CAENORHABDITIS ELEGANS DEFICIENCY MAPPING

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ABSTRACT

Six schemes were used to identify 80 independent recessive lethal deficiencies of linkage group (LG) II following X-ray treatment of the nematode *Caenorhabditis elegans*. Complementation tests between the deficiencies and ethyl methanesulfonate-induced recessive visible, lethal and sterile mutations and between different deficiencies were used to characterize the extents of the deficiencies. Deficiency endpoints thus helped to order 36 sites within a region representing about half of the loci on LG II and extending over about 5 map units. New mutations occurring in this region can be assigned to particular segments of the map by complementation tests against a small number of deficiencies; this facilitates the assignment of single-site mutations to particular genes, as we illustrate. Five sperm-defective and five oocyte-defective LG II sterile mutants were identified and mapped. Certain deficiency-by-deficiency complementation tests allowed us to suggest that the phenotypes of null mutations at two loci represented by visible alleles are wild type and that null mutations at a third locus confer a visible phenotype. A segment of LG II that is about 12 map units long and largely devoid of identified loci seems to be greatly favored for crossing over.

THE small free-living nematode *Caenorhabditis elegans* has many advantages as an experimental organism for the production and analysis of developmental mutants (BRENNER 1974; HERMAN and HOROVITZ 1980). Among the advantages are its relatively low DNA content (SULSTON and BRENNER 1974) and correspondingly small number of genes essential for normal development (BRENNER 1974). The number of genes identified by mutation is increasing rapidly; the most recent genetic map includes 437 loci mapped to the six linkage groups (M. SWANSON and D. RIDDLE, personal communication). Many different criteria, ranging from defects in behavior, morphology, cell lineage or enzymatic activity to lethal and sterile phenotypes, have been used in identifying mutants. It is important in this genetic approach to development to identify independent mutations that are allelic; such identifications bear both on the possible range of phenotypes that mutations in a given gene are capable of and on the question of total gene number and genome organization. As the number of identified mutants of similar phenotype increases, it becomes increasingly useful to map mutations fairly precisely before embarking on tests for allelism. Although the ordering of point mutations on the *C. elegans* map by two-factor and three-factor crosses is generally straightforward, the use of

multiply marked chromosomes is usually not possible because of a limitation in the number of distinct recombinant phenotypes that can be scored reliably in a single cross (BRENNER 1974). Precise mapping is also often made tedious by the tendency of many mutations on each autosome to map within a few map units of each other, in a cluster (BRENNER 1974; RIDDLE and SWANSON 1982). An alternative method for mapping point mutations involves the use of a set of overlapping deficiencies with various endpoints, which then define segments of the map; each point mutation is assigned to a given segment on the basis of complementation tests against the set of deficiencies. This approach has been used with *C. elegans* for a portion of the *X* chromosome (MENEELY and HERMAN 1979, 1981) and for small regions of LG I (ROSE and BAILLIE 1980) and LG IV (ROGALSKI, MOERMAN and BAILLIE 1982). The aim of the work reported here is to extend this general strategy to a large portion of an autosome.

We chose LG II for this work because of the existence of a good LG II balancer chromosome for maintaining deficiency heterozygotes (HERMAN 1978). To characterize the extents of the deficiencies, we extended our collection of ethyl methanesulfonate (EMS)-induced recessive lethal mutations, also maintained in balanced heterozygous stocks (HERMAN 1978). The lethals, along with visible mutations mapping in the region, were used in complementation tests against the deficiencies to develop a self-consistent genetic map. We have also studied two specific classes of LG II sterile mutants: spermatogenesis defective and oocyte defective.

Deficiencies are used not only for mapping purposes. For example, the phenotype of a particular mutation opposite a deficiency can be informative: the phenotype of a null (amorphic) mutation opposite a deficiency should be the same as that of a null homozygote, whereas an underproducing (hypomorphic) mutation or an overproducing (hypermorphic) mutation opposite a deficiency should give a more severe or less severe phenotype, respectively, than the homozygous mutant. Genetically defined deficiencies may also prove to be technically useful in the analysis of genome organization by recombinant DNA methods.

MATERIALS AND METHODS

Strains and general procedures: *C. elegans* var. Bristol (wild-type strain designated N2) was obtained from S. BRENNER. The following previously identified genes and mutations were used: *bli-1(e769), dpy-10(e128), dpy-11(e224), rol-1(e91), unc-4(e120)* and *unc-52(e444)* (BRENNER 1974); *tra-2(e1094)* (HODGKIN and BRENNER 1977); *dpy-2(e489), rol-5(sc13), rol-6(e187)* and *sqt-1(sc1)* (COX *et al.* 1980); *lin-5(e1348)* (HORVITZ and SULSTON 1980); *emb-27(g48)* (CASSADA *et al.* 1981); *vab-9(e1744)* (HODGKIN 1983); and *let-19(mn19), let-22(mn22), let-23(mn23), let-24(mn24), let-25(mn25), let-26(mn26), let-27(mn27), let-28(mn28), let-29(mn29), let-30(mn30)* and *let-31(mn31)* (HERMAN 1978). We showed that *tra-?(mn21)* (HERMAN 1978) failed to complement *tra-2(e1094)*, and *mn21* was used in this study as a *tra-2* allele. We obtained *lin-29(n836)* from V. AMBROS, *unc-104(e1265)* from the Caenorhabditis Genetics Center, *unc-105(n490)*) from J. PARK and *let-23(n1045)* from E. FERGUSON. With the exception of *dpy-11 V*, all of these genes are on LG II.

Media and culture techniques were as described by BRENNER (1974). Mating and mapping methods were as described by BRENNER (1974) and HERMAN (1978). Genetic nomenclature follows HORVITZ *et al.* (1979). Male stocks of heterozygous strains were established after generating males by heat shock (HERMAN 1978).

Induction of deficiencies by X rays: Six procedures were used to screen for recessive lethal deficiencies of LG II. All deficiencies were maintained in heterozygotes with a *C1 dpy-10 unc-52* chromosome as balancer. *C1* is a dominant crossover suppressor for the *dpy-10* to *unc-52* interval that maps very close to these markers (HERMAN 1978). All deficiency-bearing chromosomes either carried *unc-4(e120)* as a tag or were deficient for at least a part of the *unc-4* gene. Two- to three-day-old hermaphrodites were X irradiated at a dose of 7000–8000 r (MENEELY and HERMAN 1979). In those cases in which the mutagenized animals were crossed, both males and hermaphrodites were transferred to fresh plates after 1 and 2 days, and all plates were subsequently screened. To ensure that all deficiencies were independently derived, only one deficiency stock was retained from any given mating.

Deficiencies of dpy-10: *unc-4* hermaphrodites were irradiated and mated with *C1 dpy-10 unc-52/unc-4* males (in some cases these males carried *rol-1, rol-5* or *rol-6* in place of *unc-4*). The progeny were screened for Dpy non-Unc hermaphrodites (putative genotype: *C1 dpy-10 unc-52/unc-4 Df*), which were backcrossed to *C1 dpy-10 unc-52/unc-4* males; Unc-4 progeny (putative genotype: *unc-4/unc-4 Df*) were picked and crossed to *C1 dpy-10 unc-52/unc-4* males. Dpy hermaphrodite progeny were picked and used to initiate *C1 dpy-10 unc-52/unc-4 Df* stocks. Stocks were retained only if they did not segregate Dpy Unc self-progeny; this required that each deficiency extend into at least one essential gene. The frequency of recovery of a deficiency per screened chromosome was about 0.09%.

Deficiencies of rol-6: Irradiated *unc-4* or *unc-4; dpy-11* hermaphrodites were crossed with *C1 dpy-10 unc-52/rol-6* males, and the progeny were screened for Rol non-Unc hermaphrodites, which were crossed with *C1 dpy-10 unc-52/unc-4 rol-6* males. Unc Rol cross-progeny were picked (putative genotype: *unc-4 rol-6/unc-4 Df*) and mated with *C1 dpy-10 unc-52/unc-4 rol-6* males. Wild-type progeny were picked, and deficiency stocks were established from those animals that gave no Unc self-progeny, thus again requiring that the deficiency be recessive lethal. The frequency of deficiency recovery was about 0.06%.

Deficiencies of rol-5: The procedure here was the same as for *rol-6* except that *rol-5* was used instead of *rol-6*. In the case of *rol-5*, however, many of the Rol candidates proved upon subsequent testing not to carry a *rol-5* mutation; they were apparently X-ray-induced phenocopies. Deficiencies of *rol-5* were obtained at a frequency per screened chromosome of about 0.04%.

Deficiencies of unc-4: Two- to three-day-old N2 males were X irradiated (7500–9000 r) and mated with *unc-4; dpy-11* hermaphrodites. The progeny were screened for Unc non-Dpy hermaphrodites (putative genotype: *unc-4/Df*), which were crossed with *C1 dpy-10 unc-52/unc-4* males. Wild-type progeny were picked, and only those that did not segregate Unc-4 self-progeny were retained. Additional outcrosses were performed as follows. The putative *C1 dpy-10 unc-52/Df* hermaphrodites were crossed with *C1 dpy-10 unc-52/unc-4* males, and Unc-4 progeny were picked and crossed again with *C1 dpy-10 unc-52/unc-4* males. Wild-type hermaphrodite progeny that did not segregate Unc offspring were then used to establish deficiency stocks. The frequency of deficiency recovery was about 0.05%.

Deficiencies of unc-105: Animals heterozygous for *unc-105(n490)* are small and uncoordinated (homozygotes are even more severely affected). J. PARK and R. HORVITZ (personal communication) identified *unc-105(n490)* and showed that the elimination of *n490* by a deficiency results in a heterozygous wild-type phenotype. *C1 dpy-10 unc-52/unc-4 unc-105* hermaphrodites were irradiated, put on small plates in groups of four and transferred to fresh plates after 1 and 2 days. The plates were subsequently screened for wild-type progeny, which were crossed to *C1 dpy-10 unc-52/unc-4 rol-5* males. Unc-4 hermaphrodite progeny (putative genotype: *unc-4 rol-5/unc-4 Df*) were picked from broods lacking Unc-105 animals. The Unc-4 hermaphrodites were crossed to *C1 dpy-10 unc-52/unc-4 rol-5* males, and wild-type progeny were picked. Deficiency stocks were initiated from broods lacking Unc-4 Rol and Unc-4 progeny. Some revertant stocks gave viable Unc-4 progeny; these carried reversions of *unc-105(n490)* that were homozygous viable and closely linked to *unc-105* (see RESULTS).

Deficiencies recovered in a screen for recessive lethals: The final deficiency screen did not require the uncovering or elimination of a particular visible marker but was in principle capable of recovering deficiencies anywhere on LG II balanced by *C1 dpy-10 unc-52*. Hermaphrodites homozygous for *unc-4* were irradiated and mated individually with *C1 dpy-10 unc-52/unc-4* males. Five to eight wild-type progeny were picked from each mating, and their broods were screened for the

absence of Unc-4 animals, which indicated the presence of a linked recessive lethal mutation. Wild-type hermaphrodites carrying such lethals were picked and crossed with *C1 dpy-10 unc-52/unc-4* males. Unc-4 progeny (*unc-4/unc-4 let*) were picked and crossed to *C1 dpy-10 unc-52/unc-4* males. Wild-type progeny were picked, and lethal-bearing stocks were initiated from broods lacking viable Unc-4 self-progeny. Five lethal mutations were designated deficiencies because they failed to complement mutations representing at least two different complementation groups. Eighty-eight other lethals generated in this procedure were discarded.

Identification and complementation of EMS-induced recessive lethal mutations balanced by C1 dpy-10 unc-52: Young adult *unc-4* hermaphrodites were treated with EMS (HERMAN 1978) and individually mated with *C1 dpy-10 unc-52/unc-4* males. Five to eight wild-type F_1 progeny were placed individually on fresh plates, and their broods were screened. Broods lacking adult Unc-4 animals (about 5% of the viable broods) were presumed to be derived from animals that carried a recessive lethal linked to *unc-4*. Wild-type animals were picked from these broods and outcrossed twice to *C1 dpy-10 unc-52/unc-4* males, as in the procedure described for X-ray-induced lethals. Previously established lethal stocks that carried *him-1(e879) I* (HERMAN 1978) were also outcrossed, and new lethal stocks not carrying *him-1* were used in the experiments reported here. About 60% of the original lethal candidates were finally kept and used in the deficiency mapping; the principal reason for discarding candidates was poor growth as heterozygotes, but some were discarded because their patterns of complementation indicated that they carried two mutations.

Complementation tests were performed with all pairs of closely mapping recessive lethals. Males of one heterozygous stock were mated with hermaphrodites of the other, and the progeny were screened for viable Unc-4 animals, which indicated that the mutations complemented; the presence of male progeny signified a successful mating. The following gene and lethal mutation assignments were made: *let-23(mn224, mn216), let-28(mn212), let-29(mn182), let-30(mn239), let-31(mn223), let-236(mn88), let-237(mn208), let-238(mn229), let-239(mn217, mn93), let-240(mn209), let-241(mn228), let-242(mn90), let-243(mn226), let-244(mn97), let-245(mn185, mn221), let-246(mn99), let-247(mn211), let-248(mn237), let-249(mn238), let-250(mn207), let-251(mn95), let-252(mn100), let-253(mn181, mn184), let-254(mn214), let-255(mn186, mn236), let-256(mn231), let-257(mn235), let-258(mn206), let-259(mn210), let-260(mn232), let-261(mn233), let-262(mn87), let-263(mn240), let-264(mn227), let-265(mn188), let-266(mn194), let-267(mn213), let-268(mn189, mn198), let-269(mn201), let-270(mn191)* and *let-271(mn193)*.

Identification of EMS-induced recessive sterile mutations balanced by C1 dpy-10 unc-52: This procedure was identical to that for the recessive lethal screen up to the point where the broods initiated by F_1 animals were screened. When such broods contained Unc-4 animals, five to eight were picked from each brood and transferred as a group to test their fertility. When the group of Unc-4 animals proved to be sterile (about 5% of those tested), wild-type sibs (putative genotype: *C1 dpy-10 unc-52/unc-4 ste*, where *ste* signifies a recessive sterile mutation) were picked, and the fertility of their Unc-4 self-progeny was tested. In this way, 99 temporary stocks were established. We then limited our attention to two specific classes. The first class, sperm-defective mutants, was defined operationally as follows: the *ste unc-4* hermaphrodites were mated with *C1 dpy-10 unc-52/unc-4* males. If wild-type male progeny were produced, they were backcrossed to *unc-4 ste* virgin hermaphrodites; the *ste* mutation was deemed sperm defective if Unc-4 progeny issued from this latter cross. Five mutants satisfied these criteria and were outcrossed by mating *unc-4 ste* hermaphrodites with *C1 dpy-10 unc-52/unc-4* males and picking wild-type hermaphrodite progeny. The outcrossing was then repeated at least twice. The second class, oocyte-defective mutants, was defined as those that as homozygotes (recognized by their Unc-4 phenotype) laid fertilized eggs (with a hard shell; HIRSH and VANDERSLICE 1976), but the eggs either did not hatch or hatched to give defective progeny and the oocytes produced by the Unc-4 hermaphrodites were not rescuable by fertilization with N2 sperm. Seven mutants satisifed these criteria (the eggs laid by all seven mutants did not hatch); heterozygous stocks of all seven mutants were outcrossed at last twice by the procedure described for X-ray-induced lethals.

Complementation tests involving deficiencies: EMS-induced lethal mutations were assigned to segments of LG II by complementation tests against a number of deficiencies. *C1 dpy-10 unc-52/unc-4 let* males (where *let* signifies a lethal) were mated with *C1 dpy-10 unc-52/unc-4 Df* hermaphrodites; complementation was indicated by the production of viable Unc-4 progeny. This same protocol

was used for complementation tests involving pairs of deficiencies; only certain pairs were tested, and we were not successful in establishing fertile male stocks for some deficiencies.

Complementation tests between recessive sterile mutations and deficiencies were conducted as follows: *C1 dpy-10 unc-52/unc-4 ste* males were mated with *C1 dpy-10 unc-52/unc-4 Df* hermaphrodites; Unc-4 (*unc-4 ste/unc-4 Df*) hermaphrodite progeny were picked, and their fertility was checked.

The temperature-sensitive allele of *emb-27* was tested as follows: homozygous *emb-27* males were maintained at 15° and mated with different deficiency stocks at 20°. The mating plates were shifted to 25° after 2 days and incubated for another 1–2 days. F_1 L4 hermaphrodites were picked and cultured individually at 25°. The fertility of the *unc-4 Df/emb* hermaphrodite cross-progeny (all other progeny segregated Dpy Unc-52 animals) was then ascertained: sterility indicated noncomplementation.

A *C1 dpy-10 unc-52/unc-4 tra-2* male stock was used to test for complementation between deficiencies and *tra-2*; the transformation of Unc-4 hermaphrodite cross-progeny into pseudomales (HODGKIN and BRENNER 1977) indicated noncomplementation.

Recessive mutations conferring a visible phenotype were tested by crossing males heterozygous for a visible with deficiency stocks and looking among the F_1 progeny for the visible phenotype. Two of the visibles, *lin-5* and *lin-29*, were maintained in balanced stocks; *lin-5* is recessive sterile, and *lin-29* homozygotes, owing to the absence of a functional vulva, do not mate as hermaphrodites.

Because males heterozygous for *unc-105(n490)* are poor maters, *C1 dpy-10 unc-52/unc-4 Df* males were mated with *C1 dpy-10 unc-52/unc-4 unc-105* hermaphrodites for complementation testing: the presence of male progeny with the Unc-105 phenotype indicated noncomplementation.

Tests involving *sqt-1* were performed by crossing *C1 dpy-10 unc-52/unc-4 Df* males and *sqt-1/sqt-1* hermaphrodites and screening for nonroller male progeny. Since *sqt-1/+* animals roll but homozygotes do not (COX *et al.* 1980), nonroller cross-progeny can only result from a failure of the deficiency to complement *sqt-1*.

The mutation *n1045* confers a cold-sensitive vulvaless phenotype and maps between *vab-9* and *unc-4* (E. FERGUSON and R. HORVITZ, personal communication). *C1 dpy-10 unc-52/n1045 unc-4* males were mated with various deficiency hermaphrodite stocks at 20°. The mating plates were transferred to 15° after 2 days, and the vulval phenotype of the Unc-4 progeny was subsequently determined.

Recombination mapping: Linkage map distances from *unc-4* to various lethal mutations were obtained by crossing *C1 dpy-10 unc-52/unc-4 let* males with *dpy-10* or *rol-5* or *rol-6* hermaphrodites and picking wild-type hermaphrodite progeny. (For the *rol-5* and *rol-6* crosses, only those wild-type hermaphrodites carrying the *unc-4 let* chromosome were studied further.) These animals were allowed to self-fertilize, and their progeny, including viable Unc-4 recombinants, were counted. Recombination frequencies were calculated as previously reported (HERMAN 1978). The *dpy-10, rol-5* and *rol-6* markers were used for three-factor ordering of the lethal relative to *unc-4* and either *dpy-10, rol-5* or *rol-6*. The order of lethals near *unc-105* was obtained by crossing *C1 dpy-10 unc-52/unc-4 let* males with *C1 dpy-10 unc-52/unc-105* hermaphrodites and isolating *unc-4 let/unc-105* hermaphrodites. These animals, which are small and uncoordinated, owing to the presence of the semidominant *unc-105* mutation, segregate wild-type recombinants when the lethal is to the right of *unc-105* and Unc-4 recombinants when the lethal occurs to the left of *unc-105*. Map distances (and directions) of certain sterile mutations from *unc-4* were obtained by crossing *C1 dpy-10 unc-52/unc-4 ste* males with *dpy-10* hermaphrodites and picking wild-type hermaphrodite progeny. Unc-4 self-progeny were picked, five per plate. The frequency of fertile Unc-4 recombinants, R, was calculated from the fraction of plates showing no offspring, $f = (1 - R)^5$. Plates showing offspring were screened for the presence or absence of Dpy (Unc) animals.

RESULTS

Identification of deficiencies: Eighty independently derived deficiencies were identified by six different schemes, described in MATERIALS AND METHODS. Four of the schemes involved uncovering recessive visible mutations in heter-

ozygotes to give a visible phenotype. In addition, we required that each deficiency be recessive lethal and, therefore, impinge on at least one essential gene. Our collection includes 21 deficiencies from the screen for *dpy-10* deficiencies, 13 from the *rol-6* screen, seven from the *rol-5* screen and 18 from the *unc-4* screen. The frequencies of recovery of these deficiencies were about 0.04–0.09% per screened chromosome.

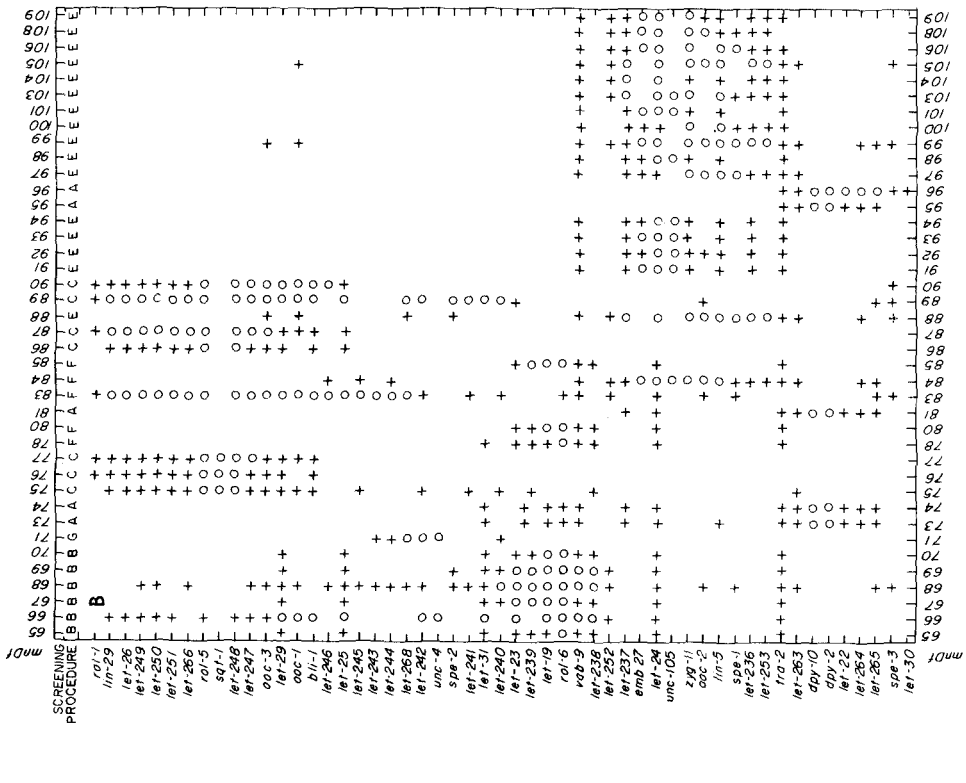
The fifth scheme was suggested to us by J. PARK and R. HORVITZ, who kindly provided the mutation *unc-105(n490)*, which is semidominant to its wild-type allele. We recovered 16 recessive lethal deficiencies by this method. An additional five mutations that eliminated the semidominant effect of *n490* and that mapped near *n490* were homozygous wild type. These mutations were apparently either intragenic point mutations or small deficiencies; the four we tested were similar to the eight recessive lethal deficiencies that were tested in that they gave a phenotype like that of the *n490* homozygote when put opposite *n490*.

The sixth scheme was simply a screen for X-ray-induced recessive lethal mutations anywhere in the region that failed to complement at least two EMS-induced mutations. Five deficiencies were recovered by this last method. The method by which each deficiency was identified is indicated in Figure 1. All of the deficiency stocks in our collection have been outcrossed at least twice, as described in MATERIALS AND METHODS.

The deficiency map: Each of the deficiencies was tested for complementation with a number of recessive lethal, sterile and visible mutations, as described in MATERIALS AND METHODS: mutations in 51 genes failed to complement at least one of the deficiencies. The results of the complementation tests, given in Figure 1, allowed us to assign mutations to specific segments, defined by one or more deficiencies. Most of the visibles and a few of the lethals had been ordered with respect to each other previously. In many cases this information in combination with the complementation results allowed us to define the extents of the deficiencies and to order previously unmapped mutations. In some cases, however, three-factor crosses were required to order point mutations and hence the deficiencies they occurred under. The results of the three-factor crosses are given in Table 1. Finally, results of deficiency-by-deficiency complementation tests (given later) were also taken into account in drawing the map of the region, which is given in Figure 2.

A few assignments of map position remain tentative. The assignment of *let-263* to the right of *dpy-10* is probably correct because if it occurred to the left of *dpy-10* it would also have to be to the left of *let-265*, based on the deficiency mapping; recombination between *dpy-10* and *let-265* was detected in the three-

FIGURE 1.—Results of complementation tests between visible, lethal and sterile mutations and deficiencies. + indicates complementation, O indicates noncomplementation; no symbol indicates the test was not performed. The screening procedure employed in the initial identification of each deficiency (see MATERIALS AND METHODS) is indicated by a letter: A, screen for deficiencies of *dpy-10*; B, *rol-6* screen; C, *rol-5* screen; D, *unc-4* screen; E, *unc-105* screen; F, screen for X-ray-induced lethals; and G, *mnDf71* was recovered after EMS treatment (see RESULTS).


B
SCREENING PROCEDURE
mnDf
rol-1
lin-29
let-26
let-249
let-250
let-251
let-266
rol-5
sqt-1
let-248
let-247
ooc-3
let-29
ooc-1
bli-1
let-246
let-25
let-245
let-243
let-244
let-268
let-242
unc-4
spe-2
let-241
let-31
let-240
let-23
let-239
let-19
rol-6
vab-9
let-238
let-252
let-237
emb-27
let-24
unc-105
zyg-11
ooc-2
lin-5
spe-1
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let-253
tra-2
let-263
dpy-10
dpy-2
let-22
let-264
let-265
spe-3
let-30

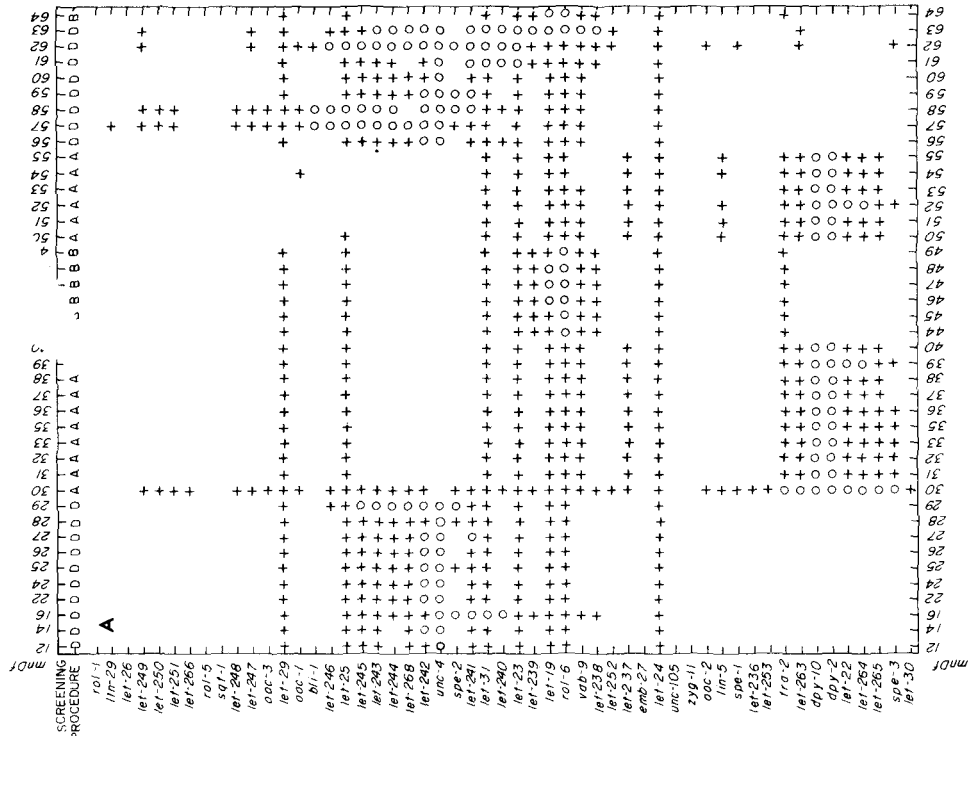
A
SCREENING PROCEDURE
mnDf
rol-1
lin-29
let-26
let-249
let-250
let-251
let-266
rol-5
sqt-1
let-248
let-247
ooc-3
let-29
ooc-1
bli-1
let-246
let-25
let-245
let-243
let-244
let-268
let-242
unc-4
spe-2
let-241
let-31
let-240
let-23
let-239
let-19
rol-6
vab-9
let-238
let-252
let-237
emb-27
let-24
unc-105
zyg-11
ooc-2
lin-5
spe-1
let-236
let-253
tra-2
let-263
dpy-10
dpy-2
let-22
let-264
let-265
spe-3
let-30

TABLE 1

Three-factor crosses

Genotype of heterozygote	Genotypes of Unc-4 recombinants (with respect to *trans* marker)	Phenotypes of non-Unc-105 recombinants [a]
let-30 unc-4-/dpy-10	15 *dpy-10*/+, 3 +/+	
let-265 unc-4/dpy-10	45 *dpy-10*/+, 4 +/+	
let-22 unc-4/dpy-10	8 *dpy-10*/+, 1 +/+	
let-264 unc-4/dpy-10	50 *dpy-10*/+, 2 +/+	
let-263 unc-4/unc-105	59 *dpy-10*/+, 0 +/+	
let-236 unc-4/unc-105		8 Unc-4, 0 WT
let-253 unc-4/unc-105		4 Unc-4, 0 WT
let-24 unc-4/dpy-10	11 *dpy-10*/+, 0 +/+	
let-237 unc-4/unc-105		0 Unc-4, 3 WT
let-252 unc-4/unc-105		0 Unc-4, 3 WT
let-252 unc-4/dpy-10	3 *dpy-10*/+, 0 +/+	
let-239 unc-4/rol-6	92 *rol-6*/+, 0 +/+	
let-23 unc-4/dpy-10	3 *dpy-10*/+, 0 +/+	
let-31 unc-4/dpy-10	6 *dpy-10*/+, 0 +/+	
let-25 unc-4/dpy-10	0 *dpy-10*/+, 3 +/+	
let-29 unc-4/dpy-10	0 *dpy-10*/+, 6 +/+	
unc-4 let-247/rol-5	64 *rol-5*/+, 0 +/+	
unc-4 let-26/rol-5	1 *rol-5*/+, 4 +/+	
unc-4 let-249/rol-5	10 *rol-5*/+, 3 +/+	
unc-4 let-250/rol-5	11 *rol-5*/+, 1 +/+	

[a] WT = wild type.

factor cross, as expected for a lethal to the left of *dpy-10*, but recombination between *let-263* and *dpy-10* was not detected (Table 1). We are less confident of our assignment of *let-239* to the right of *rol-6*. It is based on the fact that 0/92 crossovers between *let-239* and *unc-4* came between *let-239* and *rol-6*; thus, a position extremely close and to the left of *rol-6* is possible. On the basis of similar reasoning, it is possible that *let-247* is located extremely close and to the right of *rol-5*, rather than to the left, where we have tentatively placed it. If the assignment of *let-239* is incorrect, the region including *rol-6, let-19* and *let-239*, as well as the underlying deficiencies, should be rotated 180° about *rol-6*. Similarly, the placement of *let-247* affects the extents of *mnDf77* and *mnDf87*. Finally, the complementation behavior of *unc-104* (not indicated in Figure 1) deserves comment. We were able to construct *trans* heterozygotes of *unc-104* opposite *mnDf31, mnDf39, mnDf45, mnDf52* and *let-265*, all of which were wild type phenotypically, but we were unable to make animals heterozygous for *unc-104* and either *mnDf30* or *mnDf96*. This result together with the mapping of *unc-104* about 0.15 map unit left of *dpy-10* (S. BROWN, personal communication) leads us to suggest that *unc-104(e1265)* opposite a null allele is lethal and that *unc-104* maps where we have placed it in Figure 2.

The region from the left of *dpy-10* to the right of *rol-5* is not entirely covered by deficiencies; there is at least one and at most two gaps. One gap lies between *let-237* and *vab-9* (or *let-238*); *let-252* appears to occur in this gap because three-factor crosses place it between *unc-105* and *unc-4*, and it complements overlapping deficiencies representing all segments between these

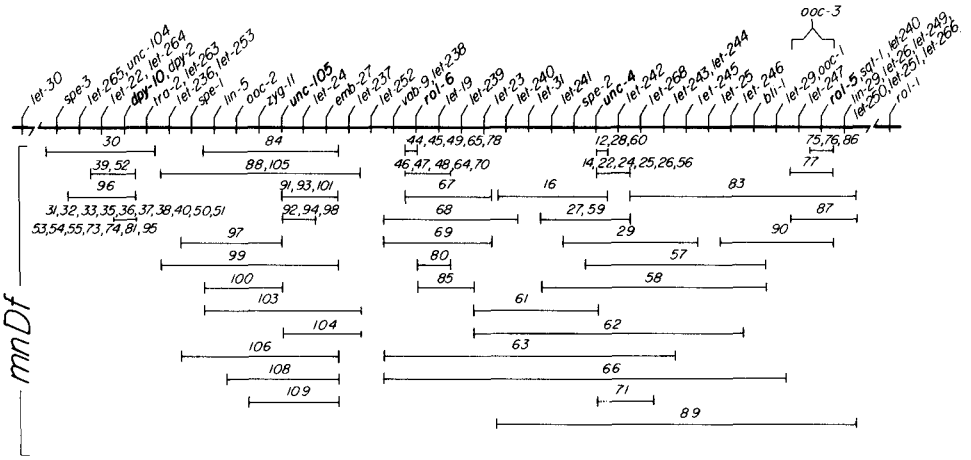

FIGURE 2.—Genetic map showing ordering of point mutations and the extents of the deficiencies on LG II. See text for discussion. The relative map order of genes *tra-2, lin-5, zyg-11, unc-105, let-24* and *vab-9* was determined by J. PARK (personal communication). V. AMBROS has positioned *lin-29* to the right of *rol-5* (personal communication). As explained in the text, it is possible that the segment including *rol-6, let-19* and *let-239* (and the underlying deficiencies) should be rotated 180° about *rol-6*; similarly, it possible that *let-247* lies extremely close and to the right of *rol-5* (see text). Distinct sites on the map were arbitrarily positioned at equal intervals. The *dpy-10* to *unc-4* distance is about 1.6 map units (HERMAN 1978).

markers except for the gap segment. An alternative possibility, which we consider unlikely, is that *let-252* opposite a deficiency gives a wild-type phenotype. There may or may not be a gap between the right end of *mnDf30* and the left ends of *mnDf88, mnDf99* and *mnDf105* (which are indistinguishable). No point mutations have been found in this region, and it is possible that *mnDf30* overlaps at least one of the other three deficiencies; we have been unable to carry out complementation tests involving any of these four deficiencies (see following data).

Complementation tests between deficiencies: The results of complementation tests involving pairs of deficiencies, which are given in Figure 3, allowed us to establish the endpoints of some deficiencies more precisely and also provided evidence pertaining to the null phenotypes of three genes. We were limited in this analysis by our inability to establish male stocks of some of the larger deficiencies.

Some of the deficiencies that were selected on the basis of their noncomplementation of *rol-6* complemented each other. Animals with *mnDf85* or *mnDf80* opposite *mnDf44, mnDf45, mnDf49, mnDf65* or *mnDf78* were fertile and non-Rol. This strongly suggests that the null phenotype of *rol-6* is wild type.

Some of the deficiencies that extended into *unc-105* also complemented each other, although in these cases complementation was generally incomplete. Animals carrying either *mnDf97* or *mnDf100* opposite *mnDf91, mnDf92, mnDf94, mnDf98* or *mnDf101* were non-Unc-105 and fertile, but their offspring were small and gave few progeny, which were sickly, although fertile. Animals with either *mnDf97* or *mnDf100* opposite *mnDf93* were non-Unc-105 and viable but sterile. One possible interpretation of these results is that the null phenotype

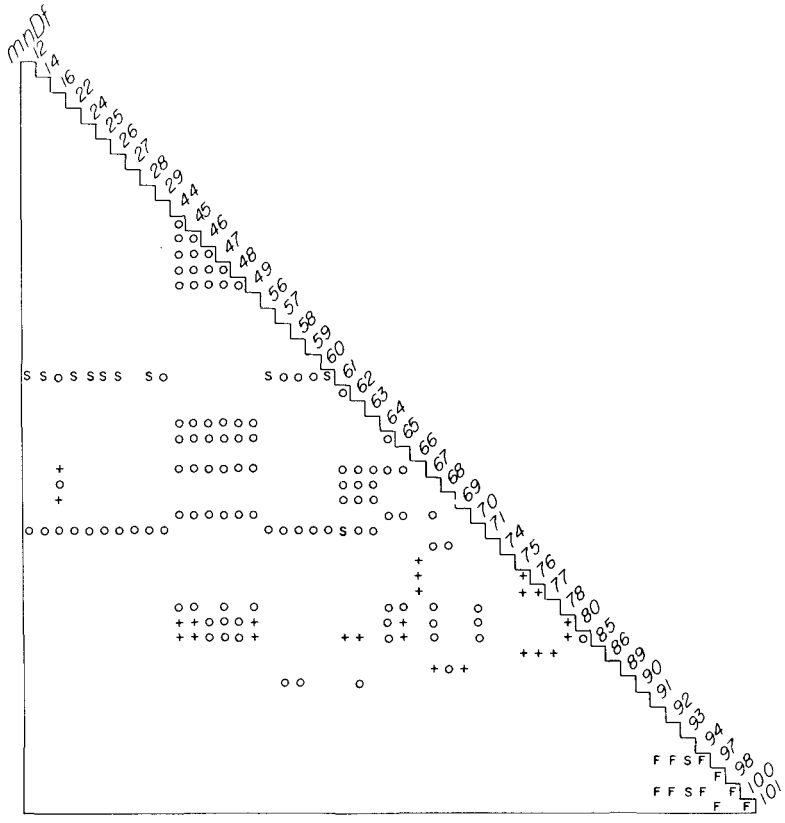

FIGURE 3.—Results of complementation tests between deficiencies. O indicates noncomplementation; S indicates that the *trans* double heterozygote was a sterile adult; F indicates that the *trans* double heterozygote was a fertile adult but that the progeny were sickly and weakly fertile; + indicates full complementation: the *trans* double heterozygote was fertile and its progeny were healthy and fertile.

of *unc-105* is wild type and that the incomplete nature of the complementations is due to overlap of the deficiencies in one or more genes near *unc-105*. This interpretation is supported by the growth properties of the five *unc-105* mutants that showed noncomplementation with *unc-105(n490)* but were homozygous viable (described in connection with the identification of *unc-105* deficiencies); three of the five grew well for several generations as homozygotes, but two when made homozygous gave progressively sicker offspring in the succeeding two generations; we suggest that the latter strains suffered from some of the same defects that affected the partially complementing deficiency strains.

Animals carrying *mnDf61* opposite *mnDf12, mnDf14, mnDf22, mnDf24, mnDf25, mnDf26, mnDf56* or *mnDf60* were Unc-4. This strongly suggests that this is the null phenotype of *unc-4*, since, unlike many of the deficiency stocks, none of these carried the *unc-4(e120)* mutation. Here too, the complementation was partial: all of the Unc-4 animals carrying two deficiencies were sterile, and

again we suggest that both members of each pair of partially complementing deficiencies extended into a gene affecting fertility, although it is also possible that the null phenotype of *unc-4* is sterile (and uncoordinated). We note that *mnDf61* opposite *mnDf71* also gave a sterile Unc-4 animal; *mnDf71* was first identified as a recessive lethal mutation, called *let-20(mn20)* (HERMAN 1978), on an *unc-4(e120)*-bearing chromosome; we discovered in the present work that it failed to complement two complementing lethals very near *unc-4*, but we do not know whether it extends into *unc-4* or still carries *unc-4(e120)*.

We encountered one discrepancy between the results of the deficiency-by-deficiency complementation tests and the map shown in Figure 1. *mnDf74* does not extend much to the right of *dpy-10* since it complements *tra-2* and *let-263*. Nonetheless, it fails to complement *mnDf67* and *mnDf68*, which map much farther to the right of *dpy-10*. The most likely explanation is that the *mnDf74*-bearing chromosome also has a lethal mutation that occurs under the other deficiencies. This was the only such anomaly we encountered in the construction of the map.

Recessive lethal mutations balanced by C1 dpy-10 unc-52: A total of 58 recessive lethal mutations, selected after EMS mutagenesis on the basis of their close coupling to *unc-4* in stocks carrying the balancer chromosome *C1 dpy-10 unc-52*, have been outcrossed, mapped and tested for complementation against various deficiencies and closely linked lethals. Some of the lethals were identified in earlier screens (HERMAN 1978). The mutations occurred in 47 genes, which are listed, with allele assignments, in MATERIALS AND METHODS. Ten genes are represented by more than one allele. Only one gene has more than two alleles: *let-23* has three lethal alleles and one allele, *n1045*, that when homozygous confers a viable and fertile but vulvaless phenotype (E. FERGUSON and R. HORVITZ, personal communication). Thirty-one of the genes were located in the region of the deficiency map (although one fell in a deficiency gap, as already noted). Eight other genes (*let-27, let-30, let-260, let-261, let-262, let-267 let-270* and *let-271*) mapped to the left of *dpy-10* (and all complemented *mnDf30*), the measured distances from *unc-4* ranging from 1.4 to 4.6 map units. The remaining eight genes (*let-28, let-254, let-255, let-256, let-257, let-258, let-259* and *let-269*) mapped to the right of *unc-4*, at distances ranging from 17 to 23 map units; this puts them to the right of all markers shown in Figure 2 since *rol-1* is only about 5 map units to the right of *unc-4*.

The vast majority of lethal mutants had a phenotype of larval arrest; only three genes (*let-22, let-239* (both alleles) and *let-262*) are represented by what appear to be embryonic arrests: no Unc-4 larvae (or adults) were found among the self-progeny of the stocks heterozygous for the *unc-4* lethal chromosome.

Recessive sterile mutations balanced by C1 dpy-10 unc-52: As a test of our deficiency mapping procedure, we have identified several EMS-induced LG II recessive sterile mutations, which have been mapped by complementation testing against the set of deficiencies. We limited our efforts to two specific classes of sterile mutant. One class, sperm-defective mutants, was defined operationally as follows: homozygous mutant hermaphrodites were self-sterile, but their oocytes could be rescued by fertilization by mutant sperm produced in heterozy-

gous males. Five sperm-defective mutants were mapped. Three, *spe-1(mn47)*, *spe-2(mn63)* and *spe-3(mn230)*, occurred in the region covered by the deficiencies and are shown in Figure 2. A fourth mutation, *mn39*, mapped 3 map units to the left of *dpy-10*, and the fifth mutation, *mn252*, was located 19 map units to the right of *unc-4*. Two of the five mutants (*spe-1* and *mn252*) lay unfertilized eggs, a property of fertilization-defective mutants previously described (WARD, and MIWA 1978; ARGON and WARD 1980). The other three mutants, however, produce self-fertilized eggs that do not hatch, except for an occasional *mn39* egg that hatches to give a sterile animal, often with a notched head.

The other class of steriles, oocyte defectives, was defined by mutant hermaphrodites that did not produce oocytes rescuable by wild-type sperm but nevertheless lay self-fertilized eggs that did not hatch. Five oocyte-defective mutants were mapped. Four, *zyg-11(mn40)*, *ooc-1(mn250)*, *ooc-2(mn249)* and *ooc-3(mn241)*, occurred in the region covered by the deficiencies and are shown in Figure 2. The fifth mutation, *mn244*, mapped about 4 map units to the left of *dpy-10*. W. WOOD (personal communication) has shown that *zyg-11(mn40)* is suppressed by *sup-7(st5)* (WATERSTON 1980), a UAG suppressor (WILLS *et al.* 1983); *mn40* has been shown by N. WOLF, K. KEMPHUES, W. WOOD and D. HIRSH (personal communication) to be an allele of *zyg-11*, of which four additional alleles have been identified; work on one allele, *b2*, has been published (HIRSH and VANDERSLICE 1976; WOOD *et al.* 1980).

DISCUSSION

We have achieved the major goal of this work, which was to manufacture a set of overlapping deficiencies covering a substantial portion of LG II that could be used as a genetic mapping kit: the extents of 80 X-ray-induced deficiencies (and one deficiency recovered after EMS treatment) have been characterized with respect to a large number of EMS-induced recessive visible, lethal and sterile mutations, which were assigned to 36 ordered sites on LG II, largely on the basis of their patterns of complementation against the deficiencies, but also on the basis of certain three-factor crosses. New visible, lethal or sterile mutations in the region can now be assigned to particular segments of the map on the basis of their patterns of complementation against a small number of deficiencies; in some cases three-factor crosses may also be required. Deficiency mapping of new point mutations may discriminate between deficiency endpoints that are currently indistinguishable and hence increase the number of distinct segments of the map. The only prerequisite for deficiency mapping is that the phenotype of the mutation opposite a deficiency must be distinct from the phenotype of the mutation opposite its wild-type allele. The mapping of recessive lethal and sterile mutations is made much easier by having an *unc-4* mutation on the lethal- or sterile-bearing chromosome. All of our deficiency-bearing chromosomes either carry *unc-4(e120)* or are deficient for the *unc-4* gene; thus, the *trans* double heterozygote can be easily recognized by its Unc-4 phenotype. We have illustrated the usefulness of the method by localizing three sperm-defective and four oocyte-defective mutants in the re-

gion covered by the deficiencies. The recessive mutation *n1045*, which confers a vulvaless phenotype, also nicely illustrates the utility of our mapping kit. This mutation had been mapped between *vab-9* and *unc-4* by E. FERGUSON (personal communication). By deficiency mapping we placed it in the same segment occupied by *let-23*, and a complementation test between *let-23* and *n1045* showed that *n1045* was a viable and fertile allele of *let-23*, for which we have three recessive lethal alleles.

There are one or two gaps in the region of the map that we have studied that have not been uncovered by any deficiency. It is possible that each such gap contains a haplo-lethal site, which would provide an impenetrable barrier for any deficiency that is to be maintained in a heterozygous stock. We have no direct evidence for the existence of haplo-lethal loci, however, and it seems quite possible that further deficiency screening would fill in the gaps.

It is curious that, of the 21 deficiencies of *dpy-10* that we recovered, 17 were indistinguishable and did not uncover any known lethal or visible mutation other than *dpy-10* and *dpy-2*. There does not seem to be a sparsity of essential loci in the neighborhood of *dpy-10* (see following data). Another possible explanation we have considered is that many of the *dpy-10* mutations are not deficiencies but simply point mutations within *dpy-10*, which are recessive lethal. There are two difficulties with this explanation (neither of which is conclusive evidence against it): first, in our experience with X-ray-induced mutations at other visible loci, it seems that the majority are extended deficiencies (*i.e.*, recessive-lethal) rather than point mutations. GREENWALD and HORVITZ (1980) have provided better documentation on this point; they found that 10 of 17 γ-ray-induced mutations of *unc-93*, the null phenotype of which is wild type, were recessive lethal. The second difficulty with the notion that *dpy-10* is an essential locus is that visible alleles of *dpy-10* are readily obtained (BRENNER 1974; COX *et al.* 1980). Haplo-lethal loci bracketing *dpy-10* would limit the size of recoverable deficiencies, but we have identified four deficiencies that uncover three lethals on the left of *dpy-10* and one lethal and *tra-2* on the right. The explanation we favor, therefore, is simply that there are preferential breakpoints near *dpy-10* that tend to produce small deficiencies.

We found several examples at three loci where two deficiencies isolated on the basis of their uncovering a visible locus complemented each other. In the case of *rol-6*, the complementing deficiencies were fertile and non-Rol, suggesting that the null *rol-6* phenotype is wild type. This suggestion is supported by the existence of an allele of *rol-6* that when homozygous confers a wild-type phenotype (COX *et al.* 1980). Examples of complementing pairs of deficiencies that extended into *unc-105* from opposite sides were also found. In these cases, however, it appeared that the deficiencies overlapped one or more subvital loci because the animals heterozygous for the two deficiencies were partially or wholly sterile. In this case too, the complementing deficiencies gave a non-Unc-105 phenotype, in support of the suggestion of J. PARK and R. HORVITZ that the null *unc-105* phenotype is wild type. Also supporting this conclusion were nonlethal mutations we identified that showed noncomplementation with *unc-105(n490)* but that were homozygous wild type. We note that *unc-105(n490)*, which is semidominant to *unc-105+*, shows the phenotype

characteristic of the *n490* homozygote when present as one copy opposite an *n490* deficiency.

We can give an approximate estimate of the number of essential loci balanced by *C1 dpy-10 unc-52* based on the fact that ten of the 47 genes identified by lethal mutations are represented by two or more alleles. Assuming a Poisson distribution of mutations per gene, we calculate a total of about 130 vital genes balanced by *C1 dpy-10 unc-52*. This is almost certainly an underestimate because of the overemphasis in such a calculation on the more mutable loci. By an independent method, BRENNER (1974) has estimated that the *X* chromosome has about 300 vital genes. LG II and *X* seem to have about the same numbers of visible loci (RIDDLE and SWANSON 1982), and *C1 dpy-10 unc-52* balances about 75–80% of LG II, based on the proportion of LG II visibles that are in the balanced region (which extends from about 3 map units to the left of *dpy-10* to *unc-52*, which seems near the right end of LG II); hence, this approach gives an estimate of approximately 230 vital loci balanced by *C1 dpy-10 unc-52*. Thirty-one of the 47 *let* genes we identified occurred within the region bounded by our deficiencies; this suggests that our deficiency map covers approximately half of the LG II genes and about 150 vital loci. The loci that we refer to as vital are those in which mutations can be recessive lethal. Another class of essential genes would be those in which null mutations give sterile phenotypes; an earlier estimate (MENEELY and HERMAN 1981) suggested that such genes may be 20% as numerous as vital genes with lethal alleles. We limited our attention in this work to two special classes of sterile mutant: sperm defectives and oocyte defectives. Five mutations in each class were mapped and tested by complementation with neighboring genes. Only one of the ten, *zyg-11(mn40)*, is an allele of a previously identified gene.

Sixteen of the genes defined by lethals and three defined by steriles, all balanced by *C1 dpy-10 unc-52*, occurred outside of the region bounded by the deficiencies. The ten of these genes that occurred to the left of the deficiencies mapped fairly close to *dpy-10*, as expected. All of the nine mapping to the right of the deficiency region, however, mapped 17 or more map units to the right of *unc-4*, even though the rightmost deficiency endpoints did not reach *rol-1*, which is only about 5 map units to the right of *unc-4*; thus, the region from about 5 to 17 map units to the right of *unc-4* seems extremely sparsely populated with vital loci. Indeed, only two of the approximately 110 genes now mapped on LG II have been located in this segment of 12 map units. By contrast, the region bounded by our deficiencies, which we estimate covers about half of the LG II genes, is only about 5 map units long. The obvious suggestion is that the region of apparent low gene density is expanded on the genetic map because it is greatly favored for crossing over.

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LITERATURE CITED

ARGON, Y. and S. WARD, 1980 *Caenorhabditis elegans* fertilization-defective mutants with abnormal sperm. Genetics **96:** 413–433.

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

CASSADA, R., E. ISNENGHI, M. CULOTTI and G. VON EHRENSTEIN, 1981 Genetic analysis of temperature-sensitive embryogenesis mutants in *Caenorhabditis elegans.* Dev. Biol. **84:** 193–205.

COX, G. N., J. S. LAUFER, M. KUSCH and R. S. EDGAR, 1980 Genetic and phenotypic characterization of roller mutants of *Caenorhabditis elegans.* Genetics **95:** 317–339.

GREENWALD, I. S. and H. R. HORVITZ, 1980 *unc-93(e1500)*: a behavioral mutant of *Caenorhabditis elegans* that defines a gene with a wild-type null phenotype. Genetics **96:** 147–164.

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

HERMAN, R. K. and H. R. HORVITZ, 1980 Genetic analysis of *Caenorhabditis elegans.* pp. 227–261. In: *Nematodes as Biological Models*, Vol. 1, Edited by B. M. ZUCKERMAN. Academic Press, New York.

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

HODGKIN, J., 1983 Male phenotypes and mating efficiency in *Caenorhabditis elegans.* Genetics **103:** 43–64.

HODGKIN, J. and S. BRENNER, 1977 Mutations causing transformation of sexual phenotype in the nematode *Caenorhabditis elegans.* Genetics **86:** 275–288.

HORVITZ, H. R., S. BRENNER, J. HODGKIN and R. K. HERMAN, 1979 A uniform genetic nomenclature for the nematode *Caenorhabditis elegans. Mol. Gen. Genet.* **175:** 129–133.

HORVITZ, H. R. and J. E. SULSTON, 1980 Isolation and genetic characterization of cell-lineage mutants of the nematode *Caenorhabditis elegans.* Genetics **96:** 435–454.

MENEELY, P. M. and R. K. HERMAN, 1979 Lethals, steriles and deficiencies in a region of the *X* chromosome of *Caenorhabditis elegans.* Genetics **92:** 99–115.

MENEELY, P. M. and R. K. HERMAN, 1981 Suppression and function of *X*-linked lethal and sterile mutations in *Caenorhabditis elegans.* Genetics **97:** 65–84.

RIDDLE, D. L. and M. M. SWANSON, 1982 *Caenorhabditis elegans.* pp. 244–258. In: *Genetic Maps 2*, Edited by S. J. O'BRIEN. National Cancer Institute, National Institutes of Health, Frederick, Maryland.

ROGALSKI, T. M., D. G. MOERMAN and D. L. BAILLIE, 1982 Essential genes and deficiencies in the *unc-22 IV* region of *Caenorhabditis elegans.* Genetics **102:** 725–736.

ROSE, A. M. and D. L. BAILLIE, 1980 Genetic organization of the region around *unc-15 (I)*, a gene affecting paramyosin in *Caenorhabditis elegans.* Genetics **96:** 639–648.

SULSTON, J. E. and S. BRENNER, 1974 The DNA of *Caenorhabditis elegans.* Genetics **77:** 95–104.

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

WATERSTON, R. H., 1980 A second informational suppressor, *sup-7 X*, in *Caenorhabditis elegans.* Genetics **97:** 307–325.

WILLS, N., R. F. GESTELAND, J. KARN, S. BOLTEN and R. H. WATERSTON, 1983 Transfer RNA-mediated suppression of nonsense mutations in *C. elegans.* Cell **33:** 575–583.

WOOD, W. B., R. HECHT, S. CARR, R. VANDERSLICE and D. HIRSH, 1980 Parental effects and phenotypic characterization of mutations that affect early development in *Caenorhabditis elegans.* Dev. Biol. **74:** 446–469.

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