-1*

(C) *mnDp1*/+; *unc-3 let-2* ♀ × *mnDp1*/+; *unc-3 let-1*/O ♂

Among Unc ♀ progeny: +/+; *unc-3 let-2*/*unc-3 let-1*

FIGURE 2.—Establishing *X*-linkage of lethals and steriles and complementation testing. Wild-type males produced in (A) were backcrossed in (B) to establish *X*-linkage—all Unc hermaphrodite progeny show the mutant phenotype—and crossed to a different mutant stock in (C) to test for complementation.

If the *let* was *X*-linked, there were no fertile Unc hermaphrodite progeny, but if the *let* was autosomal, some of the Unc progeny were viable and fertile. Only stocks carrying *X*-linked *let* mutations have been retained. Healthy looking wild-type hermaphrodite progeny in the second cross were picked to establish new stocks of each mutant. We could not be sure that these animals were produced by cross-fertilization, since wild-type self-progeny were probably also present, but this procedure sometimes resulted in a more vigorous line, perhaps as a result of eliminating secondary detrimental mutations. Independent *let* mutations were assigned sequential mutation names beginning with *mn101*.

Complementation testing between pairs of *X*-linked mutants was procedurally similar to establishing *X*-linkage, and the method used is shown in Figure 2c.

Recovery of X-ray-induced deficiencies balanced by mnDp1. The aim of this procedure was to generate *X*-chromosome deficiencies of varying extents, each extending into the *unc-3* gene and at least one neighboring essential gene, balanced by *mnDp1*. A population containing many males of about the same age was made as follows. Twenty N2 males and ten to 12 young adult hermaphrodites were put on a small plate for six to eight hr to allow the hermaphrodites to lay any eggs fertilized before exposure to the males. All worms were then transferred to a new plate, allowed to mate and lay eggs overnight, and then removed. The eggs and young worms on this plate were incubated for about 48 hr before being X irradiated at a dose of 7000 to 7500 r. Six to eight hr after irradiation, young adult males were placed on small plates with hermaphrodites homozygous for both *unc-3* and a *dpy* marker—three males and four to seven Dpy Unc hermaphrodites per plate. Two schemes that were used to recover deficiencies are shown in Figure 3. They differ in the deployment of the *dpy* marker. In the first method, the *dpy* (either of two autosomal *dpy* mutants, *dpy-5 I* or *dpy-11 V*, was used in different runs) serves only to mark the self-progeny, whereas in the second the *X*-linked *dpy-7* marks both the self-progeny and the unirradiated *X* chromosome. In each method, both males and hermaphrodites were transferred

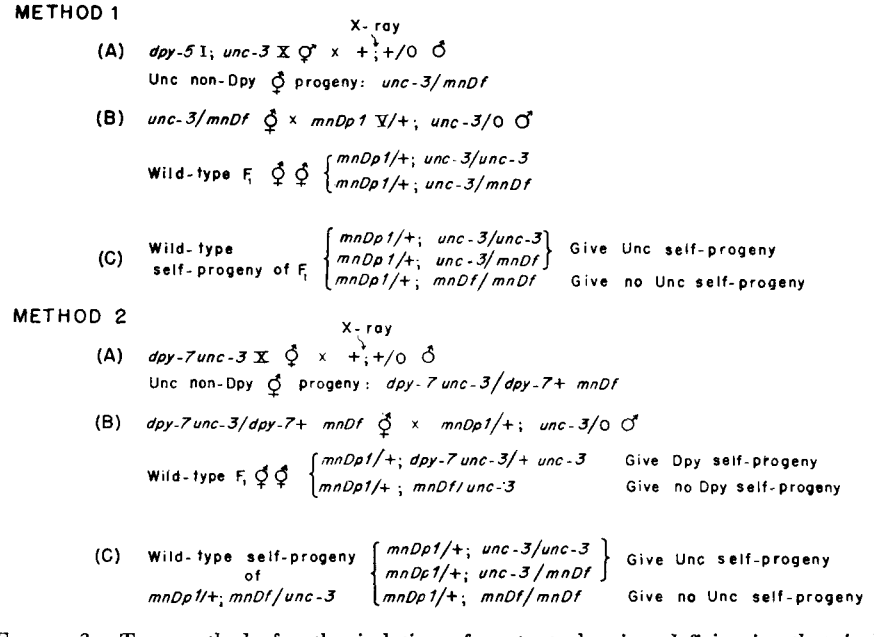

FIGURE 3.—Two methods for the isolation of mutants bearing deficiencies that include *unc-3* and at least one vital locus and are balanced by *mnDp1*. The balancer is introduced at step (B) in both methods. In method 2, recombination between *dpy-7* and *mnDf* is not illustrated.

to new plates after 24 hr, again after 48 hr, and then discarded after 72 hr. Each plate was scored for Unc-3 non-Dpy progeny, which presumably carried a mutation in the *unc-3* gene, called *mnDf* in Figure 3, transmitted by the sperm. Wild-type hermaphrodites were also counted, so that the frequency of recovery of deficiencies could be estimated. Each Unc non-Dpy animal picked was mated with six to eight *mnDp1*-containing males to balance the deficiency, if present. Eight to ten wild-type hermaphrodite progeny from each mating were plated separately. Half of these were expected to carry the newly-derived *unc-3* mutation. In the first method, six to eight wild-type hermaphrodite progeny from each plate were individually cultured. In the second method, six to eight wild-type hermaphrodite progeny were picked only from those plates that had no Dpy (Unc) animals. This tended to restrict consideration to those broods that carried the newly derived *unc-3* mutation—not exclusively so since *dpy-7* is about 17 map units away from *unc-3*—and therefore reduced the number of animals that had to be picked and cultured. The final step in each method was to select wild-type hermaphrodites that gave no Unc progeny. We inferred that these animals had the following genotype: *mnDp1*/+; *mnDf*/*mnDf*, where *mnDf* extends into *unc-3*, as well as into one or more essential genes, whose wild-type alleles are carried by *mnDp1*.

The presumed inability of each *mnDf* to complement *unc-3(e151)* was checked as follows. Each deficiency stock was mated with N2 males, and the wild-type male progeny (*mnDp1*/+; *mnDf*/0) were crossed with *dpy-11 V*; *unc-3 X* hermaphrodites. As expected, Unc non-Dpy progeny were produced. Complementation tests against *unc-7* were conducted in analogous fashion; that is, *dpy-11 V*; *unc-7 X* hermaphrodites were used in the last cross. Deficiencies of independent origin were numbered serially, starting with *mnDf1*. They are characterized further in the RESULTS.

Recombination mapping: Linkage map distances from *unc-3* to various embryonic and early larval lethals were obtained by first crossing *mnDp1*/+; *unc-3 let*/0 by *unc-7* hermaphrodites and picking wild-type hermaphrodite progeny. These animals were allowed to self-fertilize, and their adult progeny, including Unc-3 recombinants, were counted. The phenotype of homozygous *unc-3* animals is much more severe than, and easily distinguished from, that of *unc-7*. A difficulty in the analysis is that half of the *unc-3 let*/*unc-7* parents are expected to carry *mnDp1* and half are not. When no *mnDp1 is* present, the frequency of Unc-3 recombinants among total surviving progeny, R, is $(2p/3)(1-p/2)$, where p is the frequency of recombination between *unc-3* and *let*. This takes into account the fact that one-fourth of the zygotes do not reach adulthood. When the parental animal carries *mnDp1*, $R = (2p/15)(1-p/2)$. In this case only 1/16 of the zygotes do not reach adulthood. Each class of parents gave about the same number of adult progeny: the presence of *mnDp1* reduced the number of lethal zygotes and also reduced the overall fertility (HERMAN, ALBERTSON and BRENNER 1976). Hence we estimated R by taking the average of the two foregoing expressions: $R = (2p/5)(1-p/2)$, which gives $p = 1-\sqrt{1-5R}$ or $p = 5R/2$ for small R. Between 3,000 and 6,000 progeny were counted for each allele mapped. Incubations for all mapping experiments except one were at 20°: *let-8* was mapped at 25°, where it behaves as a larval lethal rather than as a sterile. Two other steriles, *let-5* and *let-9*, were not mapped by recombination, but were mapped by complementation testing against deficiencies because of the difficulty in distinguishing recombinant fertile Unc progeny from nonrecombinant sterile Unc animals.

The *unc-7* marker in *unc-3 let*/*unc-7* hermaphrodites was used for three-factor ordering of *let* mutants, as shown in Figure 4: Unc-3 recombinants were checked for the presence of the *unc-7* marker. Since the phenotype of *unc-7* cannot be recognized easily in an *unc-3* background, the Unc-3 recombinants were mated with *mnDp1*/+; *unc-7*/0 males and the progeny checked for the presence of Unc-7 hermaphrodites.

Deficiency mapping: Each EMS-induced *let* mutation was assigned to a segment of the *X*-linkage map by complementation tests against a number of deficiencies; thus, the viability or fertility of *mnDf*/*unc-3 let* (Unc) hermaphrodites was checked. Except for small deficiencies, the *mnDf*-bearing chromosome was introduced *via* sperm, since the recovery of large deficiencies through the oocyte line was less efficient than expected (see RESULTS).

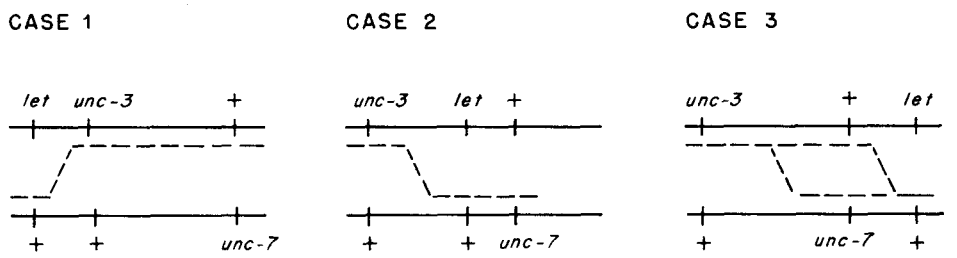

FIGURE 4.—Ordering a lethal mutation with respect to *unc-3* and *unc-7*. Viable Unc-3 recombinants are picked and checked for the possession of *unc-7*. The recombinant chromosome is symbolized by the dashed line. The expected genotypes of the recombinant animals are *let*+ *unc-3*/ *let unc-3* in case 1, *unc-3 let*+ *unc-7*/*unc-3 let unc-7*+ in case 2, and both of the foregoing genotypes in case 3.

Characterizing phenotypes: Gross morphological characterization was done with a PZO (Warsaw) dissecting stereomicroscope or with a Zeiss Universal microscope equipped with Nomarski optics. Preparation of worms for Nomarski microscopy was either by the agar-slab method of SULSTON (1976) or by suspending the worms in 0.5% 1-phenoxyl-2-propanol as an anaesthetic in *C. elegans* Ringer's solution (HIRSH, OPPENHEIM and KLASS 1976).

RESULTS

Lethals and steriles

The scheme for the generation of *X*-linked recessive lethal and sterile mutations, called *let*, balanced by *mnDp1(X;V)* is described in MATERIALS AND METHODS and illustrated in Figure 1. It consisted of treating MN-H1 with EMS and then checking the viability and fertility of the Unc progeny of wild-type F_2 hermaphrodites. Since *mnDp1* carries *unc-3*+ and *let*+, an *mnDp1*/+; *unc-3 let* animal is wild-type, but it segregates inviable or infertile Unc progeny upon self-fertilization. The heterozygous *mnDp1* stock is easily maintained because the *mnDp1* homozygote is sterile. From 310 mutagenized worms, about 4,600 fertile F_2 broods were examined. Of these, 176 did not segregate fertile Unc progeny and were backcrossed by the procedure shown in Figure 2 to establish *X* linkage. Twenty-one were found to be *X* linked, a recovery frequency of about 0.5%.

All 210 possible pairwise combinations of the 21 *X*-linked mutants were tested by complementation, by the procedure shown in Figure 2, with the result that the mutants defined 14 essential genes, as shown in Table 1. Two lethals sent to us by other labs, *e1470* and *b246*ts, were also tested by complementation and found to fall into *let-2* (Table 1).

Map distances from *unc-3* to 19 of the *X*-linked *let* mutations were measured and are shown in Figure 5. Three-factor crosses utilizing *unc-7* showed that *let-4* is located to the left of *unc-3*, that *let-1*, *let-7*, and *let-12* are to the right of *unc-3*, and that *let-2*, *let-3*, *let-6*, *let-8*, *let-10*, *let-11*, *let-14*, and *let-16* are all to the right of *unc-7*.

TABLE 1

X-linked lethals and steriles covered by mnDp1

Gene	Allele	Phenotype
let-1	*mn102*	late larval lethal
	mn115	sterile
	mn119	early larval lethal
let-2	*mn101*	ts embryonic lethal
	mn103	ts embryonic lethal
	mn109	ts embryonic lethal
	mn111	ts embryonic lethal
	b246	ts embryonic lethal
	e1470	ts embryonic lethal
let-3	*mn104*	early larval lethal
let-4	*mn105*	early larval lethal
let-5	*mn106*	progeny inviable
let-6	*mn108*	early larval lethal
	mn110	early larval ltehal
let-7	*mn112*	late larval lethal
let-8	*mn114*	ts early larval lethal
let-9	*mn107*	sterile; sperm defect
let-10	*mn113*	early larval lethal
	mn118	early larval lethal
let-11	*mn116*	early larval lethal
let-12	*mn121*	early larval lethal
let-14	*mn120*	early larval lethal
let-16	*mn117*	early larval lethal

Deficiencies

Before continuing our search for more lethals and steriles in this region, we decided that it would be wise to identify a series of *X*-linked deficiencies balanced by *mnDp1*. The lethals and steriles already identified could be used to help characterize the deficiencies, which could in turn be used to help classify new lethals and steriles into complementation groups and also to corroborate and extend the mapping analysis. The methods used to recover deficiencies that included *unc-3* and at least one essential gene in the region balanced by *mnDp1* are described in MATERIALS AND METHODS and are illustrated in Figure 3. About 135,000 hermaphrodite progeny of X-irradiated N2 males were screened, and 285 Unc hermaphrodites known to have been produced by independent events were recovered. Of these, 176 were fertile and were tested further. Forty-three of these appeared to be deficiencies of the sort desired, but of these, 13 grew very slowly and were discarded.

Each of the remaining 30 deficiencies was tested by complementation against a number of the lethals and steriles. Thirteen of the 30 deficiencies failed to complement any lethal or sterile, and all but two of these were discarded. Each of the other 17 complemented one or more of the lethals. The results of these tests

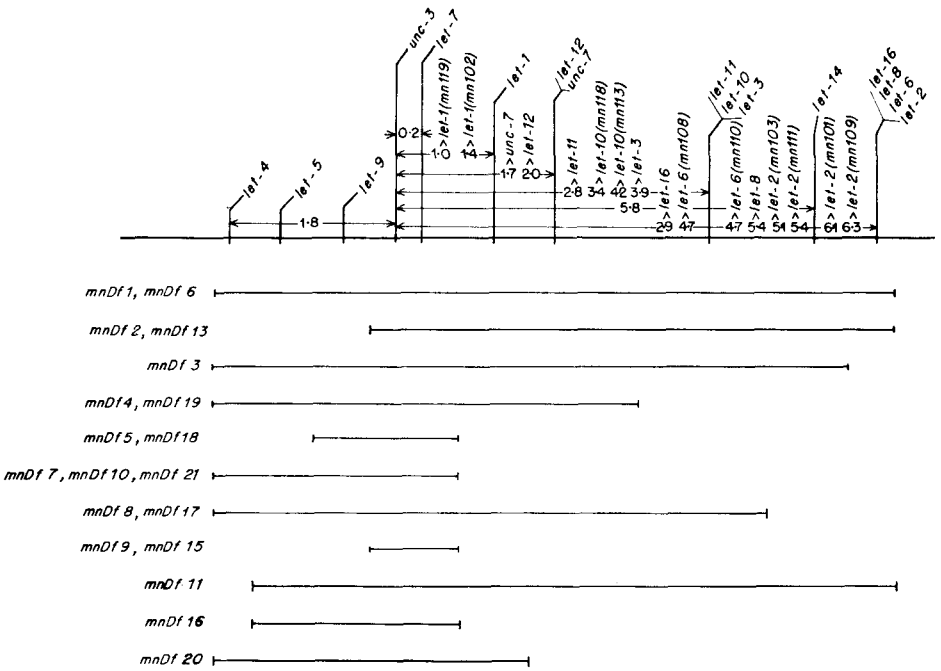

FIGURE 5.—Recombination and deficiency map of the region of the *X* chromosome covered by *mnDp1*, showing the positions of the 14 *let* genes and 19 deficiencies characterized in this work. The gene positions represented by the vertical lines are based on the results of deficiency mapping and three-factor ordering (involving *unc-3*, *unc-7*, and the *let* mutations). The data given in the horizontal arrows are percent recombination between various *let* mutations and *unc-3*.

are given in Table 2. The results allowed us to assign unambiguously each of the 14 *let* genes to one of nine contiguous segments of the region, and these segments at the same time defined the extents of the deficiencies. The resulting deficiency map agreed well with the map based on recombination distance and three-factor ordering of *let* genes (see Figure 5). The only discrepancy involves *let-16*: its map distance from *unc-3* was shorter than expected. Two genes, *let-5* and *let-9*, were mapped solely on the basis of complementation tests against deficiencies.

We observed that *unc-3 let-2/mnDf3* and *unc-3 let-6/mnDf8* and other combinations involving a large deficiency and a complementing lethal grew more slowly than the corresponding *unc-3/mnDf* stock. This effect was not seen for smaller deficiencies.

Phenotypes of lethals and steriles

The time of developmental arrest for each mutant is listed in Table 1. The early larval lethals ceased development at about the first larval (or juvenile) stage, called L1. For each gene that displayed an early larval phenotype, at least one allele was put opposite a deficiency. All such hemizygotes were found to be

TABLE 2

*Complementation tests between deficiencies and EMS-induced mutations**

Deficiencies	X-linked loci															
	let-4	*let-5*	*let-9*	*unc-3*	*let-7*	*let-1*	*unc-7*	*let-12*	*let-3*	*let-10*	*let-11*	*let-14*	*let-2*	*let-6*	*let-8*	*let-16*
mnDf1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
mnDf2	+	+	+	0		0	0	0	0	0	0	0	0	0	0	0
mnDf3	0	0	0	0				0	0	0	0	0	+	+	+	+
mnDf4	0		0	0		0		0	+	+	+	+	+	+	+	+
mnDf5	+	+	0	0	0	+	+		+	+	+		+		+	+
mnDf6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
mnDf7	0	0		0	0	+	+	+		+	+					+
mnDf8	0	0		0				0	0	0	0	+	+	+	+	+
mnDf9	+	+	+	0	0	+	+	+			+		+			
mnDf10	0	0		0	0	+	+	+	+			+	+	+		
mnDf11	+	0		0		0						0	0			
mnDf13	+	+	+	0		0			0		0		0	0		
mnDf15	+	+	+	0	0	+		+	+	+	+	+	+			
mnDf16	+	0		0	0	+	+	+	+	+		+	+	+		
mnDf17	0			0			0	0	0	0	0	+	+	+		
mnDf18	+	+	0	0	0	+		+	+	+		+	+	+		
mnDf19	0			0				0	+	+	+	+	+	+		+
mnDf20	0	0		0		0	+	+	+	+	+	+	+	+	+	
mnDf21	0	0		0	0	+		+		+	+		+	+		

* Complementation is signified by + and noncomplementation is signified by 0. No symbol means the test was not performed.

arrested at virtually the same stage as the homozygotes; that is, to take a specific example, *unc-3 let-12/mnDf3* was arrested at about the same stage as *unc-3 let-12/unc-3 let-12*. These results suggest that these alleles are amorphic or null. Except for *let-8* (see below), the early larval mutants have not been further characterized.

let-1: The three alleles of *let-1* showed distinct phenotypes, ranging from early larval lethal (*mn119*) to sterile (*mn115*). When *mn115* was put opposite *mn119* or *mn102* or was even made hemizygous opposite *mnDf20*, the resulting phenotypes were all the same: sterile adult. The heterozygote *mn119/mn102* and the hemizygotes *mn119/mnDf20* and *mn102/mnDf20* were all early larval lethals. When *unc-3 let-1* (*mn115*) hermaphrodites were mated with N2 or *mnDpl*/+; *unc-3 let-1* (*mn115*)/0 males, wild-type, but no Unc, progeny resulted. This indicates that oocytes produced by *mn115* hermaphrodites are rescuable by *let-1*$^+$ function during embryogenesis. In the absence of males, *unc-3 let-1* (*mn115*) hermaphrodites laid eggs that did not hatch. The eggs had the hard shell that characterizes zygotes and not unfertilized oocytes (HIRSH and VANDERSLICE 1976).

let-2: All six alleles of *let-2* are temperature sensitive, although only *b246* was isolated as such (HIRSH and VANDERSLICE 1976). At the restrictive temperature, 25° for *b246* and 20° for the others, all six mutants were embryonic lethals, since roughly one-fourth of the fertilized eggs laid by *mnDp*/+; *unc-3 let-2* herma-

phrodites did not hatch and no Unc animals appeared. At the permissive temperature, 20° for *b246* and 15° for the others, the Unc animals hatched, grew and reproduced, though some rather poorly. Matings for complementation between alleles were done at 20°, and the progeny were either kept at 20° or shifted to 25° shortly after hatching. In the former cases the complementation pattern for the six alleles was complex, with each allele complementing at least one other (Table 3).

let-5: The only allele of *let-5*, *mn106*, is a maternal-effect lethal: the Unc self-progeny of *mnDp1*/+; *unc-3 let-5* hermaphrodites were fertile, but their progeny grew very slowly and did not reach adulthood. The F_1 Unc animals showed no obvious abnormalities when examined by Nomarski microscopy. When they were mated with N2 males, wild-type hermaphrodites, but no Unc male adult progeny, were found; that is, the oocytes produced by homozygous mutants were rescued by *let-5*+-bearing sperm, but not by nullo-*X* wild-type sperm.

The phenotype of hemizygous F_1 Unc hermaphrodites, *unc-3 let-5/mnDf*, varied with the size of the deficiency used. For the largest deficiencies, *mnDf1* for example, hemizygotes did not survive to adulthood. The hemizygote for a smaller deficiency generally survived better; for example, *unc-3 let-5/mnDf7* had progeny that died at about the same stage as the progeny of *unc-3 let-5*. The intermediate case is illustrated by *mnDf3* and *mnDf8* in which the hemizygote was sterile.

let-7: Homozygotes for *let-7* die as early third-stage larvae. Hemizygous males, produced either by cross-fertilization or spontaneously (by nondisjunction in the heterozygous hermaphrodite parent), developed considerably farther: to late fourth-stage larvae. Nomarski microscopy showed that the male gonad was reasonably normal, with numerous sperm, but that the bursa was not fully developed (KLASS, WOLF and HIRSH 1976).

The possibility that *mn112* is a hypermorphic allele of *let-7*—that is, that *mn112* homozygotes die sooner than the hemizygotes because they have two

TABLE 3

Complementation tests between different let-2 *alleles*

	Post-hatching incubation temperature	*b246*	*e1470*	*mn111*	*mn109*	*mn103*
mn101	20°	fertile	fertile	embryonic lethal	slightly fertile	fertile
	25°	sterile	larval lethal	embryonic lethal	sterile	sterile
mn103	20°	fertile	fertile	larval lethal	larval lethal	
	25°	sterile	sterile	larval lethal	larval lethal	
mn109	20°	fertile	slightly fertile	fertile		
	25°	sterile	sterile	sterile		
mn111	20°	fertile	larval lethal			
	25°	sterile	larval lethal			
e1470	20°	fertile				
	25°	sterile				

mutant copies rather than one—was tested by making the hemizygous hermaphrodite *unc-3 let-7/mnDf5*. (Other deficiencies were used, with the same result.) This animal died at about the same stage as, or possibly earlier than, the *unc-3 let-7* homozygote. We cannot exclude the possibility that the deficiencies themselves led to an early death, but since the results were clear even with the small deficiency *mnDf5*, it seems likely that the early arrest of hermaphrodites was not due to having an extra *let-7* gene.

We next checked to see if the longer survival of *let-7* males was sex specific by examining the epistatic interactions between *let-7* and the transformer mutants *tra-1* and *tra-2*. These are autosomal recessives that transform 2*X* worms into males or pseudomales (KLASS, WOLF and HIRSH 1976; HODGKIN and BRENNER 1977). The *tra-1* males can mate successfully, but *tra-2* males are infertile and have abnormal bursae. The experiments were done as follows: *mnDp1/+*; *unc-3 let-7/0* males were mated with *tra-1/dpy-18 III* and with *tra-2/dpy-10 II*, using a high ratio of males to hermaphrodites to insure efficient cross-fertilization. Wild-type hermaphrodite progeny were picked and cultured singly. Those that gave no Dpy progeny were inferred to have the following genotype, ignoring *mnDp1* since its presence or absence is irrelevant: *tra/+*; *unc-3 let-7/+*. These animals segregated wild-type male progeny as expected, but all of the Unc progeny were arrested at about L3. This result was obtained for *tra-1* as well as for *tra-2*. When the experiments were repeated using *mnDp1/+*; *unc-3/0* males in the first crosses, the expected Unc males were found. These experiments indicate that 2*X* *let-7* animals die earlier than 1*X*, regardless of sexual phenotype.

let-8: The one *let-8* mutant is temperature sensitive. At 25° it is an early larval lethal, and *unc-3 let-8/mnDf2* animals are arrested as early larvae. At 20° (and 15°) *let-8* homozygotes become sterile adults, and their oocytes are not rescuable by wild-type sperm. Examination by Nomarski microscopy revealed a variety of gonadal abnormalities: both sperm and spermatheca were absent; although the ovary was nearly normal in shape, the oocytes were not block-shaped as in the wild type (HIRSH, OPPENHEIM and KLASS 1976), but were crammed together and had a variety of shapes, the ones farthest from the ovary being the most aberrant. The animals were not observed to discharge either fertilized or unfertilized eggs.

let-9: Homozygotes for *unc-3 let-9* were mostly sterile as self-fertilizing hermaphrodites: they laid many unfertilized eggs and occasionally gave a viable (but sterile) progeny. Their oocytes were rescuable by wild-type sperm. Moreover, when *unc-3 let-9* hermaphrodites were mated with N2 males, both wild-type hermaphrodite and Unc male progeny were produced. Furthermore, when *unc-3 let-9* hermaphrodites were mated with *mnDp1/+*; *unc-3 let-9/0* males, all four classes of cross-progeny were recovered: wild-type hermaphrodites, wild-type males, Unc males, and sterile Unc hermaphrodites. These results indicate that the *let-9*$^+$ gene is essential in hermaphrodites either for spermatogenesis or for permitting fertilization by endogenous sperm, but that it is not necessary for sperm to carry *let-9*$^+$ to be capable of fertilization. Examination of late L4 or

young adult *unc-3 let-9* hermaphrodites by Nomarski microscopy revealed numerous sperm. Indeed, *unc-3 let-9* males, which cannot copulate because they are Unc, also make large numbers of sperm.

A maternal effect on the recovery of mnDf1/unc-3 *progeny*

All of the deficiencies described in this paper were selected on the basis that *mnDf/unc-3* animals were fertile Unc-3 hermaphrodites. We found in the course of complementation experiments, however, that the recovery of *mnDf/unc-3* animals was lower than expected when the deficiency was large and derived from a mother with the genotype *mnDp1*/+; *mnDf/mnDf*. The particular example of *mnDf1* will be demonstrated.

A slight complication must first be noted: the four kinds of sperm produced by *mnDp1*/+; *unc-3*/0 animals are apparently not equally successful in producing progeny, either because they are not produced in equal numbers, owing to segregational abnormalities in males, or because some have a selective advantage over others. The first cross in Table 4 provides a measure of the relative frequencies of the four sperm genotypes. If we let q be the frequency of +; *unc-3*, r be the frequency of *mnDp1*; 0, s be the frequency of +; 0, and t be the frequency of *mnDp1*; *unc-3*, then $q{:}r{:}s{:}t$=0.26:0.30:0.20:0.24. The second cross in Table 4 is the same as the first except that the *X* chromosome of the male parent carried *mnDf1* instead of *unc-3*. The expected ratio of Unc-3 hermaphrodite progeny to wild-type male progeny is q/r=0.88. The observed ratio was 0.64, 72% of the expected value. In the third cross, *mnDf1* was carried by both *X* chromosomes of the maternal parent, and the male parent was the same as in the first cross. Segregation of *mnDp1* during oogenesis is normal (HERMAN, ALBERTSON and BRENNER 1976). We shall also assume that *mnDp1/mnDp1*; *mnDf1*/0 zygotes do not become mature males (*mnDp1/mnDp1*; *unc-3*/0 zygotes do not; unpublished experiments), although their presence would change the following calculations only slightly and the conclusions not at all. We can now reckon that the expected ratio of Unc-3 hermaphrodite progeny to wild-type male progeny in the third cross of Table 4 is $(q/2)/(r/2+s/2)$=0.53. The observed frequency was 40/266=0.15, only 28% of the expected value.

We have considered two explanations for the low recovery of *mnDf1* from ova. One was that the oocytes produced by *mnDp1*/+; *mnDf1* hermaphrodites were deficient in certain materials essential for embryogenesis—a maternal effect,

TABLE 4

Recovery of mnDf1 *from sperm and ova*

Cross	Progeny phenotypes			
	Unc-3 hermaphrodites	Wild-type males	Unc-3 males	Wild-type hermaphrodites
mnDp1/+; *unc-3*/0 × *dpy-11*; *unc-3*	254	289	188	232
mnDp1/+; *mnDf1*/0 × *dpy-11*; *unc-3*	234	368		
mnDp1/+; *unc-3*/0 × *mnDp1*/+; *mnDf1*	40	266		
mnDp1/+; *unc-3*/0 × *mnDp1*/+; *mnDf1/unc-7*	90	415		

owing to hypoploidy for the *mnDf1* region, and that the deficit was more easily overcome by *mnDp1*/+; *mnDf1*/0 zygotes than by *mnDf1/unc-3* zygotes. An alternative possibility was that the effect was not maternal, but was attributable solely to the deficiency genotype of the ovum—a haplophasic effect (HADORN 1961). We discriminated between the two interpretations by the fourth cross listed in Table 4. In this case the maternal parent carried only one copy of *mnDf1*, so that a maternal effect should have been ameliorated, whereas a haplophasic effect should have been unaffected. In the absence of a maternal effect, the expected ratio of Unc-3 hermaphrodites to wild-type males in the fourth cross is one-half that for the third cross. The observed ratio was 90/415=0.22, which is in fact greater than that observed in the third cross and 85% the expected value. We conclude that the low frequency of recovery of *mnDf1*-bearing ova from *mnDp1*/+; *mnDf1* hermaphrodites is largely a maternal effect. Other large deficiencies, *mnDf2* for example, were also recovered at low frequency from the ova of *mnDp1*/+; *mnDf*, although not as low as in the case of *mnDf1*. Smaller deficiencies, such as *mnDf5* and *mnDf20*, did not appear to show this effect.

DISCUSSION

We have identified 21 recessive lethal and sterile mutations that define 14 essential genes in a region of the *X* chromosome balanced by *mnDp1*. The genes were mapped by recombination and by complementation testing against a set of X-ray-induced deficiencies and extend the map of the *X* chromosome by about 4 map units. Twelve of the 21 mutants, in ten of the 14 genes, are early larval lethals. We suggest that many of these are null mutants because the effective lethal phases of the deficiency hemizygotes were indistinguishable from those of the homozygotes. It also seems likely that at least some of the genes identified by the early larval lethals are essential for embryogenesis, but that the contribution of the *mnDp1*-bearing mother, through oogenesis, sustains the development of mutant progeny until after hatching. This suggestion is supported by the work of HIRSH *et al.* (1977), who tested 25 temperature-sensitive zygote-defective mutants for maternal effects. At the restrictive temperature the mutants laid fertilized eggs that did not hatch. For 22 of the 25 mutants, however, a heterozygous hermaphrodite at the restrictive temperature supported the development of homozygous mutant progeny beyond hatching. It is of interest in this connection that both *mnDp1*/+; *mnDf9* and *mnDp1*/+; *mnDf15*, where *mnDf9* and *mnDf15* are deficient for *unc-3* and *let-7* (and probably other essential genes as well), occasionally give early larval Unc-3 self-progeny. One of the early larval lethals, *let-8*, is temperature sensitive, and at the permissive temperature for larval growth it is sterile, owing to defective gonadogenesis. The *let-8*$^+$ function therefore appears to be needed at different stages of development.

Two of the mutants are maternal-effect lethals: *let-1* (*mn115*) and *let-5* homozygotes grow to adulthood and give rise to defective zygotes. In the former case the zygotes do not hatch, and in the latter case they develop to about the third larval stage. In each case, oocytes produced by the homozygous mutant are

rescuable by fertilization with wild-type sperm. In each case, putting the mutation opposite a deficiency does not change the effective lethal phase, suggesting that the prolonged survival is not due to a leaky mutation. This suggestion is particularly interesting in the case of *let-1(mn115)* because two other alleles show earlier effective lethal phases: *mn119* homozygotes die as early larvae and *mn102* as late larvae. We suggest that *mn119* is a null allele, based on the reasoning already given for early larval lethals. Moreover, *mn102/mnDf20* animals also die as early larvae, as do *mn102/mn119* heterozygotes, suggesting that *mn102* is a leaky allele that is dosage sensitive. But how do we explain *mn115*? It seems that *let-1*$^+$ function is needed for at least two distinct developmental phases: a late embryonic or early larval phase, which goes unsatisfied in *mn102* and *mn119*, but which is conquered by *mn115* even in *mn115/mnDf20* hermaphrodites, and an early embryonic phase, which can be supplied either during oogenesis by a maternal *let-1*$^+$ gene—in this function *mn115* is deficient—or by a *let-1*$^+$ gene provided the zygote by the fertilizing sperm. *mn115* is then either qualitatively different from its alleles in being able to provide the first function but not the second, or it is a leaky mutation that is capable of providing, even when present in single dose, enough gene product to satisfy the late embryonic or early larval phase, but is incapable of providing during oogenesis for the early embryonic phase. The *let-1* gene brings to mind certain recently described temperature-sensitive mutants of Drosophila that are both zygotic lethals and maternal-effect lethals (FAUSTO-STERLING, WEINER and DIGAN 1977; SHEARN, HERSPERGER and HERSPERGER 1978).

Four mutants are embryonic lethals. All are temperature sensitive and allelic. Two additional temperature-sensitive mutants, given to us by other laboratories, turned out to be in the same gene, *let-2*. Many pairs of *let-2* alleles showed partial complementation: in some cases heterozygotes hatched but died as larvae and in other cases heterozygotes were fertile at 20°, but sterile at 25°. HIRSH and VANDERSLICE (1976) showed earlier that if homozygous *let-2(b246)* animals were allowed to hatch at permissive temperature, they could be rendered unable to lay either fertilized or unfertilized eggs by shifting to the restrictive temperature. This category of mutant was called *gon*, for defective gonadogenesis. Subsequently, *b246* was found to have an embryonic lethal phase as well (J. KIMBLE, personal communication). The evidence concerning *let-2* suggests that it is a highly mutable, perhaps large, locus coding for a multimeric protein that is required at more than a single stage of development, including embryogenesis, and that the embryonic requirement cannot be fully provided for during oogenesis in a *let-2*$^+$-bearing mother.

Homozygous *let-9* hermaphrodites produce defective sperm. Oocytes made by the *unc-3 let-9* homozygote can be fertilized (to give viable progeny) not only by *let-9*$^+$ sperm but also by *unc-3 let-9* sperm made in a heterozygous male. Many sperm are produced by mutant hermaphrodites, but they are generally incapable of fertilization: mutant hermaphrodites lay many unfertilized oocytes, along with an occasional fertilized egg that hatches to give a sterile adult like its parent. The *let-9* mutant is very similar to mutants of *fer-1 I* studied by WARD and MIWA (1978). As these authors have pointed out, because sperm can be

isolated relatively easily, mutants that accumulate nonfunctional sperm may be particularly useful in attempts to identify altered gene products.

The effective lethal phase for *let-7* mutants is later in males than in hermaphrodites. We attribute the difference to the number of *X* chromosomes, rather than sexual differentiation *per se*, since 2*X let-7* males, transformed by either *tra-1* or *tra-2*, died as early as *let-7* hermaphrodites. This effect is analogous to that reported by HODGKIN and BRENNER (1977) for *dpy-21 V*, which has dumpy expression in hermaphrodites but not males, and *dpy-22 X*, which has dumpy expression in hermaphrodites, but semilethal expression in males. In both cases 2*X* transformed males gave expression characteristic of the hermaphrodite. Since *dpy-21* is not an *X*-linked gene (HODGKIN, personal communication), the difference in expression between 1*X* and 2*X* individuals cannot be due to differing doses of the *dpy-21* gene itself. Similarly, the differential effect of *let-7* appears not to depend on the number of *let-7* copies, since *let-7/mnDf5* hermaphrodites died early. Possibly the enhanced survival of *let-7*/0 males is due to having only one copy of another part of the *X* chromosome. Alternatively, the difference between 1*X* and 2*X* individuals could be the cumulative effect of many *X*-linked genes, as is the case for sex determination in Drosophila (DOBZHANSKY and SCHULTZ 1934).

We identified and characterized 30 deficiencies that were balanced by *mnDp1* and that included *unc-3* and at least one essential gene. A surprisingly large fraction of both left and right endpoints of the deficiencies were outside the region defined by the *let* genes. Twenty-two (of 30) left endpoints were to the left of the left-most gene, *let-4*, and 16 right endpoints were to the right of the cluster of right-most genes. The position of one endpoint of a deficiency, inside the region or out, seemed to be independent of the position of the other endpoint: from the frequencies just given, we calculate that, of 30 deficiencies, 12 would be expected to have both endpoints outside of the region, 14 would be expected to have one endpoint outside and one in, and four would be expected to have both endpoints in. The observed numbers were 13, 12, and five, respectively. The next known gene to the left of *let-4*, about two map units away, is *unc-84* (HORVITZ, personal communication). Since *unc-84* is not covered by *mnDp1* (unpublished data), it appears that a disproportionate fraction of the left endpoints are situated between it and *let-4*. Perhaps that region, which also includes a breakpoint for *mnDp1*, is a hot-spot for X-ray-induced breaks. About the right side we can say less. No known genes map to the right of the region covered by *mnDp1*. Indeed, *mnDp1* may, or may not, extend to the right tip of the *X* chromosome. We also do not know whether terminal deletions must be capped by telomere material, as seems to be the case in Drosophila (ROBERTS 1976).

When *mnDf1*, a large deficiency, was transmitted through ova, we found that the frequency with which *mnDf1/unc-3* zygotes hatched, compared with *mnDp1*/+; *mnDf1*/0 zygotes, was lower when the maternal genotype was *mnDp1*/+; *mnDf1/mnDf1* than when it was *mnDp1*/+; *mnDf1/unc-7*. We suppose that ova produced by hypoploid hermaphrodites are deficient in materials essential for embryogenesis and that the deficit is more easily overcome by *mnDp1*/+; *mnDf1*/0 zygotes than by *mnDf1/unc-3* zygotes. This interpretation

implies that expression by *mnDp1* genes in the male is more effective than expression by the corresponding genes on the *unc-3* chromosome in the hermaphrodite. That is just what would be expected if, as in Drosophila (LUCCHESI 1973), *X* chromosome genes are less active in 2*X* animals than in 1*X*, and *X* chromosome genes translocated to an autosome behave as if still *X*-linked.

An advantage of using an unlinked duplication to balance lethals and steriles is that the region covered by the balancer is strictly delimited: a particular *X*-linked gene is either covered by *mnDp1* or it is not. We can estimate the number of indispensable genes carried by *mnDp1* by two independent methods. One estimate can be obtained from the frequency of repeat mutations, on the assumption that the number of lethal or sterile mutations per essential gene in our total sample follows a Poisson distribution. This amounts to assuming that the probabilities of mutant recovery are the same for all genes—clearly an oversimplification (for example, see JUDD, SHEN and KAUFMAN 1972 for a distribution of recovered mutants per gene in a small region), which will tend to overemphasize those genes that give more mutants and make the estimate of essential gene number low. We can at least reduce the overemphasis on the genes that give many mutants by using the following parameter: f = the fraction of mutated genes represented by more than one mutant. According to the Poisson distribution, $f = (1-e^{-m}-me^{-m})/(1-e^{-m})$, where m is the average number of mutations per essential gene. To date we have identified 14 genes, four of which have mutated more than once. By inspection of a graph depicting the relationship between f and m, we have determined that for $f = 4/14$, $m = 0.64$. The fraction of genes in the region not mutated $= e^{-m} = 0.53$; thus 14 genes represent 47% of the total number of genes in the region, so that the total number is 30.

An independent estimate of the number of essential genes carried by *mnDp1* can be derived in the following way. *mnDp1* covers about 7.5 map units on the *X* chromosome (Figure 5), which is 15% of the *X* chromosome map. BRENNER (1974) estimated earlier, on the basis of the frequency of induction of *X*-linked lethals under a standard set of conditions for EMS mutagenesis, that the *X* chromosome carries 300 essential genes. If we assigned 15% of these to *mnDp1*, that would be 45 essential genes, in good agreement with our previous estimate. Both estimates are likely to be low, however, the latter because BRENNER did not include steriles in his work. Both estimates nevertheless suggest that the number of essential genes covered by *mnDp1* is probably in the range of 30 to 80.

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LITERATURE CITED

BRENNER, S., 1974 The genetics of *Caenorhabditis elegans*. Genetics **77**: 71–94.

DEPPE, U., E. SCHIERENBERG, T. COLE, C. KRIEG, D. SCHMIDT, B. YODER and G. VON EHRENSTEIN, 1978 Cell lineages of the embryo of the nematode *Caenorhabditis elegans*. Proc. Natl. Acad. Sci. U.S. **75**: 376–380.

DOBZHANSKY, T. and J. SCHULTZ, 1934 The distribution of sex-factors in the *X*-chromosome of *Drosophila melanogaster*. J. Genet. **28**: 349–386.

FAUSTO-STERLING, A., A. J. WEINER and M. E. DIGAN, 1977 Analysis of a newly isolated temperature-sensitive maternal effect mutation of *Drosophila melanogaster*. J. Exp. Zool. **200**: 199–210.

GEHRING, W. J., 1976 Developmental genetics of *Drosophila*. Ann. Rev. Genet. **10**: 209–252.

HADORN, E., 1961 *Developmental Genetics and Lethal Factors*. Wiley, New York.

HERMAN, R. K., 1978 Crossover suppressors and balanced recessive lethals in *Caenorhabditis elegans*. Genetics **88**: 49–65.

HERMAN, R. K., D. G. ALBERTSON and S. BRENNER, 1976 Chromosome rearrangements in *Caenorhabditis elegans*. Genetics **83**: 91–105.

HIRSH, D., D. OPPENHEIM and M. KLASS, 1976 Development of the reproductive system of *Caenorhabditis elegans*. Develop. Biol. **49**: 200–219.

HIRSH, D. and R. VANDERSLICE, 1976 Temperature-sensitive developmental mutants of *Caenorhabditis elegans*. Develop. Biol. **49**: 220–235.

HIRSH, D., W. B. WOOD, R. HECHT, S. CARR and R. VANDERSLICE, 1977 Expression of genes essential for early development in the nematode *C. elegans*. pp. 347–356. In: *Molecular Approaches to Eucaryotic Systems*. Edited by G. WILCOX, J. ABELSON and C. F. FOX. Academic Press, New York.

HODGKIN, J. A. and S. BRENNER, 1977 Mutations causing transformation of sexual phenotype in the nematode *Caenorhabditis elegans*. Genetics **86**: 275–287.

JUDD, B. H., M. W. SHEN and T. C. KAUFMAN, 1972 The anatomy and function of a segment of the *X*-chromosome of *Drosophila melanogaster*. Genetics **71**: 139–156.

KLASS, M., N. WOLF and D. HIRSH, 1976 Development of the male reproductive system and sexual transformation in the nematode *Caenorhabditis elegans*. Develop. Biol. **52**: 1–18.

LUCCHESI, J. C., 1973 Dosage compensation in *Drosophila*. Ann. Rev. Genet. **7**: 225–237.

ROBERTS, P. A., 1976 The genetics of chromosome aberration. pp. 68–184. In: *The Genetics and Biology of Drosophila*, Vol. 1a. Edited by M. ASHBURNER and E. NOVITSKI. Academic Press, New York and London.

SHEARN, A., G. HERSPERGER and E. HERSPERGER, 1978 Genetic analysis of two allelic temperature-sensitive mutants of *Drosophila melanogaster*, both of which are zygotic and maternal-effect lethals. Genetics **89**: 341–353.

SULSTON, J. E., 1976 Post-embryonic development in the ventral cord of *Caenorhabditis elegans*. Phil. Trans. Roy. Soc. B. **275**: 287–297.

SULSTON, J. E. and H. R. HORVITZ, 1977 Post-embryonic cell lineages of the nematode *Caenorhabditis elegans*. Develop. Biol. **56**: 110–156.

SUZUKI, D. T., 1970 Temperature-sensitive mutations in *Drosophila melanogaster*. Science **170**: 695–706.

WARD, S. and J. MIWA, 1978 Characterization of temperature-sensitive, fertilization-defective mutants of the nematode *Caenorhabditis elegans*. Genetics **88**: 285–303.

Corresponding editor: A. CHOVNICK