Maternal-Effect Lethal Mutations on Linkage Group II of *Caenorhabditis elegans*

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ABSTRACT

We have analyzed a set of linkage group (LG) II maternal-effect lethal mutations in *Caenorhabditis elegans* isolated by a new screening procedure. Screens of 12,455 F_1 progeny from mutagenized adults resulted in the recovery of 54 maternal-effect lethal mutations identifying 29 genes. Of the 54 mutations, 39 are strict maternal-effect mutations defining 17 genes. These 17 genes fall into two classes distinguished by frequency of mutation to strict maternal-effect lethality. The smaller class, comprised of four genes, mutated to strict maternal-effect lethality at a frequency close to 5×10^{-4}, a rate typical of essential genes in *C. elegans*. Two of these genes are expressed during oogenesis and required exclusively for embryogenesis (pure maternal genes), one appears to be required specifically for meiosis, and the fourth has a more complex pattern of expression. The other 13 genes were represented by only one or two strict maternal alleles each. Two of these are identical genes previously identified by nonmaternal embryonic lethal mutations. We interpret our results to mean that although many *C. elegans* genes can mutate to strict maternal-effect lethality, most genes mutate to that phenotype rarely. Pure maternal genes, however, are among a smaller class of genes that mutate to maternal-effect lethality at typical rates. If our interpretation is correct, we are near saturation for pure maternal genes in the region of LG II balanced by *mnC1*. We conclude that the number of pure maternal genes in *C. elegans* is small, being probably not much higher than 12.

THE nematode *Caenorhabditis elegans* provides a special opportunity for the study of embryogenesis. The completely described embryonic cell lineage and the application of microsurgical techniques to its analysis are beginning to reveal the basic mechanisms by which nematode embryonic development takes place (SULSTON *et al.* 1983; reviewed by WOOD 1988). Genetic studies can be an important component of embryological analysis by identifying genes with controlling roles in specific embryonic events. Information about the contributions of these genes to embryogenesis can be obtained by studies of mutant phenotypes and by analysis of the molecularly cloned genes.

Unfortunately, genetic analysis of embryogenesis is complicated by the large numbers of genes required for viability of the embryo. While mutations in genes playing controlling roles in embryogenesis will lead to embryonic lethality, so will mutations in any of the hundreds or thousands of genes required for cell viability and proliferation. One way around this problem is to concentrate efforts on mutations that produce readily interpretable terminal phenotypes (*e.g.*, pattern mutants in Drosophila) (NUSSLEIN-VOLHARD and WIESCHAUS 1980). However, the terminal phenotypes of most *C. elegans* embryonic lethal mutations thus far identified are not readily interpretable. Therefore, we have been seeking genetic criteria that will allow us to focus on a class of mutations likely to identify genes with controlling roles in embryogenesis.

Earlier genetic studies of embryogenesis in *C. elegans* analyzed about 90 temperature-sensitive embryonic lethal mutations and identified 55 genes required for embryogenesis (HIRSH and VANDERSLICE 1976; HIRSH *et al.* 1977; WOOD *et al.* 1980; MIWA *et al.* 1980; CASSADA *et al.* 1981; ISNENGHI *et al.* 1983; DENICH *et al.* 1984). A major conclusion from these studies is that maternal gene expression plays a dominant role in *C. elegans*' embryogenesis; 54 of the 55 identified genes were defined by maternal-effect lethal mutations.

Hermaphrodites homozygous for maternal-effect lethal mutations survive when derived from heterozygous mothers, but produce inviable self-progeny. This behavior indicates that expression of the wild-type gene by the mother is sufficient for survival of the embryo, but does not necessarily mean that maternal expression of the gene is required. If maternal expression is required for survival of the embryo, then embryos produced by homozygous mothers are inviable even if they carry the wild-type allele contributed by mating to wild-type males. Maternal-effect mutations that cannot be "rescued" in this way are called strict maternal-effect mutations. Mutations capable of

rescue are referred to as partial maternal-effect mutations. Mutations in 34 of the 54 genes identified by temperature-sensitive maternal-effect lethal mutations are strict.

A second conclusion from these studies is that most of the genes identified by the temperature-sensitive embryonic lethal mutations are also required at stages of the life cycle outside of embryogenesis. This probably reflects the likelihood that many genes identified by temperature-sensitive embryonic lethal mutations encode general metabolic functions (WOOD *et al.* 1980), and thus have essential roles in cell viability or proliferation rather than having controlling roles in embryogenesis. Consistent with this possibility, estimates of the number of *C. elegans* genes being sampled by these temperature-sensitive embryonic lethal mutations range from 200 to in excess of 1000 (CASSADA *et al.* 1980; WILKINS 1986). Thus, distinguishing embryonic control genes from essential genes with primarily metabolic roles is a problem in using temperature-sensitive lethals to study embryogenesis in *C. elegans*.

One way to reduce the magnitude of this problem is to focus efforts on nonconditional maternal-effect lethal mutations. The predominant role of maternal gene activity in *C. elegans* embryogenesis argues for the existence of a set of control genes that are transcribed maternally during oogenesis and are required only during embryogenesis. Loss-of-function (null) mutations in such genes would be expected to be maternal-effect lethal, while null mutations in genes with essential metabolic roles at stages of the life cycle outside of gametogenesis and embryogenesis will be lethal in homozygotes that carry them. Thus, screens designed to identify nonconditional maternal-effect lethal mutations should eliminate from study many genes with general metabolic functions and might provide a means to enrich for mutations in maternally expressed embryonic control genes. A potential problem in this approach comes from the fact that not all mutations are null mutations. Reduced-activity or gain-of-function mutations in more generally essential genes might behave as nonconditional maternal-effect lethal mutations (PERRIMON *et al.* 1986).

In this paper we report the results of a study of 54 maternal-effect lethal mutations on LG II of *C. elegans*. Our goal in this study was to evaluate a new procedure to isolate nonconditional maternal-effect lethal mutations (PRIESS, SCHNABEL and SCHNABEL 1987; KEMPHUES *et al.* 1988). We were particularly interested in identifying genes expressed in oogenesis and required exclusively for embryogenesis (pure maternal genes). Because such genes should mutate only to strict maternal-effect lethality, we wished to determine how efficient the screen was for identifying strict maternal-effect lethal mutations, to determine what proportion of those actually identified pure maternal genes, and if possible, to estimate the number of pure maternal genes in the *C. elegans* genome.

MATERIALS AND METHODS

Strains and culture conditions: Worms were grown as described by BRENNER (1974). Genetic nomenclature conforms to the recommendations of HORVITZ *et al.* (1979). Many of the alleles for this study were obtained from the Caenorhabditis Genetics Center and included: *unc-4(e120)*, *dpy-10(e128)* (BRENNER 1974); *him-3(e1147)* (HODGKIN, HORVITZ and BRENNER 1979); *mnC1 dpy-10(e128) unc-52(e444)* (HERMAN 1978); *zyg-11(ct1)* (KEMPHUES *et al.* 1986); *sqt-1(sc13)* (COX *et al.* 1980); *lin-31(n301)* (FERGUSON and HORVITZ 1985).

Screen for maternal-effect lethal mutations: Screens for LG II maternal-effect lethal mutations used a modification of a previously described procedure (PRIESS, SCHNABEL and SCHNABEL 1987; KEMPHUES *et al.* 1988). Hermaphrodites homozygous for *lin-2(e1309)* or *egl-23(n601)* do not lay eggs (HORVITZ and SULSTON 1980; TRENT, TSUNG and HORVITZ 1983). This is not a barrier to internal self-fertilization and such hermaphrodites accumulate embryos that hatch internally and consume the mother. As a result, the hermaphrodites rapidly become immotile bags of growing larvae. The bags eventually burst, releasing the progeny. Mutations that prevent production of hatching larvae allow the survival of *lin-2* or *egl-23* homozygous hermaphrodites. Such mutations include gonadogenesis-defective, fertilization-defective, and maternal-effect lethal mutations. Only worms homozygous for maternal-effect lethal mutations accumulate refractile fertilized eggs making them easy to identify.

For the screen, hermaphrodites of genotype *unc-4/mnC1 dpy-10 unc-52 II*; *egl-23 him-3 IV* or *unc-4/mnC1 dpy-10 unc-52 II*; *him-3 IV*; *lin-2 X* were mutagenized with EMS using the procedure of BRENNER (1974) except that we varied the EMS concentration as described below. Because crosses involving hermaphrodites homozygous for *lin-2* or *egl-23* mutations are impossible or inefficient, the *him-3(e1147)* mutation was included in the strain so that we could easily obtain males for outcrosses. Homozygous *him-3(e1147)* hermaphrodites produce 3.5% self-progeny males (HODGKIN, HORVITZ and BRENNER 1979). Our initial screens using 40 mM EMS resulted in large numbers of false positives due to F_2 that grew slowly or were semisterile. We found that lowering the dose to 25–30 mM increased our efficiency of scoring; thus, some of our screening was done using the lower dose. (6349 F_1 hermaphrodites were screened after treatment with 40 mM EMS, 4894 with 30 mM EMS, and 1212 with 25 mM EMS.) The degree of sterility in F_1 progeny of mutagenized parents seemed to be a good predictor of the extent of false positives in the F_2. EMS doses that resulted in about 10% F_1 sterility proved to be optimal. The count of F_1 hermaphrodites presented above and in RESULTS does not include F_1 steriles. About 1000 F_1 *unc-4/mnC1* self-progeny were picked at 25° from each mutagenesis, and the plates were scored after 3–4 days for surviving F_2. Surviving F_2 were examined under the dissecting microscope to determine if the survivors had accumulated fertilized eggs. Cosegregation of the maternal-effect lethal mutation with the *unc-4* marker through several generations indicated that the mutations were balanced by *mnC1*. Mutations not balanced by *mnC1* were discarded. Ten mutations balanced by *mnC1* but with expressivity of less than 90% were also discarded. (One mutation, *it38*, was initially

expressed strongly but after maintenance in stock became more leaky.) Mutations were tested for temperature sensitivity by growing the strains at 16° and scoring the Unc progeny for the ability to produce viable embryos.

Outcrossing and three factor mapping: Mutations were outcrossed twice, once to eliminate the *egl-23* or *lin-2* mutations, and once more during three factor mapping (see below). For outcrossing, males of genotype *m unc-4/mnC1 II*; *him-3 IV*; *lin-2 X* or *m unc-4/mnC1 II*; *egl-23 him-3 IV* (*m* = newly identified maternal-effect lethal) were crossed to *dpy-10 zyg-11(ct1) unc-4/mnC1 dpy-10 unc-52* hermaphrodites. The phenotypically wild-type hermaphrodite progeny (*m unc-4/mnC1*; *egl-23 him-3/++*) were picked and non-Egl lines were selected. These once outcrossed lines were used for viability, brood size and male rescue tests described below. The F_1 Unc progeny from the above cross (*dpy-10 zyg-11 unc-4/m unc-4*) were plated at 25° to test for complementation with *zyg-11* because we anticipated identifying new *zyg-11* alleles in our screen.

For three-factor mapping, males of genotype *m unc-4/mnC1* were crossed to *dpy-10(e128) sqt-1(sc13)* hermaphrodites. *sqt-1(sc13)* exhibits a left roller phenotype (Rol) (KUSCH AND EDGAR 1986). F_1 progeny were picked to plates at 25° to maximize recombination frequency (ROSE and BAILLIE 1979). Because *e128 sc13/e128 +* is phenotypically identical to *e128 sc13*, we were unable to score Dpy non-Rol recombinants. We picked Rol non-Dpy recombinants and scored their self progeny for Unc and for maternal-effect lethal (Mel) phenotypes. Approximately 20 Rol non-Dpy recombinants were scored for each mutation. Analysis of these data allowed us to position each mutation into one of four regions on LG II as indicated in figure 1: region 1, left of or near but to the right of *dpy-10*; region-2, between *dpy-10* and *unc-4* or to the right and close to *unc-4*; region 3, between *unc-4* and *sqt-1* or close to and to the left of *sqt-1*; region 4, to the right of, or near but to the left of *sqt-1*.

The *zyg-1(b1)* strain was analyzed in a three-factor cross in an attempt to separate the gene(s) responsible for four of the phenotypes exhibited by this strain: uncoordinated (Unc) vulva defective (Vul) gonadogenesis defective (Gon) and maternal-effect lethal (Mel). Eleven Dpy non-Lin and 17 Lin non-Dpy progeny from hermaphrodites of genotype *lin-31(n301) dpy-10(e128)/zyg-1(b1)* were picked individually at 25° and their progeny examined for the four phenotypes. The Mel, Unc and Vul phenotypes segregated together and were found in progeny from three of the Dpy recombinants and 16 of the Lin recombinants.

Complementation: Mutations within each region were tested among themselves for complementation as follows. Reciprocal crosses of m_a *unc-4/mnC1* or m_a *unc-4 sqt-1/mnC1* with m_b *unc-4/mnC1* were carried out. Whenever possible, the hermaphrodite parent was of genotype *m unc-4 sqt-1/mnC1* and the male parent was *m unc-4/mnC1*. The test individuals are the only Unc non-Rol progeny from this cross. All complementation tests were carried out at 25°. To score for complementation, five Unc non-Rol progeny were placed on a plate and scored for the production of progeny. In crosses where the maternal chromosome was marked only with *unc-4*, we assured ourselves that we were testing outcross Unc progeny by transferring the parents to new plates each day, and testing individual Unc progeny only from those plates with approximately 50% males among the F_1. Several individual outcross progeny were tested from each cross. Mutations were assigned to complementation groups only after reciprocal crosses gave consistent results.

To test for complementation with deficiencies, males of genotype *m unc-4/mnC1* were crossed to *Df unc-4/mnC1*. Unc progeny (*Df unc-4/m unc-4*) were scored for ability to produce viable embryos. The deficiencies tested are described by SIGURDSON, SPANIER and HERMAN (1984) and are shown in Figure 1 and/or indicated in Table 1. The test for complementation with known nonconditional lethal (*let*) and sterile (*ooc*) mutations marked with *unc-4* and balanced by *mnC1* was similar to that described above for deficiencies. Mutations tested are described by SIGURDSON, SPANIER and HERMAN (1984) and included: *let-19(mn19)*, *let-23(mn23)*, *let-24(mn24)*, *let-26(mn26)*, *let-29(mn29)*, *let-30(mn239)*, *let-237(nm208)*, *let-238(mn229)*, *let-239(mn93)*, *let-240 (mn209)*, *let-247(mn211)*, *let-248(mn237)*, *let-249(mn238)*, *let-250(mn207)*, *let-251(mn95)*, *let-252(mn100)*, *let-263 (mn240)*, *let-265(mn188)*, *let-266(mn194)*, *ooc-1(mn250)*, *ooc-2(mn249)*, *ooc-3(mn241)*, *ooc(mn203)* and *ooc(mn244)*. Although failure to complement a lethal mutation or deficiency could result in either a lethal or a maternal-effect lethal phenotype, in no case did we observe lethal phenotypes. Complementation tests with temperature-sensitive embryonic lethal mutations (*zyg* or *emb*) were carried out by crossing males homozygous for the temperature-sensitive mutant to hermaphrodites of genotype *m unc-4/mnC1* and scoring several individual F_1 wild-type progeny (either *zyg/m unc-4* or *m unc-4/C1*) for production of viable embryos at 25°. In the case of mutations that complemented, absence of *mnC1* homozygotes in the F_2 validated the test results. Mutations tested were: *zyg-1(b1)*, *zyg-3(b18)*, *zyg-5(b89)*, and *emb-27(g48)*.

Male rescue tests: Strict maternal-effect mutations were identified by male rescue tests carried out as follows. Five to ten single *m unc-4* hermaphrodites were mated individually to five wild-type males at 25°. Under these conditions, outcrossing is virtually guaranteed. Eggs were counted and the percentage of hatching larvae was compared to unmated controls. In some cases, a further test was done to insure that failure to rescue was not due to a problem with mating. For this test, individual hermaphrodites were transferred daily until they had depleted their own sperm and laid only unfertilized eggs. Any fertilized eggs laid by such hermaphrodites after mating to wild-type males must be outcross. As an alternative to this often unsuccessful procedure, some rescue tests were done using *plg-1*; *him-5* males (J. HODGKIN, T. DONIACH and C. KENYON, personal communication). Because these males deposit a visible mating plug, the occurrence of mating can be verified. (In control overnight mass mating of approximately 15 *plg-1* males with 30 *unc-4* hermaphrodites, all 21 hermaphrodites with visible plugs gave outcross progeny.)

Determination of percentage viability: Between five and ten L_4 *m unc-4* hermaphrodites of each mutant strain were placed onto individual plates at 25°, and the worms were transferred daily until they had exhausted their sperm. The percentage of viable embryos was obtained by dividing the number of hatched L_1 larvae by the total eggs laid for all worms of each genotype.

Nomenclature: The screening procedure used here recovers a set of mutations that do not, as a class, fit criteria set by previous screens for lethal mutations. Unfortunately, there is considerable potential for overlap in genes defined by *zyg*, *emb*, *ooc*, *let*, and our new set of maternal-effect lethal mutations. Many, but not all, of the mutations isolated in our screen satisfy the criteria for *ooc* genes (SIGURDSON, SPANIER and HERMAN 1984). Although most *zyg* and *emb* genes (HIRSH and VANDERSLICE 1976; MIWA *et al.* 1980) are defined by maternal-effect lethal mutations, the criteria for identifying *zyg* and *emb* genes are different from those for maternal-effect lethal mutations. Therefore we have named new genes identified solely by maternal-effect lethal

alleles as *mel*. In one case, *him-14*, the mutant phenotype suggested a more appropriate name. The rationale for this choice is explained in RESULTS AND DISCUSSION.

RESULTS AND DISCUSSION

Screens for maternal-effect lethal mutations: We chose LG II as an initial target for saturation mutagenesis for three reasons: (1) The balancer chromosome *mnC1 dpy-10 unc-52* suppresses crossing over on about 75–80% of LG II and greatly facilitates handling of lethal and sterile mutations (HERMAN 1978; SIGURDSON, SPANIER and HERMAN, 1984). (2) The maternal-effect lethal mutations we identified could readily be compared by complementation with an existing set of lethal mutations and deficiencies (SIGURDSON, SPANIER and HERMAN 1984) to check for overlap between genes identified by the two mutant sets. (3) A pure maternal gene, *zyg-11*, had previously been identified on LG II (HIRSH and VANDERSLICE 1977; WOOD *et al.* 1980; KEMPHUES *et al.* 1986), and would provide a known standard for interpreting mutation frequencies.

Maternal-effect lethal mutations on LG II were obtained using the screening procedure described in MATERIALS AND METHODS. Screens of 12,455 F_1 broods resulted in the recovery of 54 LG II maternal-effect lethal mutations. Because the screen is carried out at nonpermissive temperature (25°) both nonconditional and temperature-sensitive mutations can be recovered. Seven mutations in seven genes were found to be temperature sensitive: *zyg-9(b288)*, *mel-9(b293)*, *mel-15(it7)*, *zyg-11(it11)*, *mel-4(it12)*, *zyg-1(it25)*, *him-14(it44)*. Three of these genes (*zyg-1*, *zyg-9* and *zyg-11*) had been previously identified in screens for temperature-sensitive embryonic lethal mutations.

Genetic analysis: All mutations were mapped by meiotic recombination relative to the markers *dpy-10*, *unc-4* and *sqt-1* (Figure 1). Mutations falling within each of the four regions defined by recombination were tested for complementation among themselves. The gene assignments based on these tests are indicated in Table 2. The region 1 mutations *it4* and *it52* were originally isolated on a single chromosome, but were subsequently separated showing them to be two independent mutations. The mutations *it43* and *it12* have been tentatively assigned to the same complementation group because they did not fully complement. (The double heterozygotes exhibited a maternal-effect lethal phenotype but gave more survivors than homozygotes for the leaky mutation *it12*. Furthermore, surviving progeny from double heterozygotes grew slowly.) Based on these results, the 54 mutations define 29 complementation groups.

To determine if any of the mutations were alleles of genes previously identified by lethal or maternal-effect lethal mutations, a representative from each complementation group was tested against appropri-

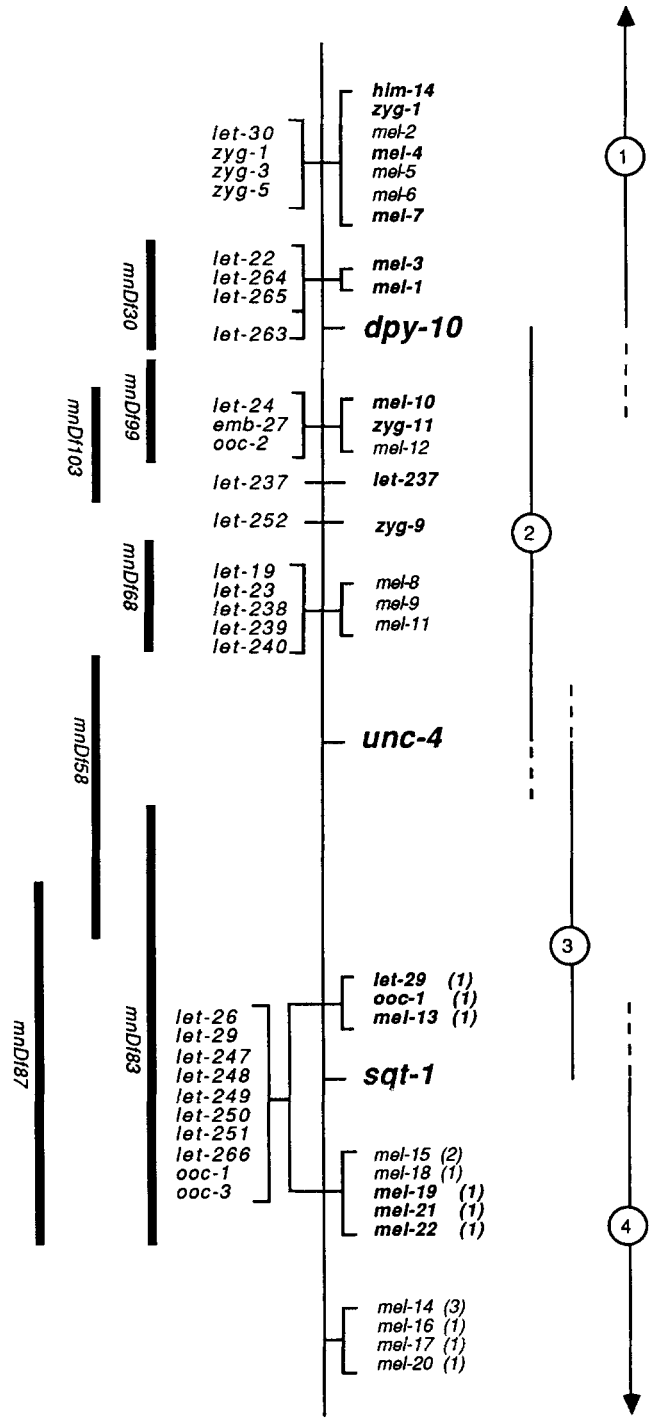

FIGURE 1.—Partial genetic map of LG II. Chromosomal regions defined by three-factor mapping are indicated by lines with circled numbers. Marker genes are shown to the right of the line in large bold print. Genes identified by our collection of maternal-effect lethal mutations are to the right of the line. Genes identified only by strict maternal-effect mutations are shown in bold. Previously identified genes that were tested for complementation with the new maternal-effect mutations are to the left of the line. Deficiencies spanning the region are indicated by bars. This map is not intended to show precise gene order but only to show the position of the genes relative to the indicated deficiencies. For example, genes covered by *mnDf30* could be on either side of *dpy-10*, and genes covered by *mnDf83* could be on either side of *sqt-1*.

TABLE 1

Complementation with deficiencies and existing LG II mutations

Gene	Maternal-effect allele tested	Fails to complement	Complements
Region 1			
mel-1	(*it19*)	*mnDf30*	*let-22, let-263, let-264, let-265*
mel-2	(*it20*)	*ooc(mn244)*	*mnDf30, let-30, zyg-1, zyg-3, zyg-5,ooc(mn203)*
mel-3	(*b281*)	*mnDf30*	Same as *mel-1*
mel-4	(*it43, it12*)		*mnDf30, let-30, zyg-1, zyg-3, zyg-5,ooc(mn203), ooc(mn244)*
mel-5	(*b314*)		Same as *mel-4*
mel-6	(*it27*)		Same as *mel-4*
mel-7	(*it36*)		Same as *mel-4*
him-14	(*it44*)		Same as *mel-4*
zyg-1	(*it4, it25,it29,it37*)	*zyg-1(b1)*	*mnDf30, let-30, zyg-3, zyg-5, ooc(mn203), ooc(mn244)*
Region 2			
mel-8	(*b309*)	*mnDf68*	*let-240, let-19, let-23, let-238, let-239*
mel-9	(*b293*)	*mnDf68*	Same as *mel-8*
mel-10	(*it10*)	*mnDf103, mnDf99*	*ooc-2, let-24, emb-27*
mel-11	(*it26*)	*mnDf68*	Same as *mel-8*
mel-12	(*it42*)	*mnDf103, mnDf99*	Same as *mel-10*
zyg-9	(*b244*)		*let-252*
let-237	(*b283*)	*mnDf103, let-237(mn208)*	*mnDf99*
Region 3			
let-29	(*b285*)	*mnDf83, let-29 (mn29)*	*mnDf58, ooc-1, ooc-3, let-26, let-247, let-248, let-250, let-249, let-251, let-266*
mel-13	(*b306*)	*mnDf83*	*mnDf58, let-29, ooc-1, ooc-3, let-26, let-247, let-248, let-250, let-249, let-251, let-266*
ooc-1	(*b316*)	*mnDf83, mnDf90, ooc-1(mn250)*	*mnDf58, let-29, let-247, let248, ooc-3*
Region 4			
mel-14	(*it24*)		*mnDf87*
mel-15	(*it38*)	*mnDf87*	*let-26, let-247, let-248, let-249, let-250, let-251, let-266, ooc-1, ooc-3*
mel-16	(*b298*)		*mnDf87*
mel-17	(*b299*)		*mnDf87*
mel-18	(*b300*)	*mnDf87*	Same as *mel-15*
mel-19	(*b310*)	*mnDf87, ooc-3(mn241)*?	Same as *mel-15*
mel-20	(*b317*)		*mnDf87*
mel-21	(*it9*)	*mnDf87*	Same as *mel-15*
mel-22	(*it30*)	*mnDf87*	Same as *mel-15*

ate deficiencies to determine a more precise chromosomal location. The relative positions of the deficiencies used and the results of these tests are shown in Figure 1 and Table 1. Mutations failing to complement a given deficiency were then tested for complementation with known lethal mutations (*let*) and maternal-effect lethal mutations (*ooc, zyg, emb*) that also failed to complement the deficiency (WOOD *et al.* 1980; CASSADA *et al.* 1981; SIGURDSON, SPANIER and HERMAN 1984). Mutations outside of the regions covered by deficiencies were tested against mutations in genes with map positions consistent with possible allelism. The results of these tests are shown in Table 1. Two larval lethal mutations, *let-29(mn29)* and *let-237(mn208)*, failed to complement the maternal-effect lethal phenotypes of *b285* and *b283*, respectively; *it20* and *it22* failed to complement *ooc(mn244)*; *b316* failed to complement *ooc-1(mn250)*, and *it4, it29, it37* and *it44* failed to complement *zyg-1(b1)*. The mutations *b310* and *ooc-3(mn241)* partially complemented and may be allelic.

Rescue tests: Male rescue tests were carried out to determine whether maternal expression of the genes identified by these mutations is necessary for embryonic viability (WOOD *et al.* 1980). Survival of m/+ heterozygous progeny from a homozygous mother

indicates that the gene is expressed by the embryonic genome at an appropriate time and in sufficient amounts to allow survival of the embryo. The results of these tests are shown in Table 2. The percentage of viable embryos from self-fertilization in unmated hermaphrodites is given as a control to evaluate whether rescue occurred. For fully expressed mutations, production of any progeny after mating indicated rescue. For some incompletely expressed mutations the results were not clear cut; these cases are indicated in Table 2 by ±. The gene *mel-14* is an interesting case that illustrates an inherent ambiguity in the male rescue test. Although a positive male rescue test does indicate embryonic expression, a negative result does not necessarily indicate a lack of embryonic expression. If embryonic expression is near a threshold level for rescue, some alleles of a single gene may appear to be strict and others may appear to be partial. Two alleles, *it24* and *b292*, gave such weak rescue that they were scored as strict, but *b291*, because it was fully expressed, gave clear evidence of rescue. However, in other ambiguous cases (± in Table 2), because we could not tell whether rescue was very weak or didn't occur, we consider the mutations to be strict, although it is possible that additional screening might recover partial maternal alleles of some of them. Based on these criteria, 17 of the 29 loci mutated exclusively to strict maternal-effect lethality. Because we are particularly interested in genes expressed only during oogenesis, we will focus primarily on these 17.

Interpretation of frequency distribution and estimation of number of pure maternal genes: The Poisson distribution is often used in genetic saturation studies to estimate the size of the gene set being sampled (GANS, AUDIT and MASSON 1975; KING and MOHLER 1975; MOHLER 1977; MENEELY and HERMAN 1979; CASSADA *et al.* 1981; SIGURDSON, SPANIER and HERMAN 1984). The Poisson distribution is strictly applicable only when the probability of mutation to the specified phenotype is the same for all genes in the set. For a set of pure maternal genes the probability of mutation to strict maternal-effect lethality should be close to 5×10^{-4}, the frequency estimated for mutations in essential genes on the *X* chromosome in *C. elegans* (BRENNER 1974). The frequency distribution of our set of strict maternal-effect mutations, shown in Figure 2, might represent a Poisson distribution, and thus, give us a means to estimate the frequency of pure maternal genes in *C. elegans*. However, the mutation rate we calculate based on the Poisson distribution is much lower than that expected for pure maternal genes and leads us to question the validity of this approach.

In applying the Poisson formula, we followed KING and MOHLER (1975), using the number of genes having one and two mutations per gene to estimate the number of genes in the zero mutation class (genes not represented in our collection). This calculation estimates the number of genes that were not identified in our screens as 20, theoretically giving a total of 37 genes balanced by *mnC1* that are capable of mutation to strict maternal-effect lethality. The mutation frequency for this set is 8×10^{-5} (37 mutations/37 genes/12,455 chromosomes). This frequency is sixfold lower than that expected for pure maternal genes. Evidence presented below leads us to believe that the basis for this low average mutation rate is the presence in our collection of many rare partial-loss-of-function or gain-of-function mutations in essential genes that typically mutate to other phenotypes (such as lethality). PERRIMON and co-workers (1986) provide evidence that many female sterile mutations in Drosophila are, in fact, rare mutations in such essential genes. If this is true for our set of mutations, calculations based on the Poisson distribution are invalid and could result in gross overestimates of the actual number of pure maternal genes in *C. elegans*.

We suggest, rather, that the frequency distribution in Figure 2 is best interpreted as representing two overlapping distributions generated by two classes of genes: a high frequency class containing genes with four, five, or six mutations each and a low frequency class containing many genes (of which we identified 13) with zero, one, or two mutations each. The high frequency class has a mutation rate of 4×10^{-4}, a rate similar to that estimated by BRENNER for *C. elegans* essential genes. The mutation rate of the low frequency class is at least sixfold lower.

Consistent with our initial expectations, the high frequency class contains two known pure maternal genes, *zyg-11* and *zyg-9*. *zyg-11* was shown by an independent set of mutations to be a pure maternal gene and *zyg-9* was shown to be a pure maternal gene, in part, based on alleles reported here (KEMPHUES *et al.* 1986). On the other hand, the low frequency class contains two genes that are allelic to known larval lethal mutations (*let-29* and *let-237*). Because the region of the LG II balanced by *mnC1* is not saturated for lethal mutations (SIGURDSON, SPANIER and HERMAN 1984), it is possible that some of the other genes in the low frequency class might also be genes that typically mutate to give nonmaternal lethal or sterile phenotypes.

If our interpretation is correct, then the low frequency class of genes might be composed primarily of genes required constitutively or at one or a few stages of the life cycle outside of embryogenesis. Null mutations in most constitutive genes would be lethals, but rare mutations that lower the activity of the product to levels tolerable for most of the life cycle might lead to maternal-effect lethality because of a more

TABLE 2

Results of male rescue tests

Gene name	Allele	Percentage viable embryos unmated control	No. of embryos	Percentage viable embryos after mating	No. of embryos	Rescue?[a]
Region 1						
him-14	*it13*	3.7	(1530)	6.5	(1071)	±
	it21	6.9	(1657)	5.6	(1167)	−
	it23	4.4	(1771)	6.9	(1298)	±
	it44ts	5.4	(1333)	5.2	(1246)	−
	it52	2.6	(1446)	7.8	(2012)	±
zyg-1	*it4*	ND[b]		ND		?
	*it25*ts	0.04	(2369)	0[e]	(1304)	−
	it29	0	(1436)	0	(965)	−
	it37	0	(770)	0[e]	(<100)[c]	−
mel-1	*b315*	0	(106)	0	(116)	−
	it19	0	(1189)	0	(565)	−
mel-2	*it20*	0	(1700)	0[f]	(726)	−
	it22	0	(2044)	0[e]	(914)	−
mel-3	*b281*	0	(1467)	0	(575)	−
	it8	0	(2210)	0[e]	(167)	−
mel-4	*it12*ts	5.8	(479)	2.3	(262)	−
	it43	0	(761)	0	(555)	−
mel-5	*b314*	0.5	(183)	9	(167)	+
mel-6	*it27*	0	(1199)	75	(923)	+
mel-7	*it36*	0	(118)	0[f]	(166)	−
Region 2						
zyg-11	*b290*	1.5	(1324)	1.5	ND[d]	−
	it1	0	(841)	0	(2445)	−
	it2	2.1	(1321)	2.6	(1593)	−
	*it11*ts	0	(1170)	0[f]	(752)	−
	it28	0	(2618)	0[f]	(1643)	−
	it34	0	(2290)	0[f]	(1823)	−
zyg-9	*b279*	0	(513)	0	(600)	−
	*b288*ts	0	(1097)	0[f]	(982)	−
	b301	0	(891)	0[e]	(<100)[c]	−
	b307	0	(1128)	0[f]	(1235)	−
	it3	0	(1973)	0[f]	(1497)	−
let-237	*b283*	0.9	(1043)	1.9	(1088)	±
mel-8	*b309*	0	(1074)	86	(666)	+
	b312	0	(602)	19	(692)	+
	it39	0	(457)	71	(808)	+
mel-9	*b293*ts	1.4	(414)	85	(311)	+
mel-10	*it10*	0.5	(955)	0	(461)	−
mel-11	*it26*	0	(1077)	86	(702)	+
mel-12	*it42*	7.2	(1420)	27	(922)	+
Region 3						
let-29	*b285*	0	(106)	0	(187)	−
ooc-1	*b316*	0	(204)	0	(483)	−
mel-13	*b306*	0	(582)	0	(860)	−
Region 4						
mel-14	*it24*	0.2	(1452)	0.5	(1409)	±
	b291	0	(480)	1.7	(621)	+
	b292	0.1	(1035)	2.6	(873)	±
mel-15	*it7*ts	0.9	(1486)	92	(2085)	+
	it38	25	(1032)	96	(1481)	+
mel-16	*b298*	ND[b]		ND		?
mel-17	*b299*	4.7	(514)	18	(325)	+
mel-18	*b300*	0	(779)	0.2	(611)	+
mel-19	*b310*	4.7	(1286)	1.5	(825)	−
mel-20	*b317*	0	(213)	94	(121)	+
mel-21	*it9*	0	(931)	0	(938)	−
mel-22	*it30*	10.8	(166)	7.8	(104)	−

[a] "+" is assigned to cases where all self progeny die but some cross progeny live and to cases where the percentage of viable cross progeny exceeds the percentage of viable self-progeny by greater than two-fold and the difference in the percentage of viable embryos is greater than 5%. "−" is assigned to cases where no viable progeny are produced after mating or where the viability of cross progeny does not exceed the viability of self-progeny. "±" is assigned to all remaining cases.

[b] These two mutations were closely linked to putative *lin* mutations that prohibited the rescue test. ND = not determined.

[c] These embryos were obtained from hermaphrodites mated after depletion of their sperm.

[d] This strain was lost before careful analysis was done; in preliminary experiments none of eight mated hermaphrodites gave noticeably greater progeny hatch than unmated controls.

[e] These results were verified (or in two cases, obtained) by sperm depletion experiments as described in MATERIALS AND METHODS.

[f] plg-1 males were used for these crosses (see MATERIALS AND METHODS.)

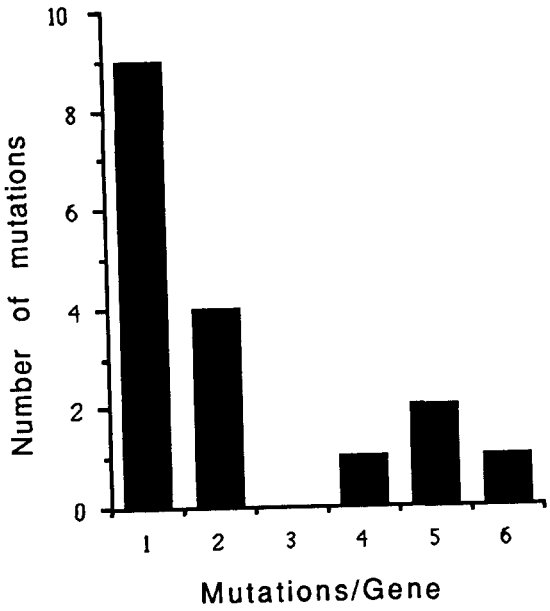

FIGURE 2.—Frequency distribution of strict maternal-effect lethal mutations.

stringent requirement for the gene function in embryogenesis. Rare mutations that affect embryonic-specific protein domains or that specifically affect regulation of maternal expression of constitutive genes could also lead to strict maternal-effect mutations. However, it is possible that the low frequency class of genes might also include mutations in pure maternal genes that are underrepresented either by chance or because of insensitivity to mutagenesis.

We have examined the phenotypes of the remaining two genes in the high frequency class. One of them appears to be a new *him* gene. Wild-type hermaphrodites (5 *AA*; *XX*) produce self-progeny males (5*AA*; *XO*) at a frequency of about one in 500; mutations in *him* genes ("high incidence of males") increase this frequency as a result of *X* chromosome nondisjunction (HODGKIN, HORVITZ and BRENNER 1979). Some homozygous *him* mutants exhibit weak maternal-effect lethal phenotypes, producing some inviable offspring as well as males, probably as a result of autosomal nondisjunction (HODGKIN, HORVITZ and BRENNER 1979). All five mutations in one of the genes in our high frequency class are leaky, producing about 5% viable self progeny. Because from 20 to 40% of the survivors are *XO* males, we have named the new gene *him-14*. Our preliminary evidence indicates that the maternal-effect lethality and the high incidence of males are due to extensive nondisjunction of all chromosomes in male and female meiosis (J. DUFFY, C. SZABO, M. BASL and K. KEMPHUES, unpublished data). If our interpretation of these phenotypes is correct, then *him-14* mutations, producing 95% embryonic inviability, are the strongest *him* mutations yet identified. Mutations in *him-14* are difficult to maintain as homozygous stocks and so would be unlikely to be recovered in screens designed to identify strains with high frequency production of males. Mutations in genes required exclusively for meiosis would be expected to exhibit maternal effects and would be expected to occur at frequencies similar to that found for *him-14*. Judging by the fact that we only identified one candidate on LG II, such genes are probably rare. Furthermore, if other such genes exist, screening for maternal-effect lethal mutations may be the best way to identify them.

The other gene with multiple alleles, *zyg-1*, appears to have a complex pattern of expression. Homozygotes for the original temperature-sensitive embryonic lethal allele, *zyg-1(b1)*, fail to lay eggs if shifted to nonpermissive temperature (25°) at the L_1 stage (VANDERSLICE and HIRSH 1976). In the original strain, this appeared to be due to a defect in gonadogenesis. We examined an existing *b1* strain and found that while some worms raised from L_1 at 25° have defective gonads, homozygotes fail to lay eggs primarily because of defective vulvae (Vul). Some homozygotes evert at the vulva; the remainder have a protruding vulva and do not lay eggs. In addition, homozygotes frequently exhibit an uncoordinated phenotype (Unc) and males have abnormal tail morphology. Unc, Vul and Mel phenotypes segregate together in outcrosses. After outcrossing, some strains have a higher or lower incidence of gonad defects. Three of the four new alleles we isolated have no detectable phenotype other than embryonic lethality. Homozygotes for the fourth mutant, *it4*, fail to produce viable progeny and move normally, but have a protruding vulva and fail to lay eggs. Heterozygotes of *b1* with any of the new alleles, when grown at 25°, exhibit wild-type movement and gonadogenesis. Worms doubly heterozygous for *b1* and *it25*, *it29*, or *it37* alleles have functional vulvae. *b1/it4* worms, however, exhibit a variable vulval defect that is less severe than either of the parental strains.

The Mel, Unc and Vul phenotypes of the *b1* strain are closely linked, since 28 recombinants between *dpy-10* and *lin-31* failed to separate them. It is possible that the Unc and Vul phenotypes are due to linked accessory mutations. However, because *it4* exhibits a Vul phenotype that is only partly complemented by *b1* and because VANDERSLICE and HIRSH (1976) were able to isolate revertants of *b1* that simultaneously restored both viability and egg laying, we think it likely that a single mutation is responsible for the Mel and Vul phenotypes.

Thus, among the genes mutating to strict maternal-effect lethality at frequencies typical for *C. elegans* genes are two pure maternal genes, *zyg-11* and *zyg-9*, one gene that may act only during meiosis, *him-14*, and one putative complex locus, *zyg-1*. Three of these genes had been identified in previous screens for

temperature-sensitive embryonic lethal mutations.

If our interpretation of the frequency distribution is correct, there are very few pure maternal genes on LG II. There are at least two, *zyg-11* and *zyg-9*. If we consider the likelihood that some pure maternal genes on LG II are not effectively balanced by *mnC1* and that some pure maternal genes are underrepresented in our collection due to chance or insensitivity to mutagenesis, it is still not likely that there are many more pure maternal genes on LG II. Therefore, assuming an even distribution of pure maternal genes over the six *C. elegans* linkage groups, we estimate that the total number of such genes could be as low as 12, and is probably not much higher.

This number is similar to the estimates in Drosophila. PERRIMON and his colleagues estimate that the number of genes on the *X* chromosome with oogenesis specific functions is 15 (PERRIMON *et al.* 1986). Of these, five mutate to strict maternal-effect lethality and are likely to be pure maternal genes. (Mutants for the other ten do not produce fertilized eggs.) Thus, since the *X* chromosome is about 1/5 of the Drosophila genome, Drosophila may have as few as 25 pure maternal genes.

Partial maternal-effect mutations: Mutations in 11 of our original set of 29 genes are rescued by embryonic expression of the wild-type allele. Therefore, the genes defined by these "partial" maternal-effect lethal mutations must be transcribed both by the maternal and the embryonic genomes. Because partial-loss-of-function alleles of constitutive genes could behave as partial maternal-effect lethals, it would be helpful to be able to identify genes in this class that might function exclusively in embryogenesis. By arguments analogous to those outlined above for pure maternal genes, genes that mutate to partial maternal-effect lethality at rates typical of essential genes are likely to have embryo-specific functions. Two genes, *mel-8*, with three partial maternal mutations, and *mel-14*, with one partial mutation and two strict mutations, might fit this category. We have not yet examined the phenotypes of these mutations in detail. Because some embryo-specific genes might mutate at typical rates to nonmaternal embryonic lethality and only rarely to partial maternal-effect lethality, it is possible that some of the genes identified by rare partial maternal-effect mutations might also be embryo-specific.

Summary and conclusions: The screening procedure is efficient for isolating strict maternal-effect mutations. Of 52 maternal-effect mutations tested, 39 are strict. Thirty-seven of these identify 17 genes that mutated only to this phenotype in our screens. Mutations in two known pure maternal genes were isolated at frequencies typical of loss-of-function alleles of *C. elegans* essential genes. Mutations in two other genes were recovered at similar frequencies but were not pure maternal genes. The remaining 13 genes are represented by only one or two mutations. Two of these are identical to genes previously identified by larval lethal mutations. We interpret these results to mean that although many *C. elegans* genes have the potential to mutate to strict maternal-effect lethality, most of these mutate only rarely to that phenotype. Pure maternal genes, on the other hand, are among a smaller class of genes for which strict maternal-effect mutations appear at much higher frequency.

After screening 12,000 chromosomes we think it likely that we have identified most of the pure maternal genes on the region of LG II balanced by *mnC1*, and therefore that there are probably not many more than 12 pure maternal genes in the *C. elegans* genome. We believe that a concerted effort to saturate the genome for such potentially informative genes is feasible.

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