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IDENTIFICATION AND CHARACTERIZATION OF 22 GENES THAT AFFECT THE VULVAL CELL LINEAGES OF THE NEMATODE *CAENORHABDITIS ELEGANS*

EDWIN L. FERGUSON AND H. ROBERT HORVITZ

Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

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ABSTRACT

Ninety-five mutants of the nematode *Caenorhabditis elegans* altered in the cell lineages of the vulva have been isolated on the basis of their displaying one of two phenotypes, Vulvaless or Multivulva. In Vulvaless mutants, which define 12 genes, no vulva is present. In Multivulva mutants, which define ten genes, one or more supernumerary vulva-like protrusions are located along the ventral side of the animal. A single recessive mutation is responsible for the phenotypes of most, but not all, of these strains. Fifteen of these 22 genes are represented by multiple alleles. We have shown by a variety of genetic criteria that mutations that result in a Vulvaless or Multivulva phenotype in six of the 22 genes most likely eliminate gene function. In addition, Vulvaless or Multivulva mutations in seven of the other genes most likely result in a partial reduction of gene function; the absence of the activity of any of these genes probably results in lethality or sterility. Our results suggest that we may have identified most, or all, genes of these two classes.

THE nematode *Caenorhabditis elegans* is well suited for studies concerning the genetic control of cell lineage. *C. elegans* consists of relatively few cells (*e.g.*, there are only 959 somatic nuclei in the adult hermaphrodite) of many different types (SULSTON and HORVITZ 1977; KIMBLE and HIRSH 1979; SULSTON *et al.* 1983). The cellular anatomy and patterns of cell divisions and cell fates of *C. elegans* are essentially invariant among individuals from the single-celled zygote to the adult (SULSTON and HORVITZ 1977; DEPPE *et al.* 1978; KIMBLE and HIRSH 1979; SULSTON, ALBERTSON and THOMSON 1980; SULSTON *et al.* 1983). A number of mutations that alter this normally invariant cell lineage have been isolated and characterized (*e.g.*, HORVITZ and SULSTON 1980; SULSTON and HORVITZ 1981). Some of these mutations affect many cell divisions; others are more specific.

We have begun an attempt to identify all genes that affect a particular set of cell lineages in *C. elegans*, those involved in the development of the vulva of the hermaphrodite. We hope to determine the number of such genes, their patterns of interaction, whether or not each of these genes also affects other cell lineages and what other features are shared by the set of lineages affected by a particular gene. We have chosen to study the cell lineages that generate the vulva for three major reasons. First, these lineages are technically easy to

study: they involve relatively few, highly visible precursor cells that undergo three rounds of divisions over a time interval of only about 5 hr (SULSTON and HORVITZ 1977). Second, the vulval precursor cells are involved in four generally interesting developmental phenomena (SULSTON and HORVITZ 1977; SULSTON and WHITE 1980; KIMBLE 1981; P. STERNBERG, personal communication): (1) determination—each of the six initially multipotential vulval precursor cells is determined to express one of three distinct fates, (2) induction—the gonadal anchor cell induces the formation of the vulva, (3) pattern formation—the six potential vulval precursor cells are of equivalent developmental potential but nonetheless express three distinct fates in a precise spatial pattern defined by their distances from the anchor cell, (4) regulation—if a vulval precursor cell is ablated, another cell can replace it. Our goal is to identify the genes and, ultimately, the molecules involved in these developmental processes.

The third reason that we chose to focus our study on the vulval cell lineages is that a number of mutants abnormal in vulval cell divisions had already been identified (HORVITZ and SULSTON 1980; SULSTON and HORVITZ 1981). These mutants were both viable and fertile and could be easily recognized with a dissecting microscope. Two classes of mutants had been characterized. In Multivulva (Muv) mutants, three ventral hypodermal cells that normally produce nonvulval progeny instead undergo vulva-like lineages to produce multiple vulva-like protrusions along the ventral side. In Vulvaless (Vul) mutants, three other ventral hypodermal cells that normally generate the cells of the vulva fail to do so. Because a vulva is not formed, the fertilized eggs of a Vul hermaphrodite are not laid. Consequently, the eggs hatch inside the body of the parent, and the young larvae eat their parent (they later escape from the parental cuticle). Vul mutants can be recognized with a dissecting microscope by the presence of "bags of worms," in which each parental cuticle encloses its progeny larvae.

This paper reports the isolation and genetic characterization of 95 mutants that display a Multivulva or Vulvaless phenotype.

MATERIALS AND METHODS

Strains and genetic nomenclature: Caenorhabditis elegans var. Bristol strain N2 and most of the mutant strains used for mapping and strain construction were obtained from BRENNER (1974) or from the *Caenorhabditis* Genetics Center, which is supported by contract number N01-AG-9-2113 between the National Institutes of Health and the Curators of the University of Missouri. Except where noted, these genes have been described by BRENNER (1974) and SWANSON, EDGLEY and RIDDLE (1984). The alleles used are either the reference alleles listed in the above publications or alleles that result in similar phenotypes. N2 is the wild-type parent of all nematode strains used in this work.

LGI: bli-3(e767); lin-6(e1466); unc-11(e47); dpy-5(e61); unc-13(e1091); sDf5; lin-28(n719) (AMBROS and HORVITZ 1984); *unc-56(e403); sup-17(n316); unc-29(e1072); nDf23; nDf24; nDf25; unc-75(e950).* *sup-17(n316)* was obtained by phenotypically reverting *lin-12(n177)* (E. FERGUSON, unpublished results). *nDf23, nDf24* and *nDf25* were obtained by crossing N2 males that had been mutagenized with γ-rays (GREENWALD and HORVITZ 1980) to hermaphrodites of genotype *dpy-5 unc-29.* F_1 Unc non-Dpy hermaphrodites, selected by their resistance to the cholinergic agonist levamisole (LEWIS *et al.* 1980), were candidates for carrying a deficiency of the *unc-29* region. These hermaphrodites

were then tested to determine whether they were heterozygous for a deficiency of the *unc-29* region, *i.e.*, whether they carried a recessive lethal mutation that failed to complement mutations in one or more genes linked to *unc-29*. *nDf23* fails to complement *lin-28*, *unc-56*, *sup-17* and *unc-29*. *nDf24* and *nDf25* fail to complement *lin-10*, *lin-28*, *unc-56*, *sup-17* and *unc-29*.

LGII: *cat-2(e1112)*; *sup-9(n180)*; *nDf3*; *unc-85(e1414)*; *bli-2(e768)*; *dpy-10(e128)*; *tra-2(n196)*; *mnDf88*; *let-253(mn184)*; *let-236(mn88)*; *lin-5(e1348)*; *mnDf68*; *vab-9(e1744)*; *rol-6(e187)*; *unc-4(e120)*; *him-9(e1487)*; *unc-52(e444)*; *C1 dpy-10(e128) unc-52(e444)*. *C1 dpy-10 unc-52* is a chromosomal abnormality of LGII that balances the right half of the chromosome (HERMAN 1978). *mnDf88*, *let-253*, *let-236* and *mnDf68* are described by SIGURDSON, SPANIER and HERMAN (1984).

LGIII: *unc-93(e1500)*; *dpy-17(e164)*; *lon-1(e185)*; *daf-4(e1364)*; *lin-16(e1743)*; *unc-36(e251)*; *unc-86(e1416,n848)*; *nDf16* (V. AMBROS and M. FINNEY, personal communication); *dpy-19(e1259)*; *sup-5(e1464)*; *sma-2(e502)*; *unc-32(e189)*; *unc-69(e587)*; *tra-1(e1099)*; *eT1(III;V)* (ROSENBLUTH and BAILLIE 1981); *let(n886)*. *n848* is a heat-sensitive allele of *unc-86* (M. FINNEY, personal communication). *let(n886)* is a mutation that confers a recessive lethal phenotype and that is linked to *eT1(III;V)* (M. FINNEY, personal communication).

LGIV: *dpy-9(e12)*; *unc-17(e245)*; *dpy-13(e184)*; *unc-5(e53)*; *unc-8(e49,n491)*; *dpy-20(e1362)*; *unc-22(e66)*; *nDf27* (H. ELLIS, personal communication); *dpy-26(n199)*; *unc-31(e169)*; *unc-30(e191)*; *ced-3(n717)* (H. ELLIS, personal communication); *dpy-4(e1166)*; *unc(n752)*. *n491* is a dominant allele of *unc-8* (J. PARK, personal communication). *unc(n752)*, which confers a recessive Unc phenotype and is linked to the translocation *nT1(IV;V)*, was identified after ethyl methanesulfate (EMS) mutagenesis of hermaphrodites of gentoype *nT1(IV)/unc-8(n491)*; *nT1(V)/unc-60*; individual F_1 hermaphrodites were picked and their progeny were examined to determine whether any mutation cosegregated with, and thus was linked to, the Vul phenotype of *nT1(IV;V)* (E. FERGUSON, unpublished results).

LGV: *unc-60(e677)*; *dpy-11(e224)*; *unc-42(e270)*; *sma-1(e30)*; *him-5(e1467)*; *unc-76(e911)*; *unc-51(e369)*; *unc(n754)*. *unc(n754)* is a mutation that confers a dominant Unc phenotype and that is linked to *nT1(IV;V)* (E. FERGUSON, unpublished results).

LGX: *unc-78(e1217)*; *dpy-23(e840)*; *lon-2(e678)*; *sup-7(st5)*; *dpy-7(e1324)*; *unc-84(e1410)*; *unc-3(e151)*; *let-15(mn127)*; *let-40(mn150)*; *let-18(mn122)*; *let-38(mn141)*; *sup-10(n183)*; *mnDp1*; *mnDf1*; *mnDf4*; *mnDf11*; *mnDf19*; *mnDf43*.

This paper follows the standardized *C. elegans* genetic nomenclature (HORVITZ *et al.* 1979). All vulval cell lineage mutations except *n300* and *n1045* were assigned *lin*, for lineage abnormal, gene names. As described in RESULTS, it is not clear whether *n300* is simply a mutation present on the translocation *nT1(IV;V)* or whether *n300* is a result of the translocation itself. For this reason we have not assigned a gene name to *n300*, although it defines a separate complementation group. Because *n1045* was shown to be an allele of the previously defined gene *let-23* (see RESULTS), it was not assigned a *lin* gene name.

General techniques: Methods for the culturing, handling and genetic manipulation of *C. elegans* have been described (BRENNER 1974). Most experiments were done at 20°, except those involving *dpy-19*, *sup-5*, *sup-7* or *lin-18*, which were done either at 20° or 25°, and *lin-12*, *lin-13* or *let-23*, which were done at 15°, 20° or 25°.

Photography: Bright-field photomicrographs were taken with Kodak Technical Pan film 2415 using a Zeiss Universal microscope equipped with a Neofluar 6.3 objective and a Zeiss microflash illuminator. For most photographs, animals were first placed on an NGM plate (BRENNER 1974) with no bacteria for approximately 5 min and then transferred to a slide containing 10–15 μl of 20% (w/v) Ficoll (Sigma) in M9 buffer (BRENNER 1974). Sephadex beads G-200-120 (Sigma) with a particle size of 40–120 μm were then placed in the solution and an 18 × 18-mm coverslip was lowered gently onto the liquid. The Ficoll increased the viscosity of the buffer solution and prevented the animals from thrashing while allowing apparently normal movement. The combination of the Sephadex beads and the viscosity of the solution supported the weight of the coverslip and prevented the animals from being crushed.

Sources of vulval cell lineage mutants: Most of the new mutants described in this manuscript were isolated by N. TSUNG during a general screen for egg-laying-defective mutants (TRENT, TSUNG and HORVITZ 1983). Other mutants were isolated either by ourselves or by the following members of our laboratory: V. AMBROS, C. DESAI, H. ELLIS, W. FIXSEN, I. GREENWALD, J. PARK and C. TRENT. *lin-1(e1026, e1275, n176)*, *lin-2(e1309, e1424, e1453)*, *lin-3(e1417)*, *lin-4(e912)*, *lin-7(e1413)*

and *lin-8(n111)*; *lin-9(n112)* were described previously by HORVITZ and SULSTON (1980) and SULSTON and HORVITZ (1981). S. BRENNER isolated *lin-18(e620)*; M. CHALFIE, *lin-15(n377)*; E. HEDGECOCK, *lin-31(e1750)*; J. HODGKIN, *lin-15(e1763)* and J. PLENEFISCH, *lin-1(n1140)*.

EMS was used as a mutagen (BRENNER 1974) to generate almost all of the mutants abnormal in vulval cell lineages. *lin-4(e912)* was obtained after P-32 decay (HORVITZ and SULSTON 1980; BABU and BRENNER 1981). *lin-7(n701)* was obtained after γ-ray mutagenesis (V. AMBROS, personal communication). The origin of *n300* is unclear. An egg-laying-defective hermaphrodite strain obtained after EMS treatment of the wild type was crossed with wild-type males, and strains with two distinct egg-laying-defective phenotypes were isolated from the F_2 progeny. These strains carried two different mutations, *unc-86(n306) III* and *n300*, which is associated with the reciprocal translocation *nT1(IV;V)* (see RESULTS). Upon reexamination, the original strain proved to carry the *unc-86* mutation but not *n300* or *nT1(IV;V)*.

Complementation: Because some mutations affecting vulval cell lineages are not expressed in males, complementation tests were scored in hermaphrodites. Males carrying the unknown mutation were mated with hermaphrodites homozygous for both a known vulval cell lineage mutation and a recessive marker used to distinguish self- from cross-progeny. If the unknown mutation was recessive and if Lin cross-progeny hermaphrodites were observed, the lineage mutations failed to complement. [Because *lin-11(n389)* hermaphrodites are unable to mate and *lin-13(n387)* hermaphrodites are sterile, complementation tests involving these two mutations were performed using hermaphrodites homozygous for a recessive marker and heterozygous for the *lin* mutation and for a closely linked *unc* mutation. *unc-32* was used to balance *lin-13*, and *unc-29* was used to balance *lin-11*.]

Suppression studies: The cell lineage mutations were tested for suppression by one of two amber suppressors, *sup-5 III* or *sup-7 X* (WATERSTON and BRENNER 1978; WATERSTON 1981; WILLS *et al.* 1983). All alleles of genes without suppressible alleles were tested. For two genes with suppressible alleles, only some alleles were tested; specifically, nine of 16 *lin-1* alleles and eight of 13 *lin-7* alleles were tested. Some mutations had been tested previously by HORVITZ and SULSTON (1980).

To help score the presence of *sup-7* in these suppression experiments, we constructed the linked double mutant *sup-7 dpy-7 X*. The presence of *sup-5* was scored using the linked double mutants *lon-1 sup-5 III* (HORVITZ and SULSTON 1980) or *dpy-19 sup-5 III* (M. FINNEY, personal communication). Table 1 describes the protocol used in testing most mutations and presents data for those mutations suppressed by a single copy of one of the suppressors. Three mutations—*lin-24(n1057)*, *lin-34(n1046)* and *let-23(n1045)*—were better suppressed by two copies of one of the suppressors than by a single copy. Details of these three suppression experiments are described below (also see RESULTS).

lin-24(n1057): As *lin-24(n1057)* results in a Vul phenotype only when heterozygous to a wild-type allele of the locus, we performed suppression experiments to examine the phenotype of *lin-24(n1057)/+* hermaphrodites. These experiments were complicated by the fact that, at 20°, some hermaphrodites of genotype *dpy-19 sup-5* can have an egg-laying-defective (Egl) phenotype similar to that of *lin-24(n1057)/+* hermaphrodites. However, no *dpy-19 sup-5* hermaphrodites are Vul; as viewed with a dissecting microscope, all *dpy-19 sup-5* hermaphrodites have a recognizable vulval structure. To compare the penetrance of the Vul defect of hermaphrodites of genotype *unc-22 lin-24(n1057)/+ +* with the penetrance of the Vul defect of hermaphrodites of genotype *dpy-19 sup-5*; *unc-22 lin-24(n1057)/+ +*, L4 hermaphrodites of both genotypes were picked. Those hermaphrodites that became Egl were examined either with a dissecting microscope or in a few cases with Nomarski optics, to determine whether a recognizable vulval structure was present. Hermaphrodites that either lacked a vulva or had a protrusion with no recognizable vulval characteristics were considered to have a Vul phenotype. The penetrance of the Vul phenotype of hermaphrodites of genotype *dpy-19 sup-5*; *unc-22 lin-24(n1057)/+ +* (3%, n = 279) was much lower than the penetrance of the Vul phenotype of hermaphrodites of genotype *unc-22 lin-24(n1057)/+ +* (22%, n = 301), demonstrating that *sup-5* suppresses *lin-24(n1057)/+*. *sup-5* was demonstrated not to be a dominant suppressor of *lin-24(n1057)/+* as 13 of 21 Vul hermaphrodites that were the progeny of hermaphrodites of genotype *dpy-19 sup-5/+ +*; *unc-22 lin-24(n1057)/+ +* segregated Dpy animals. [Hermaphrodites of genotype *dpy-19 sup-5*; *unc-22 lin-24(n1057)/+ +* were constructed by mating *dpy-19 sup-5/+ +* males with hermaphrodites of genotype *dpy-19 sup-*

5; *unc-22 lin-24(n1057)* and picking Dpy non-Unc cross-progeny. Hermaphrodites of genotype *dpy-19 sup-5*; *unc-22 lin-24(n1057)* were constructed by picking Dpy Unc progeny from animals of genotype *dpy-19 sup-5*/+ +; *unc-22 lin-24(n1057)*/+ +. These strains were sterile at 15°, confirming the presence of *sup-5*. To confirm the presence of *lin-24(n1057)*, hermaphrodites of putative genotype *dpy-19 sup-5*; *unc-22 lin-24(n1057)* were mated with wild-type males and Vul hermaphrodites were observed among the F_1 progeny.]

lin-34(n1046): From the progeny of hermaphrodites of genotype *lin-34(n1046) unc-22*/+ +; *sup-7 dpy-7*/+ +, Unc, Dpy and Dpy Unc hermaphrodites were picked. The six Dpy Unc hermaphrodites of putative genotype *lin-34(n1046) unc-22*; *sup-7 dpy-7* were not Muv and segregated very few Muv progeny. Of the 35 Unc hermaphrodites that were picked, 17 segregated Dpy Unc progeny, very few of which were Muv. Ten of the 13 Dpy hermaphrodites that were picked segregated Dpy Unc hermaphrodites. On five of the plates the Dpy Unc hermaphrodites were mostly non-Muv; on the other five plates most of the Dpy Unc hermaphrodites were Muv. All ten Dpy isolates were subsequently tested at 15° for the presence of the suppressor, and only the first five isolates were sterile and thus homozygous for *sup-7*. To confirm these results, the suppressibility of *n1046* was tested using a *dpy-19 sup-5* strain. *sup-5* also is a recessive suppressor of *n1046* (E. FERGUSON, unpublished results).

let-23(n1045): The suppression experiments of *let-23(n1045)* were complicated by the temperature-dependent phenotypes of both *n1045* and *sup-7*; at the temperatures at which *sup-7* animals are fertile, 22.5° and 25°, the majority of *n1045* hermaphrodites appear wild type, whereas at the temperature at which the Vul phenotype of *n1045* hermaphrodites is most penetrant, 15°, *sup-7* hermaphrodites are sterile. From parental hermaphrodites of genotype *let-23(n1045) unc-4*/+ +; *sup-7 dpy-7*/+ +, five Dpy Unc hermaphrodites were picked and grown at 25° and 20 Unc hermaphrodites were picked and grown at 15°. (The *cis* marker *unc-4* was used to identify animals homozygous for *n1045*.) Four of the five Dpy Unc hermaphrodites of putative genotype *let-23(n1045) unc-4*; *sup-7 dpy-7* segregated very few arrested larvae (5%, $n = 95$) and were subsequently shown to be homozygous for *sup-7* (by the criterion of sterility at 15°). The fifth Dpy Unc hermaphrodite segregated a normal number of arrested larvae (29%, $n = 60$) but generated descendants fertile at 15° and thus not homozygous for *sup-7*. The presence of *n1045* in two of the first four Dpy Unc isolates was confirmed by mating the Dpy Unc hermaphrodites with wild-type males and reisolating Unc Vul hermaphrodites in the F_2. Fifteen of the 20 Unc hermaphrodites that were grown at 15° were heterozygous for *sup-7* (as they segregated approximately ¼ sterile progeny) and segregated very few arrested larvae and many egg-laying-competent progeny. In contrast, all of the progeny of the five Unc hermaphrodites that did not segregate *sup-7* either arrested during larval development or were Vul, suggesting that at 15° *sup-7*/+ suppresses both the lethality and the vulval lineage defects that result from *n1045*.

Male mating: Male-mating experiments were carried out essentially as described by HODGKIN, HORVITZ and BRENNER (1979). Six L4 males and six L4 *dpy-11* hermaphrodites were placed on a Petri dish containing a 1-cm spot of bacteria. The males were removed after 24 hr, and the hermaphrodites were transferred to a fresh plate every 12 hr thereafter until no further cross-progeny were produced. Most males were obtained using *him-5*. *n300* males were generated using *him-9*. *lin-13* males were obtained from the progeny of fertile *lin-13; him-5* hermaphrodites, which were obtained at 15° from the balanced heterozygote strain *lin-13* +/+ *unc-32*; *him-5*. Heterozygous *lin-12(n137)* males were obtained as non-Unc males from the strain + *unc-32 lin-12(n137)*/*unc-36* + +; *him-5*.

lin-3 *complementation screen*: To obtain mutations that failed to complement the Vulvaless mutation *lin-3(e1417)*, wild-type L4 males were mutagenized with EMS and mated with L4 hermaphrodites of genotype *dpy-20 lin-3(e1417)*/*nT1(IV)*; +/*nT1(V)*. A recessive Unc mutation, *unc(n752)*, was also present on *nT1(IV;V)*. [The complementation screen was performed using heterozygous *lin-3(e1417)* hermaphrodites because 90% of *lin-3(e1417)* hermaphrodites are Vul and thus not able to mate.] The parents (six males and six hermaphrodites) were transferred to a fresh plate every day and the F_1 progeny of the mating were examined for the presence of non-Dpy, non-Unc Vulvaless hermaphrodites, which could carry a mutation that failed to complement *lin-3(e1417)*. Ninety-five matings were done, and approximately 40,000 F_1 progeny were examined, 20,000 of which were heterozygous for *lin-3(e1417)*. When this protocol was used, in addition to

TABLE 1

Dominant suppression of some vulval cell lineage mutations by the amber suppressors sup-5 *and* sup-7

	Progeny of hermaphrodites of genotype				
	lin-1(e1777)/+; sup-7 dpy-7/+ +	*lin-1(n431)/+; sup-7 dpy-7/+ +*	*lin-7(e974)/+; sup-7 dpy-7/+ +*	*lin-7(e1449)/+; sup-7 dpy-7/+ +*	*dpy-19 sup-5/+ +; lin-18(n1051) +/+ lon-2*
(1) Fraction of Lin progeny that segregated Dpy animals	0/3	1/7	1/16	0/7	0/11
(2) Fraction of Dpy progeny that segregated Lin animals	2/11	1/10	0/7	0/8	ND
(3) Fraction of phenotypically wild-type progeny that were of putative genotype *lin/lin; sup dpy/+ +*	6/30	2/20	3/33	2/29	12/65
	Progeny of hermaphrodites of putative genotype				
	lin-1(e1777); sup-7 dpy-7/+ +	*lin-1(n431); sup-7 dpy-7/+ +*	*lin-7(e974); sup-7 dpy-7/+ +*	*lin-7(e1449); sup-7 dpy-7/+ +*	*dpy-19 sup-5/+ +; lin-18(n1051)*
(4) Fraction of Lin progeny that segregated Dpy animals	1/9	0/4	0/17	0/15	0/13
(5) Fraction of wild-type progeny that were of parental genotype	8/9 (1/9 was *lin; + +*)	9/9	18/21 (3/21 were *lin; + +*)	14/14	36/36
(6) Fraction of Dpy non-Lin progeny of putative genotype *lin; sup dpy* that were sterile at 15°	3/3	2/2	4/4	3/3	3/3

From the progeny of hermaphrodites of genotype *lin/+; sup dpy/+ +*, Lin animals and Dpy animals were picked (*dpy-19* was used in *cis* to *sup-5*; *dpy-7* was used in *cis* to *sup-7*). In most cases the *lin* mutation was not suppressed, and about ⅔ of the Lin hermaphrodites segregated Dpy animals and about ⅔ of the Dpy hermaphrodites segregated Lin animals. Dpy Lin animals were picked, for example, to establish a putative *lin; sup-7 dpy-7* strain, which was placed at 15°. If the strain became sterile (*sup-7* homozygotes are sterile at 15°; WATERSTON 1981), the strain was confirmed to be homozygous for *sup-7* and the mutation was considered to be nonsuppressed. [For some Muv strains, the sickness of the Muv mutant prevented the establishment of the *lin; sup-7 dpy-7* strain. In these cases, *lin/+; sup-7 dpy-7* hermaphrodites were placed at 15°; the presence of Lin hermaphrodites among the sterile progeny of these animals indicated that the *lin* mutation was not suppressed. The alleles of *lin-3* and *let-23* that result in lethality or sterility were determined not to be suppressed based on the observations that hermaphrodites of genotype *unc-8 lin-3/+ +; sup-7 dpy-7* (or *let-23 unc-4/+ +; sup-7 dpy-7*) segregated Unc Lin (or Unc Let) progeny.]

If a *lin* mutation was suppressed by a single copy of the amber suppressor, none or few of the Lin hermaphrodites segregated Dpy animals (line

1), and none or few of the Dpy hermaphrodites segregated Lin animals (line 2). (The Dpy hermaphrodites that segregated Lin animals and the Lin hermaphrodites that segregated Dpy animals were recombinants between the *dpy* and *sup* mutations. The distance betwen *sup-7* and *dpy-7* is approximately 4 map units. However, because *sup-7 dpy-7* hermaphrodites grow more slowly than hermaphrodites of genotype *sup-7 dpy-7/+ dpy-7*, the frequency of Dpy non-Sup recombinants among the Dpy animals picked tended to be higher than expected from the recombination frequency between these two genes.) In these cases, from the same parent, about 30 phenotypically wild-type progeny were also picked. Of these animals, approximately $^{2}/_{11}$ were of genotype *lin/lin; sup dpy/+ +*, as they segregated ¼ Lin non-Dpy, ¼ Dpy non-Lin and ½ wild-type progeny (line 3). From the progeny of phenotypically wild-type hermaphrodites of putative genotype *lin/lin; sup dpy/+ +*, three classes of animals were picked, Lin, wild type, and Dpy. None or few of the Lin animals segregated Dpy progeny (line 4). All of the wild-type hermaphrodites were of parental genotype, as evidenced by the phenotypes of their progeny (line 5). The Dpy progeny were of putative genotype *lin; sup dpy* and were tested at 15° for sterility to confirm the presence of the suppressor (line 6). To confirm the presence of the *lin* mutation in the putative *lin; sup dpy* strain, Lin hermaphrodites were reisolated from the strain after crossing with wild-type males. The Lin hermaphrodites were confirmed to be of genotype *lin; + +* by noting the absence of Dpy animals among their progeny (E. FERGUSON, unpublished observations). ND, not determined.

six partially dominant Vul or Egl mutations—including *lin-24(n1057)*, *lin-33(n1043)* and *lin-33(n1044)*—two additional alleles of *lin-3*, *n1058* and *n1059*, were obtained.

Construction of the double mutant lin-24(n432) ced-3(n717): From the progeny of hermaphrodites of genotype *lin-24(n432) unc-31* +/+ + *ced-3*, many Vul non-Unc hermaphrodites were picked. As *lin-24(n432)* results in a partially dominant Vul phenotype, most hermaphrodites were of the parental genotype; however, a few hermaphrodites were recombinants of genotype *lin-24(n432) unc-31* +/*lin-24(n432)* + *ced-3* and segregated very few egg-laying-competent progeny. The progeny of these hermaphrodites that failed to segregate Unc progeny were of putative genotype *lin-24(n432) ced-3*. Three such putative *lin-24 ced-3* animals were saved, and the presence of *ced-3* in these strains was confirmed using Nomarski optics (H. ELLIS, personal communication).

RESULTS

Isolation of mutants: We have identified 95 independently derived mutants abnormal in the vulval cell lineages. These mutants fall into two general classes, Multivulva (Muv) and Vulvaless (Vul), both of which have been described previously (HORVITZ and SULSTON 1980; SULSTON and HORVITZ 1981). In a Muv hermaphrodite, one or more supernumerary vulva-like protrusions are located along the ventral side of the animal. Some Muv hermaphrodites lack a functional vulva. The ventral protrusions are readily visible when viewed with a dissecting microscope and have been used as the basis for the isolation of the Muv strains described in this manuscript. Like those Muv mutants obtained previously (SULSTON and HORVITZ 1981), the majority of these new Muv mutants generate supernumerary vulva-like structures as a consequence of the expression of vulval cell lineages by cells not normally involved in vulval development (P. STERNBERG, personal communication). However, in Muv mutants defective in two newly identified genes, *lin-17* and *lin-18*, an abnormality in the lineage of the posterior-most of the three vulval precursor cells generates a single supernumerary vulva-like structure immediately posterior to the vulva (P. STERNBERG, personal communication).

In a Vul hermaphrodite, no vulva is present. Some Vul hermaphrodites express some vulval cell divisions resulting in a single ventral protrusion at the normal location of the vulva. A Vul hermaphrodite does not lay eggs and its progeny hatch internally, causing the parent to become a "bag of worms." (A bag of worms comprises the cuticle of the adult hermaphrodite enclosing its progeny larvae.) Most of the new Vul mutants reported in this manuscript initially were identified on the basis of their defects in egg laying when viewed with a dissecting microscope, *i.e.*, (1) these Vul strains produced many bags of worms and (2) individual Vul hermaphrodites generally laid no eggs. Egg-laying-defective mutants with these characteristics were examined using Nomarski optics for the presence of a vulva. Those strains in which the vulva was clearly absent in a majority of hermaphrodites were designated Vul mutants and studied further to establish that they were defective in vulval cell lineages (P. STERNBERG, personal communication).

Our classification of a strain as either a Muv strain or a Vul strain implies that most, but not necessarily all, of the animals of that strain have either a Muv or a Vul phenotype. In some incompletely penetrant Muv or Vul strains, individual hermaphrodites can be phenotypically wild type or can have the

superficially opposite phenotype. Specifically, in certain Vul strains, some hermaphrodites lack a vulva but have more than one ventral protrusion at or near the site of the vulva; other hermaphrodites can have a functional vulva and one or possibly two supernumerary ectopic vulva-like structures. In certain Muv strains, some hermaphrodites lack a functional vulva and have a single ventral protrusion at the normal site of the vulva. Thus, the terms "Muv" and "Vul" refer to the most common phenotypes resulting from a mutation in a strain but do not necessarily accurately describe the phenotypes of all individual hermaphrodites of that strain.

Complementation and mapping: To manipulate genetically Muv and Vul mutations, it is necessary to be able to mate either hermaphrodites or males carrying these mutations. A hermaphrodite without a vulva can reproduce, but it cannot mate with males. However, most of the vulval cell lineage mutants we have studied are of incomplete penetrance, *i.e.*, some hermaphrodites form a functional vulva and are able to mate. In these cases genetic studies could be performed by mating males with egg-laying-competent hermaphrodites. In contrast, in some strains the vulval defect is 100% penetrant. Males of such strains were induced by heat shock (HODGKIN 1983). If these males were able to mate, a doubly mutant strain containing *him-5* was constructed, and the males produced by this strain were used for subsequent genetic manipulations. These two procedures enabled us to mate relatively easily all but three mutant strains. To mate hermaphrodites carrying the Vul mutation *n300*, we examined 3×10^5 hermaphrodites before a single egg-laying-competent hermaphrodite was obtained. To mate strains carrying either the Vul mutation *n676* or the Muv mutation *n177*, we mutagenized these strains and obtained extragenic mutations that permitted mating.

A new mutation was first tested for complementation against alleles of known genes of the same phenotypic class. As detailed below, Muv mutations in different genes result in different and often distinctive phenotypes, so that in most cases it was necessary to test a new Muv mutation for complementation against Muv alleles of only one or a few genes. However, most Vul mutations result in similar phenotypes, and, thus, it was necessary to test most new Vul mutations for complementation against Vul alleles of all genes. If a new mutation complemented alleles of those genes with mutations resulting in a similar phenotype, the mutation was mapped to one of the six linkage groups by testing for linkage to a standard marker on each chromosome using the protocol described in TRENT, TSUNG and HORVITZ (1983). Three-factor crosses were then performed to position the gene on the linkage group (Table 2). We have assigned new complementation groups, defined by the Muv and Vul mutants, *lin* (lineage abnormal) gene names. These genes are distributed fairly uniformly throughout the genome (Figure 1). Some details concerning these mapping and complementation experiments are presented below.

Mutant phenotypes and genetic characterization: This section, which is summarized in Table 3, describes characteristics of mutants defective in the genes affecting the vulval cell lineages. Phenotypes as visualized with a dissecting microscope are presented first, followed by data detailing genetic tests or strain

TABLE 2

Three- and four-factor crosses

Gene	Genotype of heterozygote	Phenotype of selected hermaphrodites	Genotype of selected hermaphrodites (with respect to unselected marker)
lin-3	+ *unc-8* +/*unc-5* + *lin-3*	Lin	24/30 *unc-8*/+
	+ + *unc-31*/*unc-5* *lin-3* +	Lin	0/12 *unc-31*/+
	+ *dpy-20* +/*lin-3* + *unc-31*	Lin	8/27 *dpy-20*/+
lin-8	*sup-9* + + +/+ *lin-8* *unc-85* *dpy-10*; *unc-93* *lin-9*	Sup	2/40 *lin-8* *unc-85* *dpy-10*/+ + +
	+ *lin-8* + +/*sup-9* + *lin-31* *unc-85*; *unc-93* *lin-9*	Sup	11/17 *lin-8*/+
lin-9	*lin-8*; + *lin-9* +/*dpy-19* + *unc-32*	Dpy	16/16 *lin-9*/+
		Unc	0/15 *lin-9*/+
	lin-8; + + *unc-32*/*dpy-19* *lin-9* +	Dpy	15/15 *unc-32*/+
		Lin	0/16 *unc-32*/+
	+ *lin-9(n942)* +/*dpy-19* + *unc-32*	Dpy	5/5 *lin-9*/+
		Unc	1/10 *lin-9*/+
	+ *lin-9(n942)* +/*sma-2* + *unc-32*	Sma	3/4 *lin-9*/+
		Unc	1/13 *lin-9*/+
lin-10	+ + *lin-10*/*dpy-5* *unc-13* +	Dpy	10/10 *lin-10*/+
		Unc	0/7 *lin-10*/+
	+ *lin-10* +/*dpy-5* + *unc-56*	Dpy	13/15 *lin-10*/+
		Unc	2/9 *lin-10*/+
	+ *unc-13* +/*dpy-5* + *lin-10*	Lin	3/19 *unc-13*/+
lin-11	+ *lin-11* +/*dpy-5* + *unc-75*	Dpy	11/21 *lin-11*/+
		Unc	3/7 *lin-11*/+
	+ + *lin-11*/*dpy-5* *unc-29* +	Dpy	15/15 *lin-11*/+
		Unc	0/15 *lin-11*/+
	+ *unc-29* +/*dpy-5* + *lin-11*	Dpy	8/10 *unc-29*/+
		Lin	4/21 *unc-29*/+

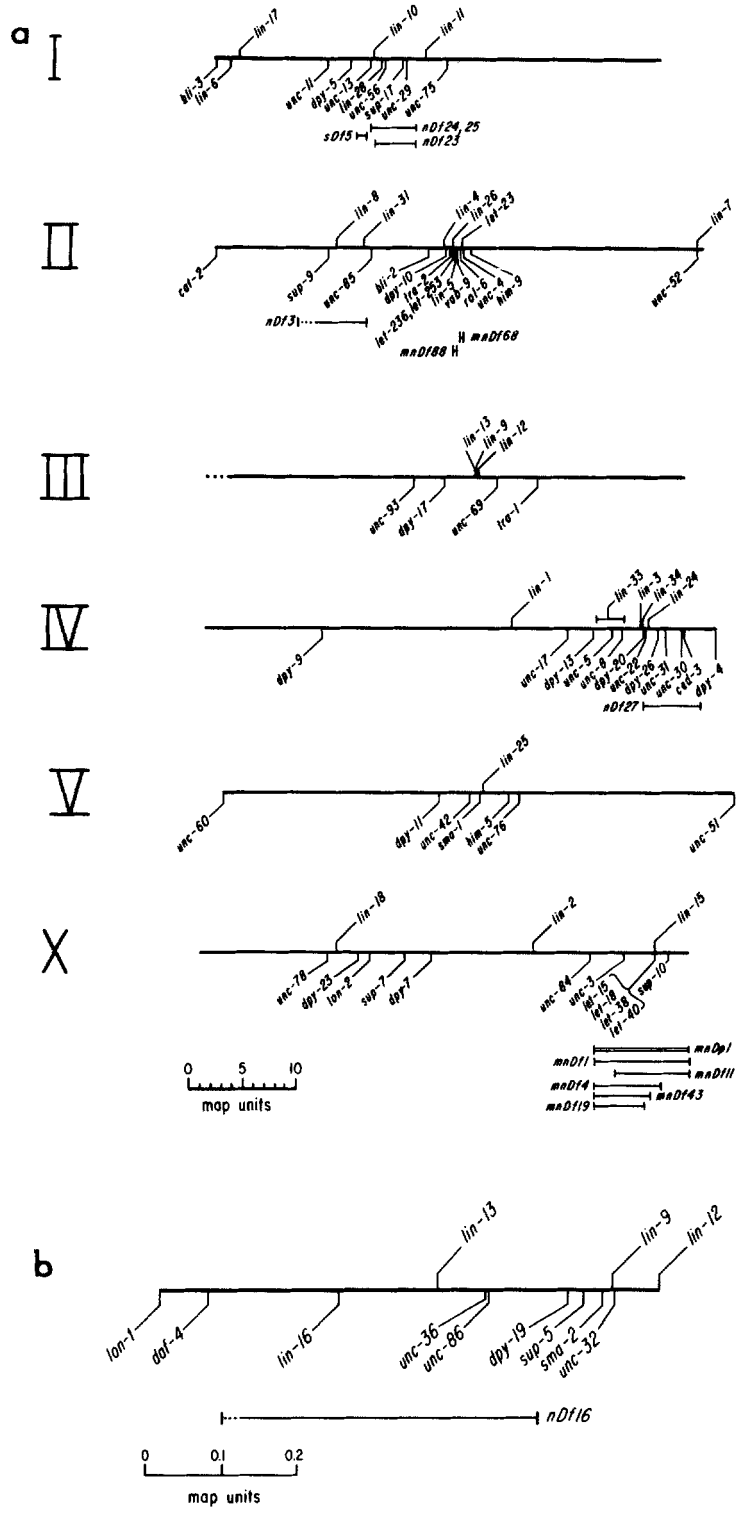
lin-13	*lon-1 + +/+ lin-13 unc-32*	Unc	12/12 *lon-1/+*
	+ lin-13 +/lon-1 + unc-32	Unc	4/7 *lin-13/+*
		Lon	5/12 *lin-13/+*
	+ lin-13 +/lon-1 + unc-36	Lon	30/34 *lin-13/+*
		Unc	1/16 *lin-13/+*
	+ lin-13 +/daf-4 + unc-32	Unc	11/25 *lin-13/+*
	+ lin-13 unc-36 +/lin-16 + + unc-86	Unc-86	7/13 *lin-13 +/+*
			1/13 *lin-13 unc-36/+*
	+ dpy-19 +/lin-13 + unc-32	Unc	3/21 *dpy-19/+*
lin-15	*unc-93; + lin-15 +/unc-3 + sup-10*	Sup	5/21 *lin-15/+*
lin-17	*lin-17 + +/+ unc-11 dpy-5*	Dpy	4/4 *lin-17/+*
	bli-3 + +/+ lin-17 dpy-5	Lin	1/21 *bli-3/+*
	+ + unc-13/bli-3 lin-17 +	Lin	1/17 *unc-13/+*
	+ lin-6 +/bli-3 + lin-17	Lin-17	4/14 *lin-6/+*
lin-18	*unc-78 + +/+ lin-18 lon-2*	Lin Lon	1/41 *unc-78/+*
	unc-78 lin-18 lon-2/+ + +	Lon non-Unc	6/11 *lin-18/lin-18*
			5/11 *lin-18/+*
	+ dpy-23 +/lin-18 + lon-2	Lin	4/4 *dpy-23/+*
lin-24	*+ + lin-24/unc-8 dpy-20 +*	Dpy	0/24 *lin-24/+*
(n432)		Unc	25/25 *lin-24/+*
	+ lin-24 +/dpy-20 + unc-31	Dpy	6/11 *lin-24/+*
		Unc	4/6 *lin-24/+*
	+ + lin-24/dpy-20 unc-22 +	Unc	0/3 *lin-24/+*
	+ unc-22 +/dpy-20 + lin-24	Unc Lin	2/2 *lin-24/+*
	+ lin-24 +/dpy-20 + unc-30	Dpy	2/9 *lin-24/+*
		Unc	9/10 *lin-24/+*
	+ lin-24 +/unc-22 + dpy-26	Unc	3/10 *lin-24/+*
lin-24	*+ + lin-24/dpy-20 unc-22 +*	Unc	0/5 *lin-24/+*
(n1057)	*+ lin-24 +/unc-22 + unc-30*	Unc-22	1/7 *lin-24/+*
		Unc-30	17/18 *lin-24/+*
	+ lin-24 +/unc-22 + dpy-26	Unc	1/5 *lin-24/+*

ABLE 2—Continued

Gene	Genotype of heterozygote	Phenotype of selected hermaphrodites	Genotype of selected hermaphrodites (with respect to unselected marker)
lin-25	*+ + lin-25/dpy-11 unc-42 +*	Dpy	7/7 *lin-25/+*
		Unc	0/3 *lin-25/+*
	+ lin-25 +/dpy-11 + unc-76	Dpy	3/6 *lin-25/+*
		Unc	6/9 *lin-25/+*
	+ unc-42 +/dpy-11 + lin-25	Dpy	3/5 *unc-42/+*
		Lin	3/10 *unc-42/+*
	+ him-5 +/lin-25 + unc-76	Lin	2/9 *him-5/+*
		Unc	4/9 *him-5/+*
	+ sma-1 +/unc-42 + lin-25	Lin	4/6 *sma-1/+*
		Unc	11/14 *sma-1/+*
lin-26	*+ lin-26 +/dpy-10 + unc-4*	Unc	3/11 *lin-26/+*
	lin-26 + +/+ vab-9 unc-4	Unc	11/11 *lin-26/+*
		Vab	0/3 *lin-26/+*
	+ vab-9 +/lin-26 + unc-4	Unc	15/21 *vab-9/+*
	+ lin-26 +/tra-2 + unc-4	Unc	12/17 *lin-26/+*
	+ rol-6 +/lin-26 + unc-4	Unc	10/13 *rol-6/+*
		Lin	0/2 *rol-6/+*
	+ lin-5 +/lin-26 + unc-4	Unc	46/49 *lin-5/+*
lin-31	*+ unc-85 +/lin-31 + bli-2*	Lin	3/4 *unc-85/+*
	lin-31 + +/+ unc-85 bli-2	Lin	1/49 *unc-85 bli-2/+ +*
	sup-9 + +/+ lin-31 unc-85; unc-93	Sup	17/125 *lin-31 unc-85/+ +*
lin-33	*lin-33 + +/+ unc-22 unc-30*	Unc-30	13/13 *lin-33/+*
(n1043)	*(lin-33 +) +/(+ unc-8) dpy-20*	Unc	0/13 *lin-33/+*
	+ + lin-33/unc-17 dpy-13 +	Unc	3/3 *lin-33/+*
		Dpy	0/3 *lin-33/+*
		non-Lin, non-Unc, non-Dpy	1/20 *lin-33/+ + +*
			1/20 *unc-17 dpy-13 lin-33/+ + lin-33*

lin-33	*lin-33* + +/+ *unc-22 unc-30*	Unc-30	44/44 *lin-33*/+
(n1044)	*(lin-33* +) +/(+ *unc-8) dpy-20*	Unc	0/10 *lin-33*/+
	+ (+ *lin-33)/unc-17 (dpy-13* +)	Unc	15/16 *lin-33*/+
		Dpy	0/19 *lin-33*/+
lin-34	+ *(lin-34* +)/*unc-8* (+ *dpy-20)*	Unc	4/4 *lin-34*/+
	(lin-34 +) +/(+ *dpy-20) unc-22*	Unc	7/7 *lin-34*/+
	+ *(lin-34* +)/*lin-3* (+ *dpy-20)*	Dpy	0/16 *lin-34*/+
let-23	+ + *let-23/dpy-10 lin-26* +	Dpy	4/4 *let-23*/+
(n1045)	+ *let-23* +/*lin-5* + *unc-4*	Unc	10/11 *let-23*/+
	+ *let-23* +/*vab-9* + *unc-4*	Unc	4/5 *let-23*/+

Three-factor crosses were performed as described by BRENNER (1974). From heterozygotes of genotype *ab/c* recombinants A non-B and B non-A were picked. The progeny of each recombinant hermaphrodite were examined for the expression of the *trans* marker, *c*. In occasional crosses either C or AB hermaphrodites were picked and scored for the segregation of the *trans* marker(s). *lin-8(n111); lin-9(n112)* is a synthetic Muv strain; *n111* and *n112* each results in a wild-type phenotype when isolated, and, thus, each requires the presence of the other for scoring in mapping experiments. *sup-9* and *sup-10* are recessive suppressors of *unc-93(e1500)* (GREENWALD and HORVITZ 1980). The mapping of *lin* genes relative to these two genes was performed in the presence of *unc-93(e1500).*


a
I
lin-17
lin-10
lin-11
bli-3
lin-6
unc-11
dpy-5
unc-13
lin-28
unc-56
sup-17
unc-29
unc-75
sDf15
nDf24, 25
nDf23
II
lin-8
lin-31
lin-4
lin-26
let-23
lin-7
cat-2
sup-9
unc-85
bli-2
dpy-10
rol-6
unc-4
him-9
unc-52
nDf31
let-236, let-563
lin-3
rol-9
H
mnDf68
mnDf88 H
III
lin-13
lin-9
lin-12
unc-93
dpy-17
unc-69
tra-1
IV
lin-1
lin-33
lin-3
lin-34
lin-24
dpy-9
unc-17
dpy-13
unc-5
dpy-8
dpy-20
unc-22
dpy-26
unc-31
unc-30
cad-3
dpy-4
nDf27
V
lin-25
unc-60
dpy-11
unc-42
sma-1
him-5
unc-76
unc-51
X
lin-18
lin-2
lin-15
unc-78
dpy-23
lon-2
sup-7
dpy-7
unc-84
unc-3
let-16
let-18
let-38
let-40
sup-10
mnDp1
mnDf1
mnDf11
mnDf4
mnDf43
mnDf19
0 5 10
map units
b
lin-13
lin-9
lin-12
lon-1
daf-4
lin-16
unc-36
unc-86
dpy-19
sup-5
sma-2
unc-32
nDf16
0 0.1 0.2
map units

manipulations. We constructed double mutants between all alleles of genes that were not already known to have amber alleles and one of the amber suppressors, *sup-5* or *sup-7* (see MATERIALS AND METHODS). Only data from those suppression experiments in which we observed partial or complete suppression are presented below (or in MATERIALS AND METHODS).

lin-1(e1026, e1275, e1777, n176, n303, n304, n383, n430, n431, n746, n753, n757, n1047, n1054, n1140, n1141) IV: *Multivulva*: The pattern of ventral protrusions in *lin-1* hermaphrodites ranges from four large fairly evenly spaced protrusions to a single vast ventral protrusion at the vulva (Figure 3a). Many of the most severely affected hermaphrodites rupture at the vulva after the L4 molt. Males do not have ventral protrusions. One allele, *e1275*, is weaker and slightly heat sensitive. *e1275* males mate if grown at low temperatures. We have maintained some alleles as balanced heterozygotes, *lin-1/nT1(IV)*; *+/nT1(V)*. *lin-1* was mapped by HORVITZ and SULSTON (1980).

As detailed in Table 1, two of nine *lin-1* alleles tested, *e1777* and *n431*, were suppressed by the amber suppressor *sup-7* and thus probably eliminate *lin-1* gene activity. Both were suppressed by a single copy of the suppressor, *i.e.*, hermaphrodites of genotype *lin-1*(amber)/*lin-1*(amber); *sup-7/+* were phenotypically wild type. To further reduce the amount of suppressed *lin-1* product by a factor of two, hermaphrodites of genotype *lin-1*(amber)/*lin-1*(null, nonamber); *sup-7/+* were constructed. Of 36 hermaphrodites of genotype *lin-1(e1777)/lin-1(e1026)*; *sup-7/+*, 35 had small protrusions at or near the vulva; however, the remainder of the ventral hypodermis appeared normal. The *lin-1* alleles *e1026*, *e1275*, *n176*, *n303*, *n304*, *n383* and *n430* are not suppressed by *sup-7*.

lin-2(e1309, e1424, e1437, e1453, n105, n167, n305, n380, n397, n670, n674, n768, n1052) X: *Vulvaless*: The penetrance of the Vul defect in *lin-2* mutants ranges from a high of 90–95%, in *e1309* and *n1052* animals, to a low of about 21%, in *n768* animals (Table 4). One allele, *n105*, is somewhat heat sensitive: at 15°, 24% of *n105* hermaphrodites are Vul, whereas at 25°, 90% of *n105* hermaphrodites are Vul. One or rarely two ventral protrusions are evident in 15% of the Vul hermaphrodites of genotype *lin-2(e1309)*. Males are phenotypically wild type as viewed with a dissecting microscope. *lin-2* was mapped by HORVITZ and SULSTON (1980).

Weak alleles of *lin-2* (*n768*, *n167*, *n105* at 15°) result in two distinct phenotypes not seen in hermaphrodites carrying stronger alleles of *lin-2* (*e1309*, *n1052*). First, some of these hermaphrodites are severely egg-laying defective (Egl) but not Vul; these hermaphrodites do not lack a vulva but rather have

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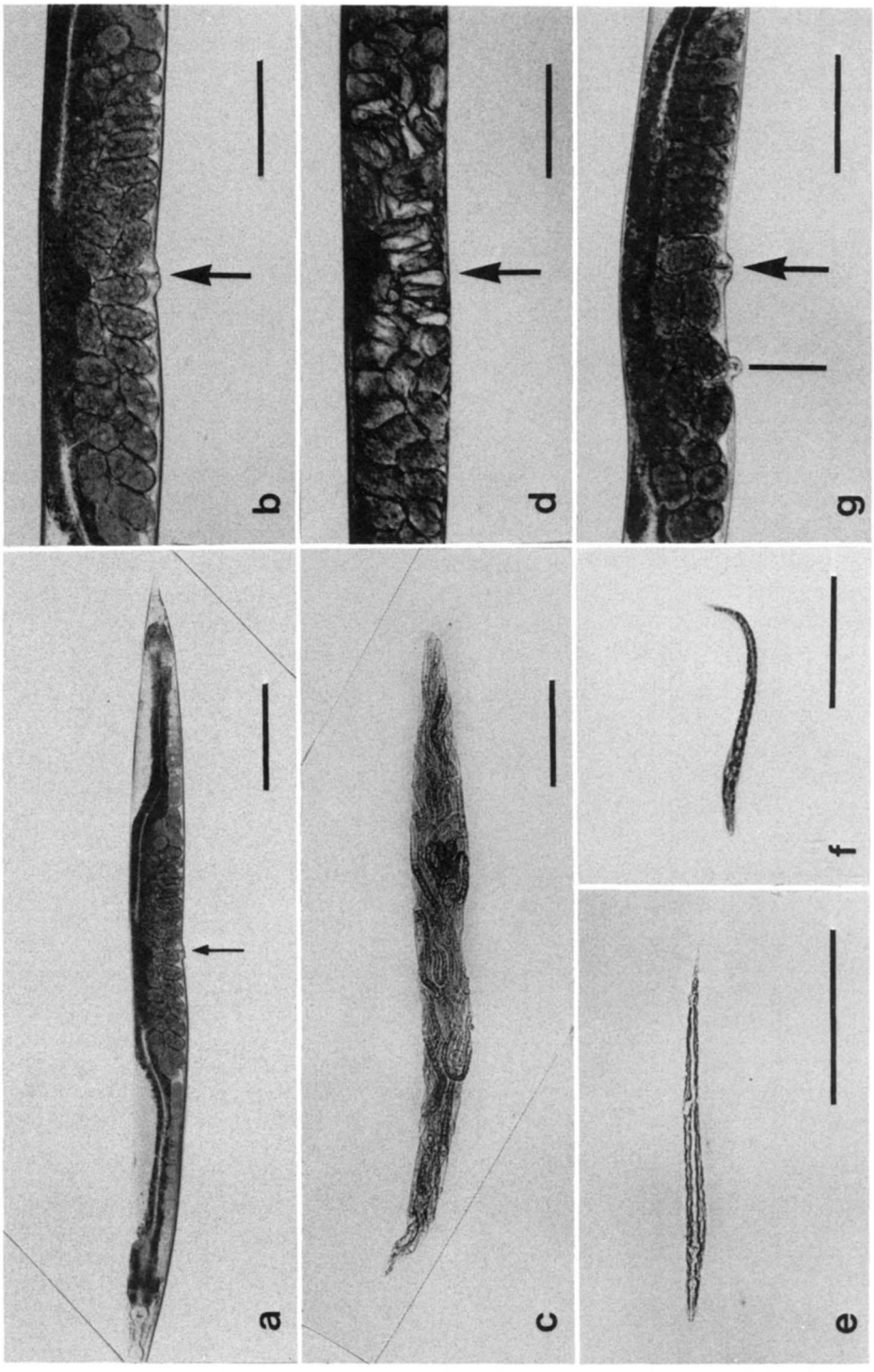
FIGURE 1.—a, Partial genetic map of *C. elegans* indicating the markers used in this study. Genes that affect the vulval cell lineages are drawn above the lines representing the *C. elegans* linkage groups. The extents of deficiencies (*Df*s) and duplications (*Dp*s) are indicated below the lines. *mnDf88* fails to complement *lin-5* and *lin-26*. *mnDf68* fails to complement *vab-9*, *rol-6* and *let-23*. *nDf27* fails to complement the genes in the interval between *dpy-20* and *ced-3*. b, Expanded genetic map of *C. elegans* showing the region of LGIII extending from *lon-1* through *lin-12*. The precise map positions of *lin-16*, *unc-86*, *sup-5* and *sma-2* were determined by M. FINNEY (personal communication).


a
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e
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g

FIGURE 2.—Bright-field photomicrographs of a wild-type hermaphrodite, representative Vul hermaphrodites, and of non-Vul hermaphrodites carrying mutations in genes with Vul alleles. a, Wild-type hermaphrodite; the arrow indicates the vulva (bar, 200 μm). b, The midsection of the same wild-type hermaphrodite as in "a"; the arrow indicates the vulva (bar, 100 μm). c, A Vul hermaphrodite of genotype *lin-24(n432)* in which the progeny have hatched internally (bar, 200 μm); this phenotype of a "bag of worms" is common to all Vul mutants and allows the scoring of the Vul phenotype in the dissecting microscope. d, The midsection of a Vul hermaphrodite of genotype *lin-12(n302)*; the arrow indicates where the vulva is located in wild-type hermaphrodites (bar, 100 μm). e, A hermaphrodite of genotype *let-23(n1045)* that has arrested during larval development (bar, 200 μm); *let-23(n1045)* hermaphrodites that do not arrest during larval development display a cold-sensitive Vul phenotype. f, A hermaphrodite of genotype *lin-3(n1059)* that has arrested during larval development (bar, 100 μm); two other alleles of *lin-3*, *e1417* and *n378*, result in a Vul phenotype. g, The midsection of an egg-laying-competent *lin-7(n701)* hermaphrodite with a functional vulva, indicated by an arrow, and an additional ventral protrusion, indicated by a line (bar, 100 μm); although most *lin-7(n701)* hermaphrodites are Vul in phenotype, about 45% are not.

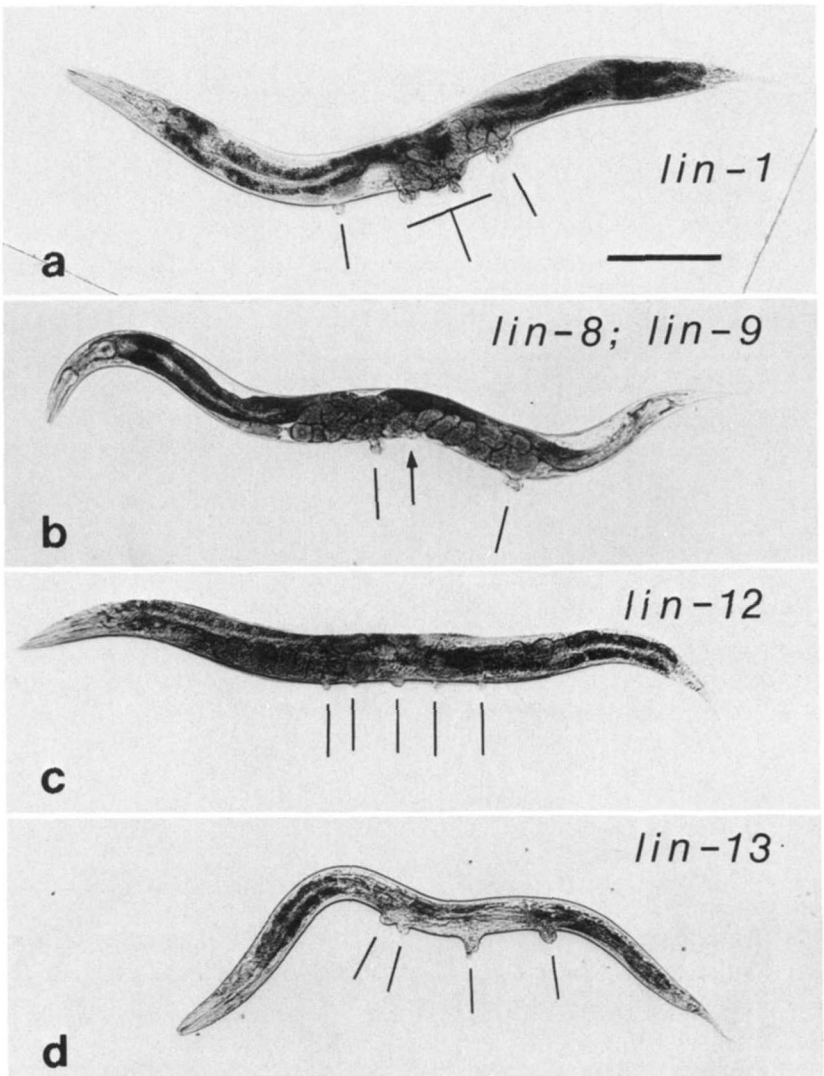

FIGURE 3a–d.—Bright-field photomicrographs of representative Muv hermaphrodites. All photographs are to the same scale (bar, 200 μm). a, A *lin-1(e1777)* hermaphrodite with two small ventral protrusions and a single large ventral protrusion, indicated by lines. The phenotype of *lin-1(e1777)* hermaphrodites ranges from one very large protrusion at the vulva to four evenly spaced smaller protrusions. The majority of *lin-1(e1777)* hermaphrodites do not have functional vulvae. b, A *lin-8(n111); lin-9(n112)* hermaphrodite with a functional vulva, indicated by an arrow, and two ventral protrusions indicated by lines. *lin-8(n111); lin-9(n112)* hermaphrodites usually have a normal vulva and between one and four ventral protrusions. c, A *lin-12(n137)* hermaphrodite that lacks a functional vulva and has five small ventral protrusions, indicated by lines. The middle protrusion is slightly larger than the other four protrusions. The phenotypes of *lin-12(n137)* hermaphrodites do not vary extensively. d, A *lin-13(n387)* hermaphrodite grown at 25° that is sterile and has four ventral protrusions, indicated by lines. *lin-13* hermaphrodites that are grown at 25° are slightly smaller than the wild type, are sterile and usually have between two and four ventral protrusions.

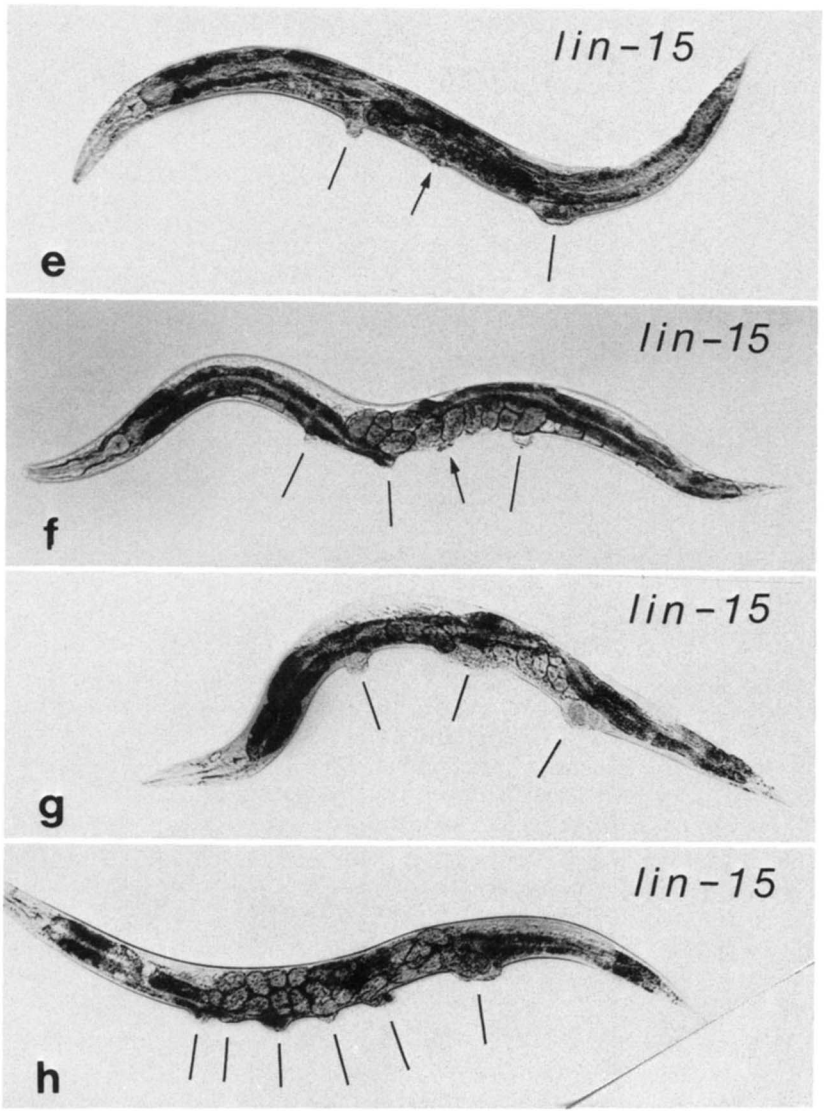

FIGURE 3e–h.—Bright-field photomicrographs of representative Muv hermaphrodites. Four hermaphrodites carrying *lin-15(n309)* showing the variability of the Muv phenotype. All photographs are to the same scale as in Figure 3a–d. e, A *lin-15(n309)* hermaphrodite with two ventral protrusions, indicated by lines, and a functional vulva, indicated by an arrow. f, A *lin-15(n309)* hermaphrodite with three ventral protrusions, indicated by lines, and a functional vulva, indicated by an arrow. g, A *lin-15(n309)* hermaphrodite with three ventral protrusions, indicated by lines. h, A *lin-15(n309)* hermaphrodite with six ventral protrusions, indicated by lines.

an abnormal vulva and are able to release some eggs or larvae. Second, some egg-laying-competent hermaphrodites are Muv; these animals have a functional vulva and one or two ectopic supernumerary vulva-like structures (Table 4 and Figure 2g).

The penetrance of the Vul defect in *e1309* hermaphrodites that pass through

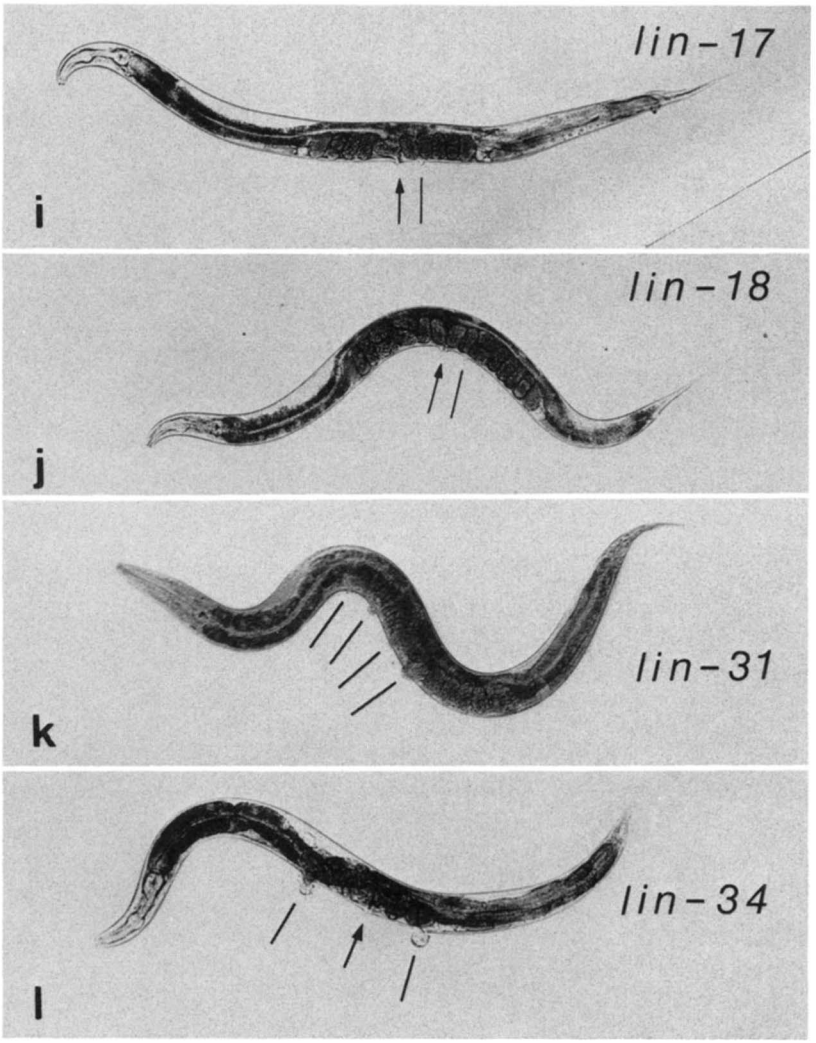

FIGURE 3i–l.—Bright-field photomicrographs of representative Muv hermaphrodites. All photographs are to the same scale as in Figure 3a–d. i, A *lin-17(n671)* hermaphrodite with a single ventral protrusion, indicated by a line, posterior to a functional vulva, indicated by an arrow. *lin-17(n671)* hermaphrodites are slightly longer than the wild type and have a single protrusion immediately posterior to the vulva; ventral protrusions are never observed in other locations. j, A *lin-18(n1051)* hermaphrodite with a single ventral protrusion, indicated by a line, posterior to a functional vulva, indicated by an arrow. *lin-18* hermaphrodites have a single ventral protrusion immediately posterior to the vulva; ventral protrusions are never observed in other locations. k, A *lin-31(n301)* hermaphrodite with four ventral protrusions, indicated by lines. *lin-31(n301)* hermaphrodites have between zero and four small ventral protrusions and some *lin-31* hermaphrodites lack a functional vulva. l, A *lin-34(n1046)* hermaphrodite with two ventral protrusions, indicated by lines, and a functional vulva, indicated by an arrow. *lin-34(n1046)* hermaphrodites usually have between zero and three variably sized ventral protrusions either anterior or posterior to a functional vulva.

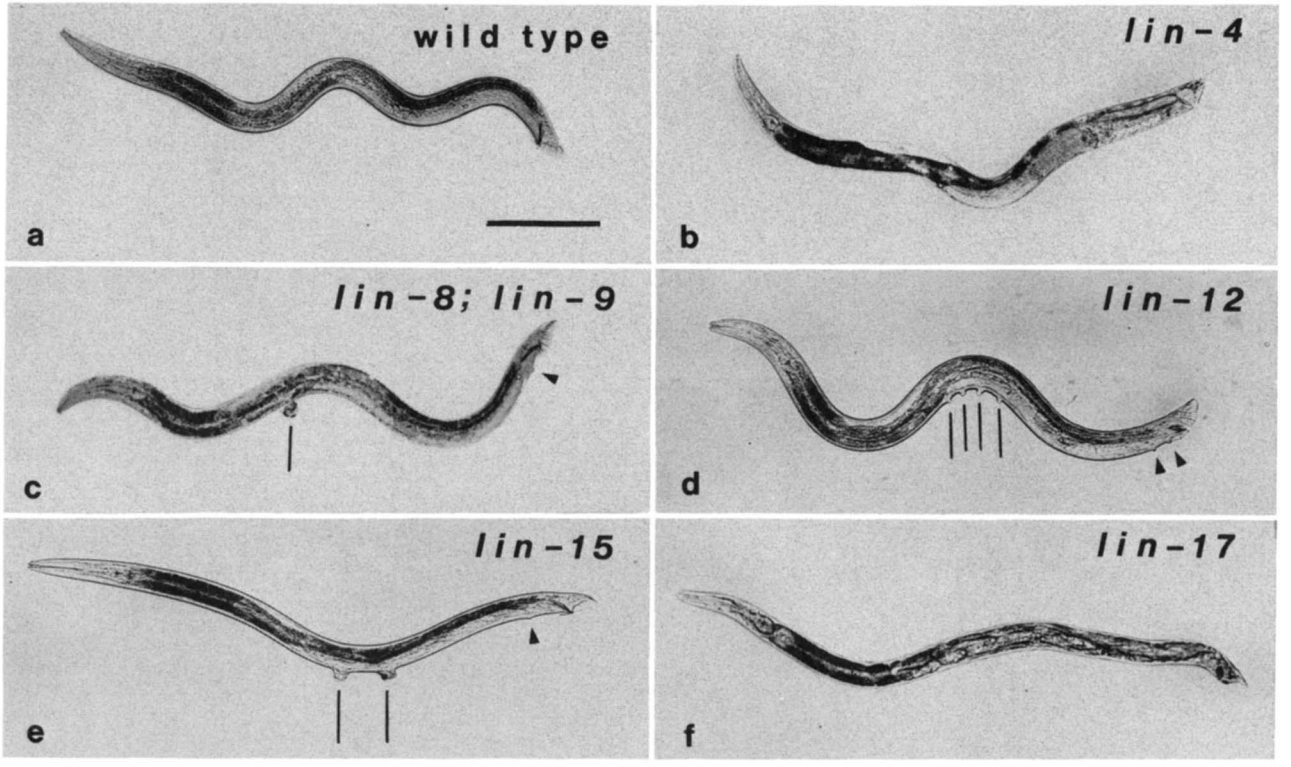

FIGURE 4.—Bright-field photomicrographs of males carrying mutations that affect the vulval cell lineages. Only visibly mutant males are shown. All photographs are to the same scale (bar, 200 μm). a, Wild type. b, A *lin-4(e912)* male with a deformed tail. c, A *lin-8(n111); lin-9(n112)* male that has a single ventral protrusion, indicated by a line, and an ectopic hook-like structure in the tail, indicated by an arrowhead. *lin-8(n111); lin-9(n112)* males have between zero and two ventral protrusions. The ectopic hook is not usually visible in the dissecting microscope. Nomarski optics were used to determine that these tail structures have hook-like morphology and are generated by a lineage pattern similar to that that generates the hook (SULSTON and HORVITZ 1981; P. STERNBERG, personal communication). d, A *lin-12(n137)* male with four small ventral protrusions, indicated by lines, and two ectopic hooks, indicated by arrowheads. The phenotype of *lin-12(n137)* males does not vary extensively. Only one ectopic hook is usually visible in the dissecting microscope. Nomarski optics were used to determine that these tail structures have hook-like morphology and are generated by a lineage pattern equivalent to that that generates the hook (GREENWALD, STERNBERG and HORVITZ 1983). e, A *lin-15(n309)* male with two ventral protrusions, indicated by lines, and an ectopic hook-like structure, indicated by an arrowhead. *lin-15(n309)* males have between one and three ventral protrusions. The ectopic hook is not usually visible in the dissecting microscope. Nomarski optics were used to determine that these tail structures have hook-like morphology and are generated by a lineage pattern similar to that that generates the hook (P. STERNBERG, personal communication). g, A *lin-17(n671)* male with a deformed tail.

TABLE 3

Genes that affect the vulval cell lineages

Genes	No. of alleles	Types of alleles	Reference allele(s)	Hermaphrodite phenotype	Male phenotype	Nature of alleles (evidence)
lin-1 IV	16	*e1777am, n431am, e1275hs*	*e1777am, e1275hs*	Muv: 1 large to 4 small protrusions.	Wild-type morphology. Mating abolished.	Recessive, null (no. of alleles, 2 amber alleles)
lin-2 X	13	*e1453am* (non-null), *n105hs*	*e1309, e1453am*	Vul*: incompletely penetrant.	Wild-type morphology. Efficient mating.	Recessive, null? (no. of alleles)
lin-3 IV	2 + 2 non-Vul		*e1417*	Vul: incompletely penetrant. Non-Vul alleles result in larval arrest or sterility.	Wild-type morphology. Efficient mating.	Recessive, partially decreased function (phenotype enhanced by non-Vul alleles)
lin-4 II	1		*e912*	Vul: highly penetrant. Thin and slightly long.	Very abnormal. Mating abolished.	Recessive, unknown
lin-7 II	13	*e974am, e1413am, e1449am, n308cs, n701cs*	*e1413am*	Vul*: incompletely penetrant.	Wild-type morphology. Efficient mating.	Recessive, null (no. of alleles, 3 amber alleles)
lin-8 II	1		*n111*	WT: Muv with *lin-9* (strain CB1322).	Wild-type morphology. Efficient mating.	Recessive, non-null? (phenotype possibly enhanced by Df)
lin-9 III	1		*n112*	WT: Muv with *lin-8* (strain CB1322).	Wild-type morphology. Efficient mating.	Recessive, partially decreased function (phenotype enhanced by sterile alleles)
CB1322				Muv: variable numbers of large protrusions. Slightly heat sensitive.	Ventral protrusions. Efficient mating.	
lin-10 I	3		*e1439*	Vul: incompletely penetrant.	Wild-type morphology. Efficient mating.	Recessive, null (phenotype not enhanced by Df)
lin-11 I	4		*n389*	Vul: 3 alleles highly penetrant. (*n566* 100% Egl but can mate.) Slightly Unc.	Wild-type morphology. Inefficient mating.	Recessive (*n672* partially dominant), unknown

lin-12 III	7		*n137*(Muv) *n302*(Vul)	Muv: 5 small ventral protrusions. No vulva. Vul: highly penetrant as homozygotes, incompletely penetrant as heterozygotes.	Muv: homozygous: 5 ventral protrusions. Mating abolished. Heterozygous: 1–2 ventral protrusions. Efficient mating. Vul: wild-type morphology. Efficient mating.	Partially dominant, increased activity (dosage studies)
lin-13 III	2	*n387hs, n388hs*	*n387hs*	Muv: sterile, 0–4 ventral protrusions. Maternal effects.	Sterile, occasional ventral protrusions. Mating abolished.	Recessive, partially decreased function (phenotype enhanced by Df)
lin-15 X	5	*n765hs*	*n309, n765hs*	Muv: variable numbers of large protrusions. *n765* can display maternal effect.	Ventral protrusions. Mating abolished.	Recessive, partially decreased function? (phenotype possibly enhanced by Df)
lin-17 I	5		*n671*	Muv: small protrusion posterior to vulva.	Very abnormal tail. Mating abolished.	Recessive, unknown
lin-18 X	2	*e620hs, n1051hs am*	*e620 hs, n1051hs am*	Muv: small protrusion posterior to vulva. Maternal effects.	Wild-type morphology. Efficient mating.	Recessive, null? (amber allele results in heat-sensitive phenotype)
lin-24 IV	2	*n1057am* (non-null)	*n432*	Vul*: *n432*—incompletely penetrant, partially dominant. *n1057*—wild type as homozygote; incompletely penetrant as heterozygote.	Wild-type morphology. Efficient mating.	Partially dominant, novel function ?
lin-25 V	2	*n545hs*	*e1446, n545hs*	Vul: highly penetrant, some sterility.	Wild-type morphology. Mating abolished.	Recessive, unknown
lin-26 II	1		*n156*	Vul: highly penetrant. Slightly dumpy.	Very abnormal tail. Mating abolished.	Recessive, partially decreased function (phenotype enhanced by Df)
lin-31 II	11		*n301*	Muv: 0–4 small ventral protrusions.	Wild-type morphology. Mating abolished.	Recessive, null (no. of alleles, phenotype not enhanced by Df)
lin-33 IV	2		*n1043*	Vul*: incompletely penetrant.	Wild-type morphology. Efficient mating.	Partially dominant, novel function?
lin-34 IV	1	*n1046am* (non-null)	*n1046am*	Muv: variable numbers of large protrusions.	Wild-type morphology. Mating greatly reduced.	Partially dominant, unknown

TABLE 3—Continued

Genes	No. of alleles	Types of alleles	Reference allele(s)	Hermaphrodite phenotype	Male phenotype	Nature of alleles (evidence)
let-23 II	1 + 3 non-Vul	*n1045cs am* (non-null)	*n1045cs am*	Vul*: incompletely penetrant. Some *n1045* animals arrest during larval development. Non-Vul alleles result in fully penetrant larval arrest.	Wild-type morphology. Mating greatly reduced.	Recessive, partially reduced function (phenotype enhanced by Df, other alleles)
[a]	1		*n300*	Vul: highly penetrant.	Slightly abnormal tail. Mating abolished.	Recessive, unknown, associated with translocation *nT1(IV;V)*

Reference alleles are those alleles used in mapping studies and/or studies of gene interactions (E. FERGUSON and P. STERNBERG, unpublished observations). Hermaphrodite and male phenotypes are described as viewed with a dissecting microscope. Data concerning the penetrances and expressivities of the Vul mutations are presented in Table 4. An "*" following "Vul" indicates that one or more alleles of the gene result in a phenotype described in the legend of Table 4. Data concerning male mating ability are presented in Table 13. Some genes have alleles that were not identified in our screen for Muv and Vul mutants. These alleles result in lethal or sterile phenotypes. The number of such alleles, if any, is noted following the number of Vul or Muv alleles of that gene, *e.g.*, *let-23*, which has one allele that results in a Vul phenotype and three alleles that result in a larval lethal phenotype, has "1 + 3 non-Vul" alleles. am, amber; cs, cold sensitive; hs, heat sensitive; WT, wild-type.

[a] *n300* has not been assigned to a gene (see RESULTS).

a dauer larval stage (CASSADA and RUSSELL 1975; RIDDLE, SWANSON and ALBERT 1981) is equivalent to that of unstarved animals (95% Vul, $n = 227$); however, the penetrance of the Vul phenotype markedly decreases in *e1309* hermaphrodites that do not pass through a dauer larval stage but have been starved before reaching adulthood (36% Vul, $n = 416$).

The allele *e1453* does not result in the strongest *lin-2* phenotype (Table 4) and, thus, probably does not totally eliminate *lin-2* gene activity. Nonetheless, *e1453* is an amber mutation. *e1453* is suppressed in a *sup-5* heterozygote, *i.e.*, hermaphrodites of genotype *sup-5/+*; *lin-2(e1453)/lin-2(e1453)* are wild type (HORVITZ and SULSTON 1980). To reduce the amount of suppressed *lin-2* gene product, hermaphrodites of genotype *sup-5/+*; *lin-2(e1453)/lin-2*(nonamber) were constructed. The nonamber allele used was *e1309*, the *lin-2* allele of highest penetrance. Of 23 hermaphrodites of genotype *sup-5/+*; *lin-2(e1453)/lin-2(e1309)*, two did not have functional vulvae. Although hermaphrodites of genotype *sup-5/+* can have a vulval defect (three of 257 such hermaphrodites had abnormal vulvae), the penetrance of the vulval defect in hermaphrodites of genotype *sup-5/+*; *lin-2(e1453)/lin-2(e1309)* is probably greater, suggesting that the vulval defect of these animals results from the incomplete suppression of the Lin-2 phenotype.

lin-3(e1417, n378, n1058, n1059) IV: *Vulvaless*: The penetrance of the Vul defect of *e1417* hermaphrodites is 89%, whereas that of *n378* hermaphrodites is 97% (Table 4). In both cases 15% of the Vul hermaphrodites have a single ventral protrusion. Males are phenotypically wild type as viewed with a dissecting microscope. *lin-3* was mapped between *unc-5* and *dpy-20* by HORVITZ and SULSTON (1980).

The penetrance of the Vul phenotype in *n378* hermaphrodites that pass through a dauer larval stage decreases from 97 to 70% ($n = 389$). In addition, the percentage of Vul hermaphrodites that have one or more ventral protrusions increases from 14 to 42%, suggesting that in these animals a greater number of vulval precursor cells have divided. However, the penetrance of the Vul defect in *n378* hermaphrodites that do not pass through a dauer larval stage but have been starved before reaching adulthood is equivalent to that of unstarved animals (99% Vul, $n = 180$).

In an effort to determine the null phenotype of *lin-3*, a complementation screen to obtain new *lin-3* alleles was performed against *lin-3(e1417)*, as described in MATERIALS AND METHODS. Two additional alleles of *lin-3*, *n1058* and *n1059*, were obtained. *n1059* animals arrest during early larval development with a rigid, rod-like phenotype (Figure 1f). Although most *n1058* hermaphrodites are sterile adults, some arrest with a phenotype similar to that caused by *n1059*. To determine whether it is the *lin-3* mutation in the *lin-3(n1058)* strain that results in the observed sterility, 30 Unc non-Dpy recombinants were picked from among the progeny of hermaphrodites of genotype *unc-8(e49)* + *dpy-20/+ lin-3(n1058)* +. These recombinant hermaphrodites were allowed to produce self-progeny for 1 day and then were mated wtih *lin-3(e1417)* males. The self-progeny of these hermaphrodites were scored for the presence of sterile Unc animals, and the cross-progeny were scored for the

TABLE 4

Penetrance and expressivity of Vul alleles

Gene	Allele	%Vul		% Vul hermaphrodites with given no. of ventral protrusions			
				0	1	2	3
lin-2							
	e1309 (n = 286)	93		85	13	2	0
	e1424 (n = 358)	69		46	40	13	1
	e1437 (n = 221)	54					
	e1453 (n = 348)	50		38	53	8	1
	15° *n105* (n = 261)	24*					
	25° *n105* (n = 175)	90					
	n167 (n = 278)	41*					
	n305 (n = 251)	85					
	n380 (n = 267)	75					
	n397 (n = 308)	77					
	n670 (n = 339)	71					
	n674 (n = 295)	79					
	n768 (n = 358)	21*	Vul	12	46	34	8
			non-Vul	68	27	5	0
	n1052 (n = 190)	89					
lin-3							
	e1417 (n = 280)	89		84	16	0	0
	n378 (n = 266)	97		86	13	1	0
lin-4							
	e912 (n = 204)	100		96	4	0	0
lin-7							
	e974 (n = 272)	94		75	23	2	0
	e1413 (n = 341)	98		73	21	6	1
	e1449 (n = 214)	95					
	n106 (n = 362)	33*	Vul	50	36	12	2
			non-Vul	79	19	2	0
	15° *n308* (n = 253)	91					
	20° *n308* (n = 276)	65*	Vul	37	40	20	3
			non-Vul	67	16	16	1
	25° *n308* (n = 179)	28*					
	n385 (n = 272)	91					
	n673 (n = 312)	94		80	17	3	0
	n699 (n = 387)	91		70	21	9	0
	15° *n701* (n = 196)	96					
	20° *n701* (n = 345)	62*					
	25° *n701* (n = 236)	56*					
	n759 (n = 240)	91					
	n760 (n = 229)	94					
	n763 (n = 176)	93					
	n764 (n = 208)	94					
lin-10							
	e1438 (n = 220)	93		55	34	11	0
	e1439 (n = 282)	95		76	20	4	0
	n299 (n = 211)	97		84	13	3	0

TABLE 4—Continued

Gene	Allele	%Vul		% Vul hermaphrodites with given no. of ventral protrusions 0	1	2	3
lin-11							
	n382 (*n* = 182)	100		2	98	0	0
	n389 (*n* = 115)	100		3	97	0	0
	n566 (*n* = 233)	100		0	100	0	0
	n672 (*n* = 122)	100		3	97	0	0
lin-24							
	n432 (*n* = 232)	97*		92	8	0	0
	n1057 (*n* = 203)	0					
	n1057/+ (*n* = 314)	33*					
lin-25							
	e1446 (*n* = 279)	100		19	81	0	0
		11% are sterile					
	15° *n545* (*n* = 305)	8					
	25° *n545* (*n* = 234)	100		9	91	0	0
		18% are sterile					
lin-26							
	n156 (*n* = 268)	100		88	12	0	0
lin-33							
	n1043 (*n* = 221)	96*		95	5	0	0
	n1044 (*n* = 253)	95*		93	7	0	0
let-23							
	15° *n1045* (*n* = 219)	50					
		49% undergo larval arrest					
	20° *n1045* (*n* = 763)	14*	Vul	14	64	22	0
			non-Vul	74	25	1	0
		51% undergo larval arrest					
	25° *n1045* (*n* = 310)	2*	Vul	14	72	14	0
			non-Vul	62	36	2	0
		28% undergo larval arrest					
[a]	*n300* (*n* = 202)	100		100	0	0	0

To determine the penetrance and expressivity of the phenotype of each allele of the Vul genes, about ten plates each containing 25 L2 hermaphrodites were placed at 20° or at the indicated temperature. The percentage of hermaphrodites that turned into bags of worms was then determined. An "*" following the percentage of Vulvaless hermaphrodites indicates that some hermaphrodites carrying this Vul allele have an abnormal vulva and are severely Egl but are able to release some progeny. To determine the penetrance of these mutations, we measured the percentage of animals that were severely egg-laying defective without regard to their abilities to release progeny, and we report these data here as "% Vul." Most mutations in most Vul genes do not completely prevent vulval cell divisions (P. STERNBERG, personal communication). The vulval cells that are produced can form one or more ventral protrusions. For at least one allele of each gene, the percentage of Vul hermaphrodites with given numbers of ventral protrusions was determined. For weak alleles of three genes, *lin-2*, *lin-7* and *let-23*, some egg-laying-competent hermaphrodites also have ventral protrusions. The frequency of ventral protrusions in such hermaphrodites is prefaced by the phrase "non-Vul." For *let-23(n1045)*, the phrase "non-Vul" refers only to adult hermaphrodites and not to *let-23(n1045)* hermaphrodites that arrest during larval development.

[a] *n300* has not been assigned to a gene (see RESULTS).

TABLE 5

Phenotypes of lin-3 *homozygous and heteroallelic strains*

lin-3 allele	*n378*	*e1417*	*n1058*	*n1059*
n378	97% Vul (n = 266)	88% Vul (n = 226)	78% Vul (n = 364)	100% Vul (n = 665)
e1417		89% Vul (n = 351)	59% Vul (n = 414)	99.8% Vul (n = 471)
n1058			Sterile; occasional arrested larvae	Arrested larvae
n1059				Arrested larvae

Interactions among *lin-3* alleles. Either the penetrance of the Vul phenotype (expressed as "% Vul") or a statement of the phenotype ("sterile" or "arrested larvae") is presented. Animals heteroallelic for different *lin-3* mutations were obtained in the following ways. *n378/e1417*: non-Unc progeny from the mating of *lin-3(n378); him-5* males with *unc-32; lin-3(e1417)* hermaphrodites. *n378/n1058*: non-Unc, non-Dpy progeny from hermaphrodites of genotype + *lin-3(n378) dpy-20/unc-8(e49) lin-3(n1058)* +. *n378/n1059*: non-Dpy progeny from hermaphrodites of genotype *lin-3(n378) dpy-20/lin-3(n1059)* +. *e1417/n1058*: non-Unc, non-Dpy progeny from hermaphrodites of genotype + *lin-3(e1417) dpy-20/unc-8(e49) lin-3(n1058)* +. *e1417/n1059*: non-Dpy progeny from hermaphrodites of genotype *lin-3(e1417) dpy-20/lin-3(n1059)* +. The phenotype of hermaphrodites of genotype *lin-3(n1058)/lin-3(n1059)* was determined by mating males of genotype + *lin-3(n1059)* +*/unc-8(e49)* + *dpy-20* with hermaphrodites of genotype *unc-8(e49) lin-3(n1058)* +*/unc-8(e49)* + *dpy-20*. Many arrested larvae were seen among the progeny of the mating. The genotypes of 44 non-Unc progeny were determined; all were of genotype + *lin-3(n1059)* +*/unc-8(e49)* + *dpy-20*.

presence of Vul animals. In all cases the sterility cosegregated with *lin-3(n1058)*. *unc-8* and *dpy-20* are approximately 2 map units apart. Thus, the sterility is linked to within 0.07 map units of *lin-3(n1058)* and probably results from the same mutation.

The phenotypes of hermaphrodites heterozygous for various *lin-3* alleles are described in Table 5. *n1059* increases the severity of the other *lin-3* alleles in *trans* and, thus, probably results in a phenotype more closely resembling the null phenotype of *lin-3*. However, although *n1058* results in a more severe phenotype than *e1417*, *i.e.*, some *n1058* animals undergo larval arrest, as do all animals of genotype *n1058/n1059*, *n1058* reduces the penetrance of the Vul phenotype of *e1417* in *trans*. These results are consistent with the hypothesis that these three mutations—*e1417*, *n1058*, *n1059*—result in successively greater reductions of a single *lin-3* activity and that *e1417* and *n1058* display partial intragenic complementation. However, these results are also consistent with an alternate hypothesis that *lin-3* has two activities, an early function essential during larval development, which may also be necessary for fertility, and a later function needed during vulval development.

lin-4(e912) II: *Vulvaless*: Greater than 99% of *lin-4* hermaphrodites form bags of worms and about 95% do not have ventral protrusions (Table 4). *lin-4* hermaphrodites are thinner and slightly longer than the wild type. Some *lin-4* males die before adulthood; others have incompletely formed tail structures (Figure 4b). *lin-4* was mapped by HODGKIN (1974). The phenotype of *lin-4*

animals has been described by SULSTON and HORVITZ (1981) and CHALFIE, HORVITZ and SULSTON (1981) and interpreted as reflecting temporal transformations in the fates of certain cells by AMBROS and HORVITZ (1984). For ease of genetic manipulation *lin-4* is maintained in two balanced heterozygote strains, *lin-4(e912)/C1 dpy-10 unc-52* and *lin-4(e912)/C1 dpy-10 unc-52; him-5.*

e912 results in a slightly heat-sensitive phenotype. Although no egg-laying-competent *e912* hermaphrodites were observed at any temperature, at 15° 4% (n = 326) of *e912* hermaphrodites have recognizable vulval structures as viewed with a dissecting microscope. Although 4% of *e912* hermaphrodites grown at 20° have a ventral protrusion (Table 4), no such vulval structure is observed in these hermaphrodites.

lin-7(e974, e1413, e1449, n106, n308, n385, n673, n699, n701, n759, n760, n763, n764) II: *Vulvaless*: The penetrance of the Vul phenotype of all but one of the *lin-7* mutants is about 95% (Table 4). The Vul phenotype of *n106* hermaphrodites is much less penetrant (33%, n = 362). In addition, two alleles, *n701* and *n308*, are cold sensitive: at 15° both result in a Vul phenotype of about 95% penetrance, whereas at 25° the Vul phenotype resulting from both mutations is much less penetrant (62%, *n701*; 28%, *n308*). In general 20–30% of *lin-7* hermaphrodites have one, or rarely two, ventral protrusions. Males are phenotypically wild type as viewed with a dissecting microscope. *lin-7* was mapped by HORVITZ and SULSTON (1980).

Weak alleles of *lin-7* (*n106*, *n308* at 25°, *n701* at 25°) result in two distinct phenotypes not seen in hermaphrodites carrying a stronger allele of *lin-7* (*e.g.*, *e1413*). First, some of these hermaphrodites are severely Egl but not Vul; these hermaphrodites do not lack a vulva but rather have an abnormal vulva and are able to release some eggs or larvae. Second, some egg-laying-competent hermaphrodites are Muv; these animals have a functional vulva and one or two ectopic supernumerary vulva-like structures (Table 4).

The penetrance of the Vul phenotype in *e1413* hermaphrodites that pass through a dauer larval stage is probably equivalent to that of unstarved animals (87% Vul, n = 171); however, the penetrance of the Vul phenotype markedly decreases in *e1413* hermaphrodites that do not pass through a dauer larval stage but have been starved before reaching adulthood (22% Vul, n = 273).

e1413 is an amber mutation (HORVITZ and SULSTON 1980) and, thus, probably eliminates *lin-7* gene activity. It is suppressed by a single copy of *sup-7*, *i.e.*, hermaphrodites of genotype *lin-7*(amber)/*lin-7*(amber); *sup-7*/+ are phenotypically wild type (HORVITZ and SULSTON 1980). As detailed in Table 1, two of the eight other *lin-7* alleles tested, *e974* and *e1449*, were also suppressed by a single copy of *sup-7*. To further reduce the amount of suppressed *lin-7* product by a factor of two, hermaphrodites of genotype *lin-7*(amber)/*lin-7*(null, nonamber); *sup-7*/+ were constructed. Of 55 hermaphrodites of genotype *lin-7(e1413)/lin-7(n385); sup-7*/+, six were either egg-laying defective or lacked a functional vulva. Although hermaphrodites of genotype *sup-7*/+ can have a vulval defect (two of 226 such hermaphrodites had abnormal vulvae), the penetrance of the vulval defect in hermaphrodites of genotype *lin-7*(amber)/*lin-7*(null, nonamber); *sup-7*/+ is much greater, suggesting that the vulval de-

fect of these animals results from the incomplete suppression of the Lin-7 phenotype. The alleles *n308*, *n385*, *n673*, *n701*, *n699* were not suppressed by *sup-7*.

CB1322: *Multivulva*: The Muv strain CB1322 carries two unlinked mutations, *lin-8(n111) II* and *lin-9(n112) III*. Each of these mutations alone results in a wild-type phenotype (HORVITZ and SULSTON 1980). Many CB1322 hermaphrodites have two ventral protrusions, one anterior and one posterior to the vulva (Figure 3b). Some hermaphrodites have an abnormal vulva and are egg-laying defective. The males of this strain have zero to two ventral protrusions (Figure 4c).

lin-8(n111) II: *Wild-type*: When separated from *lin-9(n112)*, *lin-8(n111)* results in a wild-type phenotype in both hermaphrodites and males. *lin-8* was mapped between *cat-2* and *unc-85* by HORVITZ and SULSTON (1980).

We have established that *nDf3* fails to complement *lin-8*. Males of genotype *lin-8(n111) dpy-10/+ +*; *lin-9(n112)/+* were mated with hermaphrodites of genotype *nDf3 +/lin-31 bli-2*. [*nDf3* fails to complement *lin-31* (see below) but complements *bli-2* and *unc-85* (GREENWALD and HORVITZ 1980).] From F_1 progeny hermaphrodites of genotype *lin-8(n111) dpy-10/nDf3 +*; *lin-9(n112)/ +*, Muv non-Dpy hermaphrodites of putative genotype *lin-8(n111) dpy-10/nDf3 +*; *lin-9(n112)* were picked. These hermaphrodites segregated approximately ¼ dead eggs, confirming the presence of *nDf3*.

The phenotype of hermaphrodites of genotype *lin-8(n111)/nDf3*; *lin-9(n112)* suggests that *n111* may not eliminate *lin-8* gene activity. The phenotype of these hermaphrodites is similar to the phenotype of *lin-8(n111)*; *lin-9(n112)* hermaphrodites except that, relative to *lin-8(n111)*; *lin-9(n112)* hermaphrodites, hermaphrodites of genotype *lin-8(n111)/nDf3*; *lin-9(n112)* have a higher incidence of sterility and general sickness. This sterility and sickness is not seen in either hermaphrodites of genotype *lin-8(n111)/nDf3*, which have a wild-type phenotype, or in hermaphrodites of genotype *nDf3/+*; *lin-9(n112)*. [Hermaphrodites of the former genotype were constructed by picking progeny of hermaphrodites of genotype *lin-8(n111) dpy-10/nDf3 +*; *lin-9(n112)/+* that segregated approximately ¼ dead eggs and did not segregate any Muv progeny. Hermaphrodites of the latter genotype were constructed by mating males of genotype *unc-85/+*; *dpy-17 lin-9(n112)/+ +* with hermaphrodites of genotype *nDf3 +/lin-31 bli-2*, and, from F_1 progeny of genotype *nDf3 +/unc-85*; *dpy-17 lin-9/+ +*, picking F_2 Dpy non-Unc animals of genotype *nDf3 +/unc-85*; *dpy-17 lin-9*.]

lin-9(n112) III: *Wild-type*: When separated from *lin-8(n111)*, *lin-9(n112)* results in a wild-type phenotype in both hermaphrodites and males. *lin-9* was shown to map near *unc-32* by HORVITZ and SULSTON (1980). A second allele of *lin-9*, *n942*, that results in a sterile phenotype and will be described elsewhere was used to map *lin-9* between *sma-2* and *unc-32*.

n112 results in a temperature-dependent maternal effect, which is evident at 15° but not at 20° or 25°. At 15°, from parental hermaphrodites of gentoype *lin-8(n111)*; *dpy-17 lin-9(n112)/+ +*, only 2% (n = 150) of the progeny hermaphrodites of genotype *lin-8(n111)*; *dpy-17 lin-9(n112)* were Muv.

lin-10(e1438, e1439, n299) I: *Vulvaless*: The penetrance of the Vul phenotype of all three alleles of *lin-10* is about 95% (Table 4). Sixteen percent (n = 211) of *n299* hermaphrodites that are Vul have one, or occasionally two, ventral protrusions. However, even though 93% (n = 220) of *e1438* hermaphrodites are Vul, 45% of the Vul hermaphrodites have one or more ventral protrusions. Males are phenotypically wild type as viewed with a dissecting microscope.

Unlike the six other genes with incompletely penetrant Vul mutations (*lin-2*, *lin-3*, *lin-7*, *lin-24*, *lin-33* and *let-23*), the penetrance of the Vul phenotype of *lin-10(n299)* hermaphrodites is not decreased either by starvation or by passage through the dauer larval stage (E. FERGUSON, unpublished results).

A series of deficiencies in the *unc-13* region were used to further map *lin-10*. The deficiencies *sDf5* and *nDf23* complement *lin-10*, whereas the deficiencies *nDf24* and *nDf25* fail to complement *lin-10*. [*sDf5* complements both *nDf24* and *nDf25*; *nDf23*, *nDf24* and *nDf25* all fail to complement *lin-28* (E. FERGUSON, unpublished results).] The penetrance of the Vul phenotype in hermaphrodites of genotype *lin-10(e1439)/Df* is not enhanced relative to the penetrance of the Vul phenotype in *e1439* hermaphrodites: 92% (n = 177) of hermaphrodites of genotype *dpy-5 lin-10(e1439)/+ nDf25* were Vul, and 88% (n = 139) of hermaphrodites of genotype *dpy-5 lin-10(e1439)/+ nDf24* were Vul. These results are consistent with the hypothesis that *e1439* reduces or eliminates *lin-10* activity and that lack of *lin-10* gene activity results in a Vul phenotype.

lin-11(n382, n389, n566, n672) I: *Vulvaless*: The egg-laying defect of all four mutants defective in this gene is 100% penetrant; greater than 95% of all hermaphrodites have a single ventral protrusion and all form bags of worms. No hermaphrodites of genotypes *n382*, *n389* or *n672* have been observed to form a functional vulva. However, *n566* hermaphrodites can form a functional vulva, as some animals are able to mate. Hermaphrodites and males carrying any of the four alleles are slightly uncoordinated. Males have an otherwise wild-type phenotype as viewed in a dissecting microscope.

Males of genotypes *n382*, *n389* or *n682* were originally obtained by heat shock and mated to *unc-32*; *him-5* hermaphrodites to generate *lin-11*; *him-5* strains, which were used for subsequent genetic manipulations. Because *lin-11* males mate inefficiently (Table 13), about 20 L4 *lin-11*; *him-5* males were generally used in each mating.

n382, *n389* and *n566* are recessive mutations. *n672* is slightly semidominant, as approximately 5% of heterozygous hermaphrodites are Vul. *n672* was assigned to *lin-11* (*i.e.*, interpreted as allelic with the three recessive mutations) based on its map position and on complementation data. *n672; him-5* males were mated to hermaphrodites of genotype *dpy-5* + *lin-11(n389)/dpy-5 unc-29* +. Eleven F_1 Lin non-Dpy hermaphrodites were picked. All of their progeny were Vul, indicating that they were of genotype *dpy-5 lin-11(n389)/+ lin-11(n672)*. Sixteen F_1 non-Dpy egg-laying-competent hermaphrodites were also picked. The progeny of these hermaphrodites indicated that their genotype was + + *lin-11(n672)/dpy-5 unc-29* +. From one of these hermaphrodites three Unc non-Dpy and eight Dpy non-Unc animals were picked. Zero of three Unc

animals and eight of eight Dpy animals segregated *n672*, confirming that *n672* is close to or right of *unc-29*, as is *lin-11(n389)*.

lin-12(n137, n177, n427, n302, n379, n676, n769) III: *Multivulva and Vulvaless*: Seven partially dominant mutations affecting the vulval cell lineages map to the same region of LGIII: all seven of these mutations are closely linked to *unc-32 III*, and most have been shown to map slightly to the right of *unc-32* (Table 6). These mutations result in three different phenotypes: *n137*, *n177* and *n427* are semidominant Muv mutations; *n302*, *n379* and *n676* are semidominant Vul mutations; *n769* is a dominant Vul and a recessive Muv mutation. The three semidominant Muv mutations are slightly cold sensitive (Table 7).

Homozygous Muv hermaphrodites lack a functional vulva, have a small brood size and generally have five ventral protrusions (Figure 3c). Heterozygous Muv hermaphrodites are similar but more fertile (Table 8). The penetrance of the Vul defect is greater than 95% in all three homozygous Vul strains but is lower in heterozygous strains (Table 9).

Homozygous Muv males (Figure 4d) have four midbody ventral protrusions. In addition, Muv males have two ventral protrusions just anterior to the tail; however, only the anterior of these two protrusions is usually visible as viewed with a dissecting microscope. A single protrusion just anterior to the tail is observed in about 70% of the heterozygous Muv males at 20°. About 25% of these males have either one of two protrusions midbody. Vul males have a wild-type phenotype as viewed with a dissecting microscope.

The lack of a functional vulva is a highly penetrant defect in all of the homozygous Vul and Muv *lin-12* strains. To permit genetic manipulations, these mutations are maintained in a number of different genetic backgrounds. Because the homozygous Vul males mate efficiently, the three Vul strains are maintained as *lin-12(Vul)*; *him-5* strains. However, because neither homozygous males nor heterozygous hermaphrodites of the other four *lin-12* alleles are able to mate, heterozygous males of two different genotypes [+ *lin-12/unc-32* +; *him-5* or *lin-12/eT1(III)*; *him-5/eT1(V) him-5*] are used to transfer these mutations. [*n886*, a recessive lethal mutation linked to *eT1(III;V)* (M. FINNEY, personal communication) is also present in the latter strain. *him-5* was placed on *eT1(V)* by recombination (V. AMBROS, personal communication).]

Heteroallelic *lin-12* strains were constructed to examine the interactions between the different mutations. Three classes of heteroallelic strains were constructed: *lin-12(Muv)/lin-12(Muv)*, *lin-12(Muv)/lin-12(Vul)* and *lin-12(Vul)/lin-12(Vul)*. The heteroallelic interactions of the Muv alleles with each other and with the Vul alleles could not be differentiated in hermaphrodites of different genotypes because all such heteroallelic hermaphrodites have a Muv phenotype. However, these interactions could be quantified in males by counting the number of ventral protrusions. The interactions between the Vul mutations were quantified by measuring the penetrance of the Vul defect in the heteroallelic strain. [*n769*, which results in a totally penetrant Vul phenotype as a heterozygote, was examined in *trans* only with a *lin-12(Muv)* allele.] To generate males carrying different pairs of Muv alleles in *trans*, heterozygous Muv

males (*lin-12(n177)*/+ or *lin-12(n427)*/+) were mated wtih *sup-17(n316); lin-12(n137)* hermaphrodites. [*sup-17(n316) I* is an extragenic suppressor of partially dominant *lin-12* alleles isolated by phenotypically reverting *lin-12(n177)*; E. FERGUSON, unpublished results.] In both cases approximately one-half of the progeny males were phenotypically indistinguishable from homozygous *n137* males, indicating that *n177* and *n427* interact with *n137*. Similarly, the penetrance of the Vul defect in hermaphrodites bearing two different Vul mutations in *trans* is similar to the penetrance of the homozygous Vul strains (Table 9). In addition, the Vul mutations enhance the phenotype of the Muv mutation *n137* in *trans*: *lin-12(Muv)/lin-12(Vul)* is more mutant than *lin-12(Muv)*/+ but less mutant than *lin-12(Muv)/lin-12(Muv)* (Tables 7 and 8). This pattern can be seen both in the fertility of hermaphrodites and in the number of ventral protrusions of males.

A detailed genetic analysis of these and other partially dominant *lin-12* alleles has been reported (GREENWALD, STERNBERG and HORVITZ 1983). These studies have (1) led to the identification of amber alleles of *lin-12* and shown that loss of *lin-12* function does not result in a Vul or Muv phenotype and (2) demonstrated that the Muv and Vul mutations we are describing in this paper are alleles of *lin-12* and result in increases in *lin-12* gene activity. The differing phenotypes (Muv and Vul) caused by these alleles appear to reflect differences in the magnitude of the increase in *lin-12* activity. The elevated level of *lin-12* activity resulting from all seven partially dominant *lin-12* alleles is sufficient to prevent the formation of the gonadal anchor cell, which is necessary for the generation of a functional vulva. Thus, all seven mutants fail to form a functional vulva. However, only in the three Muv strains is the increase in the level of *lin-12* activity apparently sufficient to alter directly the lineages of the cells of the ventral hypodermis and to thereby result in a Muv phenotype.

lin-13(n387,n388) III: *Multivulva*: Both alleles of *lin-13* result in a heat-sensitive sterile Muv phenotype and a temperature-dependent maternal effect. The protrusions of the Muv hermaphrodites are variably sized and evenly spaced along the ventral side. The phenotype of homozygous *lin-13* hermaphrodites segregating from a heterozygous strain depends on the temperature at which the strain was grown. At 25°, *n387* hermaphrodites segregating from a heterozygote are both Muv and sterile (Figure 3d). At 20°, about half of the *n387* hermaphrodites segregating from a heterozygote are sterile, but only a few of the animals are Muv. At 15°, *n387* hermaphrodites segregating from a heterozygote are almost wild type in appearance and fertility. However, if the progeny of these 15° animals are grown at 15°, all are sterile and some are Muv. If the progeny of these 15° animals are grown at 25°, some animals arrest during larval growth, and the rest are both sterile and Muv. The male phenotype similarly is heat sensitive; only males that are the progeny of *lin-13* hermaphrodites and are grown at 20° or 25° have ventral protrusions. The *lin-13* alleles are maintained in the balanced strains + *lin-13* +/*dpy-17* + *unc-86, lin-13* +/+ *unc-32; him-5* or *lin-13/eT1(III); him-5/eT1(V) him-5.*

lin-13 acts both maternally and zygotically. The maternal activity of *lin-13* can be observed at 15°: *lin-13* homozygous hermaphrodites that are the prog-

TABLE 6

Map data for lin-12 *alleles: three- and four-factor crosses*

Allele	Genotype of heterozygote	Phenotype of recombinant	Genotype of selected recombinants
lin-12(n137)			
	+ + *lin-12/dpy-19 unc-32* +	Unc	10/10 + *unc-32* +/*dpy-19 unc-32* +
		Dpy	11/11 *dpy-19* + *lin-12/dpy-19 unc-32* +
		WT	8/8 + + +/*dpy-19 unc-32* +
	unc-36 + +/+ *unc-32 lin-12*	WT	3/19 + *unc-32* +/*unc-36* + +
			16/19 + + +/*unc-36* + +
	+ *dpy-19* + +/*unc-36* + *unc-32 lin-12*	WT	1/1 *unc-36* + + +/+ *dpy-19* + +
		Unc-36 Lin	2/3 *unc-36* + + +/*unc-36* + *unc-32 lin-12*
			1/3 *unc-36 dpy-19* + +/*unc-36* + *unc-32 lin-12*
		Unc-32 Lin	2/4 + *dpy-19 unc-32 lin-12/unc-36* + *unc-32 lin-12*
			2/4 + + *unc-32 lin-12/unc-36* + *unc-32 lin-12*
	unc-36 + +/+ *dpy-19 lin-12*	WT	3/3 + *dpy-19* +/*unc-36* + +
lin-12(n302)			
	+ (+ *lin-12*)/*dpy-19* (*unc-32* +)	Unc	9/9 + + +/*dpy-19* (*unc-32* +)
		Dpy	2/2 *dpy-19* (+ *lin-12*)/*dpy-19* (*unc-32* +)
lin-12(n379)			
	+ + *lin-12/dpy-19 unc-32* +	Unc	7/7 + + +/*dpy-19 unc-32* +
		Dpy	5/5 *dpy-19* + *lin-12/dpy-19* + *unc-32*
	+ *unc-32* + +/*dpy-19* + *lin-12 unc-69*	Dpy	5/9 *dpy-19 unc-32* + +/*dpy-19* + *lin-12 unc-69*
			4/9 *dpy-19* + + +/*dpy-19* + *lin-12 unc-69*
lin-12(n676)			
	+ + *lin-12/dpy-19 unc-32* +	Dpy	1/1 *dpy-19* + *lin-12/dpy-19 unc-32* +
		Unc	3/3 + *unc-32* +/*dpy-19 unc-32* +
	+ *lin-12* +/*dpy-19* + *unc-69*	Unc	6/11 + *lin-12 unc-69/dpy-19* + *unc-69*
			5/11 + + *unc-69/dpy-19* + *unc-69*
	dpy-19 + +/+ *unc-32 lin-12*	Unc	3/3 + *unc-32* +/+ *unc-32 lin-12*

lin-12(n769)		
+ *lin-12* +/*dpy-19* + *unc-69*	Dpy	2/7 *dpy-19 lin-12* +/*dpy-19* + *unc-69*
		5/7 *dpy-19* + +/*dpy-19* + *unc-69*
	Unc	10/11 + *lin-12 unc-69*/*dpy-19* + *unc-69*
		1/11 + + *unc-69*/*dpy-19* + *unc-69*
+ *unc-32* +/*dpy-19* + *lin-12*	WT	2/2 + *unc-32* +/*dpy-19* + +
	Unc Lin/+ (Vul)	1/1 + *unc-32* +/+ *unc-32 lin-12*
	Dpy Lin/+ (Vul)	1/1 *dpy-19 unc-32* +/*dpy-19* + *lin-12*
+ *unc-32* +/*dpy-17* + *lin-12*	WT	1/1 + *unc-32* +/*dpy-17* + +

Three-factor crosses that indicate the map position of most *lin-12* alleles. In these crosses *n137* was treated as a strictly dominant mutation, and *n769* was treated as a dominant mutation with different phenotypes as a heterozygote and as a homozygote. Because of the incompletely penetrant dominant phenotypes of the *lin-12* Vul alleles *n379*, *n302* and *n676*, the progeny of recombinant hermaphrodites were examined for the segregation of these markers. WT, wild type.

TABLE 7

Interactions among lin-12 *mutations: male ventral hypodermis*

Genotype	Temperature	% males with given no. of protrusions						
		0	1	2	3	4	5	Other
n427/+	15° (*n* = 128)	26	51	14	2	1		6
	25° (*n* = 132)	75	20	2				3
n137/+	15° (*n* = 182)	20	47	16	10	2		5
	20° (*n* = 159)	29	52	11	4	1		3
	25° (*n* = 160)	63	34					3
n769/n769	15° (*n* = 170)	39	28	14	3	3	1	12
n137/n769	15° (*n* = 84)		3	7	6	25	43	16
	25° (*n* = 85)	13	18	8	13	24	13	11
n137/n302	15° (*n* = 65)			1	11	28	53	7
	20° (*n* = 71)			13	17	24	35	11
	25° (*n* = 65)	1	9	20	29	29	3	7
n137/n379	15° (*n* = 87)				3	6	78	13
	20° (*n* = 71)		1	8	15	37	37	2
	25° (*n* = 54)	4	9	11	21	24	9	22
n137/n676	20° (*n* = 86)			1	7	27	58	7
n137/n137	15° (*n* = 70)					9	86	5
	20° (*n* = 68)					9	85	6
	25° (*n* = 83)				4	10	82	4

The numbers of ventral protrusions of males were counted using a dissecting microscope. Males homozygous for the Muv alleles of *lin-12*—*n137* and *n427*—or for *lin-12(n769)* have one or more midventral protrusions. Males homozygous for the Vul alleles of *lin-12*—*n379*, *n302*, and *n676*—or heterozygous for *lin-12(n769)* are phenotypically wild type. When numbers of protrusions are specified, one protrusion is directly anterior to the tail; the remaining protrusions are located in the midsection of the animal. These numbers do not include a second protrusion just anterior to the tail that is usually not visible as viewed with a dissecting microscope. The "Other" category contains animals with unusual numbers or positions of protrusions. These data are presented in increasing order of severity of phenotypic effect upon the male hypodermis. Except for *lin-12(n769)*, the alleles used in this study have also been ranked according to the severity of their effect upon the gonadal anchor cell by GREENWALD, STERNBERG and HORVITZ (1983). The relative orders of the two rankings are equivalent, except that *lin-12(n379)* appears to result in a higher level of *lin-12* activity in the hypodermis than does *lin-12(n302)*, opposite to their relative order of elevation of *lin-12* activity in the gonad. The following strains were used. *n427/+*: non-Unc male cross-progeny from the mating of + *lin-12(n427)/unc-32* +; *him-5* males with *unc-32* hermaphrodites. *n137/+*: non-Unc males from the strain + *unc-32 lin-12(n137)/unc-36* + +; *him-5*. *n769/n769*: males from the strain *dpy-19 lin-12(n769); him-5*. *n137/n769*: Dpy male cross progeny from the mating of *dpy-17* + *lin-12(n769)/+ unc-32* +; *him-5* males with *dpy-17* + *lin-12(n137)/+ unc-32 lin-12(n137 n720)* hermaphrodites. *lin-12(n137 n720)* is a null allele of *lin-12* (GREENWALD, STERNBERG and HORVITZ 1983); approximately 10% of the hermaphrodites of genotype *lin-12(n137)/lin-12(n137 n720)* form an anchor cell and are able to mate. *n137/n302*: non-Unc males from the strain + *unc-32 lin-12(n137)/unc-86(e1416)* + *lin-12(n302); him-5*. *n137/n379*: non-Unc males from the strain + *unc-32 lin-12(n137)/unc-86(e1416)* + *lin-12(n379); him-5*. *n137/n676*: Unc male cross-progeny from the mating of + *unc-32 lin-12(n137)/dpy-19* + +; *him-5* males with + *unc-32 lin-12(n676)/dpy-19* + + hermaphrodites. *n137/n137*: Unc-32 males from the strain + *unc-32 lin-12(n137)/unc-36* + +; *him-5*.

TABLE 8

Interactions among lin-12 *mutations: brood sizes of hermaphrodites*

Genotype	Average no. of progeny
n379/+	53 (n = 23)
n302/+	58 (n = 34)
n137/+	57 (n = 47)
n137/n379	38 (n = 54)
n137/n302	20 (n = 49)
n137/n137	11 (n = 54)

Progeny produced at 25° were counted. In the majority of *lin-12* strains, all hermaphrodites lack functional vulvae and turn into bags of worms. However, in a few *lin-12* strains, some hermaphrodites are egg-laying competent. These hermaphrodites have a much larger brood size than the egg-laying-defective individuals of the same strain. Thus, to compare the brood sizes of differing *lin-12* strains, only progeny from hermaphrodites unable to lay eggs were counted. The following strains were used. *n379/+*: non-Unc Vul cross-progeny from the mating of *lin-12(n379); him-5* males and *unc-32* hermaphrodites. *n302/+*: non-Dpy Vul cross-progeny from the mating of wild-type males and hermaphrodites of genotype *dpy-19* + *lin-12(n302)/dpy-19 unc-32* +. *n137/+*: non-Unc, non-Dpy hermaphrodites from the strain + *unc-32 lin-12(n137)/dpy-19* + +. *n137/n379:* non-Unc hermaphrodites from the strain + *unc-32 lin-12(n137)/unc-86(e1416)* + *lin-12(n302). n137/n302:* non-Unc, non-Dpy hermaphrodites from the strain + *unc-32 lin-12(n137)/dpy-19* + *lin-12(n302).*

eny of a *lin-13/+* parent are phenotypically wild type, whereas their progeny, which lack the maternal component of *lin-13* activity, are sterile and sometimes Muv. However, the zygotic activity of *lin-13* is sufficient to generate a wild-type phenotype: if fertile *lin-13* hermaphrodites are mated with wild-type males, cross-progeny hermaphrodites of genotype *lin-13/+* lack the maternal component of *lin-13* activity but are phenotypically wild type.

Both *lin-13* alleles were obtained by discovering sterile Muv animals among the F_2 progeny of mutagenized hermaphrodites and picking many phenotypically wild-type hermaphrodites from the same plate. A few such hermaphrodites proved to be heterozygous for the Muv mutation. To map (and balance) these mutations, hermaphrodites heterozygous for the Muv mutations were mated with wild-type males and the male progeny (one-half of which were heterozygous for the Muv mutation) were mated with hermaphrodites of the two mapping strains described in MATERIALS AND METHODS. Both mutations were balanced by *unc-32.*

The deficiency *nDf16* (V. AMBROS and M. FINNEY, personal communication) fails to complement *lin-13.* The phenotype of hermaphrodites of genotype *lin-13(n387)/nDf16* was determined by mating *lin-13(n387)* + *unc-32/+ unc-86(n848)* + males with hermaphrodites of genotype *nDf16 +/unc-86(e1416) unc-32.* The non-Unc-32 non-Unc-86 progeny were of genotype *lin-13(n387) unc-32/nDf16* +. (*nDf16* fails to complement *unc-86* but complements *unc-32.*) The phenotype of *lin-13(n387)/nDf16* hermaphrodites depended upon the temperature at which the cross was performed. At 15° these hermaphrodites were

TABLE 9

Interactions among lin-12 *mutations: percentages of Vulvaless hermaphrodites*

	+	*n379*	*n302*	*n676*
n379	9 (n = 172)	97.6 (n = 427)		
n302	66 (n = 221)	99.1 (n = 426)	99.8 (n = 419)	
n676	75 (n = 387)	99.1 (n = 451)	100 (n = 488)	100 (n = 482)
n769	100 (n = 425)			

L3 hermaphrodites of each genotype were picked and later scored for their ability to lay eggs. The following strains were used. *n379/+*: non-Unc hermaphrodites from the strain *unc-86(e1416) + + lin-12(n379)/+ dpy-19 unc-32 +*. *n302/+*: non-Unc hermaphrodites from the strain *unc-86(e1416) + + lin-12(n302)/+ dpy-19 unc-32 +*. *n676/+*: non-Unc hermaphrodites from the strain *+ + unc-32 lin-12(n676)/unc-36 dpy-19 + +*. *n769/+*: non-Unc cross-progeny from the mating of *+ lin-12(n769)/unc-32 +* males and *unc-32* hermaphrodites. *n302/n379:* non-Dpy, non-Unc hermaphrodites from the strain *+ dpy-19 lin-12(n302)/unc-86(e1416) + lin-12(n379)*. *n676/n379:* non-Dpy non-Unc hermaphrodites from the strain *dpy-19 + lin-12(n379)/+ unc-32 lin-12(n676)*. *n676/n302:* non-Dpy non-Unc hermaphrodites from the strain *dpy-17 + lin-12(n302)/+ unc-32 lin-12(n676)*.

sterile and had a single protrusion at the vulva. At 25° these hermaphrodites arrested during what appeared by size to be the L2 stage. Because *nDf16* in *trans* to a *lin-13* allele increased the severity of the *lin-13* phenotype at all temperatures tested, it is likely that the two Muv alleles of *lin-13* do not result in the null phenotype of the locus.

lin-15(e1763,n309,n377,n765,n1139) X: *Multivulva*: All alleles of *lin-15* result in a similar Muv phenotype (Figure 3e–h): some hermaphrodites have a normal vulva and two or three large protrusions (one or two anterior to the vulva and one posterior), and other hermaphrodites have similar patterns of protrusions but lack a functional vulva. A number of hermaphrodites rupture at the vulva shortly after the L4 molt. Males have between one and three ventral protrusions (Figure 4e). One allele, *n765*, is heat sensitive and has a temperature-dependent maternal effect. *n765* animals are phenotypically wild type at 15° but are mutant at 20° and 25°. At 20°, but not at 25°, *n765* hermaphrodites that are the progeny of parents of genotype *lin-15(n765)/+* have fewer and generally smaller ventral protrusions than do *n765* hermaphrodites that are the progeny of *n765* animals. Hemizygous *n765* males mate at 15° and 20° but do not mate at 25°.

The presence of F_1 Muv males and non-Muv hermaphrodites from a cross between wild-type males and *n309* hermaphrodites suggested that the mutation was *X* linked. Because these males do not mate, males of genotype *tra-1(e1099); lin-15(n309)/+* were used to perform complementation tests; *tra-1* transforms genotypically *XX* hermaphrodites into phenotypic males that are able to mate, although inefficiently (HODGKIN and BRENNER 1977).

A three-factor cross (Table 2) localized *lin-15* to the right end of LGX. A series of deficiencies for this region (MENEELY and HERMAN 1979, 1981) was used to map *lin-15* further. These deficiencies are maintained in balanced strains, *mnDp1/+*; *mnDf*. (*mnDp1* is a duplication of the right end of the *X* chromosome attached to LGV; *mnDp1* is homozygous sterile or larval lethal.)

Wild-type males were crossed with these balanced strains, and F_1 males (genotype *mnDp1/+*; *mnDf/0*) were crossed with *unc-17; lin-15(n309)* hermaphrodites. The presence of Muv non-Unc hermaphrodite progeny (genotype *unc-17/+*; *lin-15/mnDf*) established that the deficiency failed to complement *lin-15*. *mnDf1*, *mnDf4* and *mnDf11* failed to complement *lin-15*; *mnDf43* and *mnDf19* complemented *lin-15* (Figure 1).

The phenotype of *lin-15* in *trans* to a deficiency of the locus suggests that the known alleles of *lin-15* may not result in a total lack of gene activity. Specifically, when *unc-84 lin-15(n765)* males were mated with hermaphrodites of genotype *mnDp1/+*; *mnDf4* at 25°, the great majority (95%, $n = 79$) of F_1 Muv hermaphrodites of genotype *unc-84 lin-15(n765)/+ mnDf4* were the size of L3 larvae and were sterile. (At 25°, *n765* results in a phenotype equivalent to that of *n309*.) However, at 20°, the deficiency did not appear to grossly enhance the *lin-15* phenotype.

Four genes (*let-15*, *let-18*, *let-38* and *let-40*) with sterile or lethal alleles have been mapped to this region (MENEELY and HERMAN 1979, 1981). To test whether any of these *let* mutations failed to complement *lin-15* for the Muv phenotype, males of genotype *mnDp1/+*; *unc-3 let/0* were mated with *unc-17; lin-15(n309)* hermaphrodites. No Muv hermaphrodites were observed among the non-Unc progeny. To confirm the results of the above experiments and to test whether *lin-15/let* was Let, *mnDp1/+*; *unc-3 let/0* males were mated with *unc-3 lin-15(n309)* hermaphrodites. For all four *let* genes, Unc non-Muv hermaphrodite progeny (genotype *unc-3 + lin-15(n309)/unc-3 let +*) were observed. Thus, *lin-15* appears to complement all known lethal mutations in the region.

lin-17(e1456,n669,n671,n677,n698) I: *Multivulva*: About half of *n671* and *n677* hermaphrodites have a single protrusion posterior to the vulva (Figure 3i). The other three *lin-17* mutations may be of somewhat lower penetrance for this phenotype. Hermaphrodites are slightly uncoordinated and have a long irregularly shaped tail. In some *lin-17* hermaphrodites, one of the two arms of the gonad does not develop. A few *lin-17* hermaphrodites are sterile. *lin-17* males have undeveloped tails and some rupture at the anus after the L2 molt (Figure 4f).

lin-18(e620,n1051) X: *Multivulva*: Fewer than half of *lin-18* hermaphrodites have a single protrusion posterior to the vulva [30% ($n = 157$), *e620* at 25°] (Figure 3j). Both *lin-18* alleles are slightly heat sensitive and result in a slight maternal effect. *lin-18* males are phenotypically wild type as viewed with a dissecting microscope.

n1051 is an amber mutation (Table 1) and, hence, is likely to eliminate *lin-18* gene activity. However, *n1051* results in a heat-sensitive phenotype, which suggests that an altered but potentially functional *lin-18* gene product may be synthesized in *lin-18(n1051)* animals. Alternatively, the heat-sensitive phenotypes resulting from both *lin-18* alleles may reflect a temperature-sensitive process revealed or induced by eliminating *lin-18* activity (other *C. elegans* temperature-sensitive mutants of this type have been identified; SULSTON and

HORVITZ 1981; GOLDEN and RIDDLE 1984; W. FIXSEN, personal communication).

n1051 was suppressed by a single copy of the amber suppressor *sup-5, i.e.*, hermaphrodites of genotype *sup-5*/+; *lin-18(n1051)/lin-18(n1051)* were phenotypically wild type. To reduce the amount of suppressed *lin-18* product, hermaphrodites of genotype *sup-5*/+; *lin-18(n1051)/lin-18*(nonamber) were constructed. The nonamber allele used, *e620*, results in a phenotype similar to that of *n1051*. Of 148 hermaphrodites of genotype *sup-5*/+; *lin-18(n1051)/lin-18(e620)* at 25°, two were either egg-laying defective or had an abnormal vulva. However, the severity of the vulval defect in these hermaphrodites was not very different from that observed in hermaphrodites of genotype *sup-5*/+ (three of 257 such hermaphrodites had abnormal vulvae), suggesting that the vulval defect of hermaphrodites of genotype *sup-5*/+; *lin-18(n1051)/lin-18(e620)* is completely suppressed.

lin-24(n432,n1057) IV: *Vulvaless:* Most *lin-24* hermaphrodites that turn into bags of worms are Vulvaless. However, a minority of the egg-laying-defective (Egl) hermaphrodites that become bags of worms are nonetheless able to release some progeny. This observation suggests that in these animals at least some vulval cells are generated. In determining the penetrance of the Vul phenotype that results from these two mutations (and the penetrance of the two alleles of *lin-33*; see below), we measured the percentage of hermaphrodites that were severely Egl without regard to their ability to release progeny.

n432 results in a partially dominant Vul phenotype: 95% (n = 458) of *n432* homozygous hermaphrodites are Vul, whereas 57% (n = 396) of *n432*/+ heterozygotes are Vul. *n1057* results in a wild-type phenotype when homozygous ($^{0}/_{203}$ were Vul), but results in an Vul phenotype when heterozygous with a wild-type allele (33% Vul, n = 314). The males of both strains are phenotypically wild type as viewed with a dissecting microscope. The penetrance of the Vul defect markedly decreases in *n432* hermaphrodites that have passed through a dauer larval stage (29% Vul, n = 455).

nDf27 is a deletion that fails to complement *unc-22* and *unc-31*, visible markers flanking *lin-24* (H. ELLIS, personal communication). Hermaphrodites heterozygous for *nDf27* have no obvious vulval abnormalities in that $^{0}/_{105}$ hermaphrodites of genotype *unc-8(e49)*/+ *nDf27* were Vul. (*nDf27* complements *unc-8*.) Thus, it is unlikely that the Vul phenotype of the *lin-24* alleles results from a loss of *lin-24* activity. The phenotype of *lin-24(n432)/nDf27* hermaphrodites was determined by examining the non-Unc progeny from hermaphrodites of genotype *unc-8(e49) lin-24(n432)*/+ *nDf27*. Fifty-four percent (n = 452) of these hermaphrodites were Vul. Thus, the presence or absence of a wild-type *lin-24* allele does not alter the penetrance of the Vul defect in hermaphrodites heterozygous for *n432*. The phenotype of *lin-24(n1057)/nDf27* was established by mating *n1057* males with hermaphrodites of genotype *nDf27* +/ *unc-30 dpy-4*. (*nDf27* fails to complement *unc-30* but complements *dpy-4*.) Non-Unc cross-progeny were picked and scored for their ability to lay eggs, and their progeny were tested to determine their genotypes. One hundred and three of 103 hermaphrodites of genotype *lin-24(n1057)/nDf27* were phenotyp-

ically wild type, suggesting strongly that the mutant phenotype of *lin-24(n1057)/+* animals results from an interaction between the mutant and wild-type products of the *lin-24* gene.

The vulval precursor cells of the hermaphrodite, P(5–7).p, are a subset of the ventral hypodermal cells (P1–12).p present in both males and hermaphrodites (SULSTON and HORVITZ 1977). Observations using Nomarski optics have shown that in *n432* animals some of the P(1–12).p ventral hypodermal cells die during the L1 and early L2 stages (P. STERNBERG, personal communication). These cell deaths are not suppressed in the double mutant *lin-24(n432) ced-3(n717)*. [*ced-3(n717)* blocks the onset of the normal program of cell death in *C. elegans* (HORVITZ *et al.* 1983; H. ELLIS, personal communication).] In addition the P(1–12).p cells that die in *n432* animals do not have the appearance of cells in which the normal program of cell death has been activated (H. ELLIS, personal communication). Thus, it is possible that the aberrant cell deaths in *n432* hermaphrodites may be the result of the action of an altered, cytotoxic form of the *lin-24* gene product. *lin-24* may encode a product utilized in the cells P(1–12).p, and the mutation *n432* may produce an altered *lin-24* product that kills these cells. A similar phenomenon has been observed for the dominant mutation *mec-4(e1611)*, which causes the death of the six microtubule cells that mediate touch sensitivity in *C. elegans* (CHALFIE and SULSTON 1981; M. CHALFIE, personal communication).

We have tentatively made an assignment of allelism between the two partially dominant Vul mutations *n432* and *n1057* based on three criteria. First, both mutations map to the interval between *unc-22* and *dpy-26* on LGIV (Table 2). Second, both mutations result in similar defects in the vulval cell lineages and in the presence of ectopic cell deaths in the ventral hypodermal cells during the L1 stage (P. STERNBERG, personal communication). Third, 92% (n = 350) of hermaphrodites of genotype *unc-22 lin-24(n1057)/+ lin-24(n432)* were Vul. The penetrance of the vulval defect of this strain is equivalent to the penetrance of the vulval defect of homozygous *n432* hermaphrodites, suggesting that the two mutations may be allelic. However, we have also observed a similar increase in the penetrance of the Vul phenotype between two partially dominant Vul mutations in different genes: the penetrance of the Vul defect in hermaphrodites of genotype *lin-33(n1043) +/+ lin-24(n432)* is 99% (n = 442). Thus, the possibility remains that *n432* and *n1057* are mutations of separate, closely linked genes.

Although *n1057* does not result in the loss of *lin-24* gene function, this allele is an amber mutation as it is suppressed by the amber suppressor *sup-5* (see MATERIALS AND METHODS). Suppression requires two copies of the suppressor. Specifically, the penetrance of the Vul phenotype of hermaphrodites of genotype *unc-22 lin-24(n1057)/+ +* (22%, n = 301) was much higher than the penetrance of the Vul phenotype of hermaphrodites of genotype *dpy-19 sup-5; unc-22 lin-24(n1057)/+ +* (3%, n = 279). *n1057* is not suppressed by a single copy of *sup-5*, as 13 of 21 Vul hermaphrodites that were the progeny of hermaphrodites of genotype *dpy-19 sup-5/+ +; unc-22 lin-24(n1057)/+ +* segregated Dpy animals.

lin-25(e1446,n545) V: *Vulvaless*: *n545* is heat sensitive. The phenotype of *n545* at 25° is similar to that of *e1446*. *n545* hermaphrodites at 25° and *e1446* hermaphrodites have never been observed to lay eggs: approximately 85% form bags of worms, whereas the rest are sterile [18% (n = 234), *n545* at 25°; 11% (n = 279), *e1446*]. Nonetheless, a few *lin-25* hermaphrodites are able to mate, indicating that an occasional hermaphrodite forms an abnormal but functional vulva. Most [91% (n = 234), *n545* at 25°; 81% (n = 279), *e1446*] *lin-25* hermaphrodites have a single protrusion at the vulva. At the permissive temperature of 15°, all *n545* hermaphrodites are fertile and only 8% (n = 305) are Vul. *lin-25* males are phenotypically wild type as viewed with a dissecting microscope. *e1446* is maintained in the balanced strain *+/nT1(IV); lin-25(e1446)/unc(n754) nT1(V)*. [*unc(n754)* is a dominant mutation resulting in uncoordinated locomotion linked to *nT1(IV;V)* (E. FERGUSON, unpublished results).]

lin-26(n156) II: *Vulvaless*: The Vul phentoype of *n156* is highly penetrant, as greater than 99% (n = 268) of the hermaphrodites form bags of worms. A single ventral protrusion is seen in 14% (n = 268) of the hermaphrodites. However, a small percentage (1–2%) of the hermaphrodites can mate, indicating that an occasional hermaphrodite forms an abnormal but functional vulva. *lin-26* hermaphrodites have a smaller, slightly fatter body than does the wild type. Males are very small and scrawny with a rounded tail. For genetic manipulations, the mutation is maintained in two balanced strains, *lin-26(n156)/ C1 dpy-10 unc-52; him-5* and *+ lin-5 unc-4/lin-26(n156) + unc-4*.

Hermaphrodites of genotype *lin-26(n156)/mnDf88* arrest during larval development (K. EDWARDS, personal communication), suggesting that *n156* does not result in the total loss of *lin-26* activity and that the null phenotype of *lin-26* is probably lethal. SIGURDSON, SPANIER and HERMAN (1984) have mapped two *let* genes, *let-253* and *let-236*, to the same region of LGII to which *n156* has been localized. *n156* complements mutations in both of these *let* genes (K. EDWARDS, personal communication).

lin-31(e1750,n301,n376,n428,n429,n435,n762,n1048,n1049,n1050,n1053) II: *Multivulva*: Hermaphrodites have between zero and four small, widely spaced ventral protrusions (Figure 3k). In some hermaphrodites the vulva is nonfunctional, causing these animals to become bags of worms. Males do not have ventral protrusions.

GREENWALD and HORVITZ (1980) isolated four deletions of *lin-31, nDf2* through *nDf5*, and showed that *lin-31(n301)/Df* animals are of the same gross phenotype as *lin-31(n301)* animals. This fact and the large number of alleles isolated suggest that some, if not all, of the known alleles of *lin-31* eliminate *lin-31* gene activity. [At the time of the above paper, we had erroneously assigned *n301* to the gene *lin-8*. However, *lin-31(n301)* is not allelic to *lin-8(n111)*, as these mutations complement and are separated by approximately 1 map unit (Table 2).]

lin-33(n1043,n1044) IV: *Vulvaless*: Most *lin-33* hermaphrodites that turn into bags of worms are Vulvaless. However, a minority of the Egl hermaph-

rodites that become bags of worms are nonetheless able to release some progeny. This observation suggests that in these animals at least some vulval cells are generated. In determining the penetrance of the Vul phenotype resulting from these two mutations (as in determining the penetrance of the two alleles of *lin-24*; see above), we measured the percentage of hermaphrodites that were severely Egl without regard to their ability to release progeny.

n1043 results in a partially dominant Vul phenotype: 95% (n = 502) of *n1043* homozygous hermaphrodites are Vul, whereas 77% (n = 447) of *n1043*/+ heterozygotes are Vul. *n1044* also results in a partially dominant Vulvaless phenotype: 96% (n = 425) of *n1044* homozygous hermaphrodites are Vul, whereas 79% (n = 411) of *n1044*/+ heterozygotes are Vul. Males are phenotypically wild type as viewed with a dissecting microscope.

The penetrance of the Vul defect of *n1043*, like that of *lin-24(n432)*, is lower in animals that have recovered from the dauer larval stage. Fifty-three percent (n = 235) of *n1043* hermaphrodites that had recovered from dauer larvae were Vul. In *n1043* hermaphrodites, as in *lin-24* hermaphrodites, some of the cells P(1–12).p die, suggesting that *n1043* also may result in the production of a toxic product (P. STERNBERG, personal communication).

n1044 maps to a position similar to that of *n1043* (Table 2). The penetrance of the Vul defect in hermaphrodites of genotype *lin-33(n1043)/lin-33(n1044)* is 98% (n = 248), equivalent to the penetrance of the Vul defect in homozygous *n1043* hermaphrodites, which suggests that these mutations may be allelic. However, the penetrance of the Vul defect in hermaphrodites of genotype *lin-33(n1043)* +/+ *lin-24(n432)* is 99% (n = 442), even though *n1043* and *n432* are mutations in different genes. Nonetheless, we have decided on the basis of the similarity of the map positions and phenotypes of *n1043* and *n1044* to consider them provisionally to be alleles of the same gene. However, the possibility remains that they are mutations of separate, closely linked genes.

lin-34(n1046) IV: *Multivulva*: *n1046* results in an incompletely penetrant but partially dominant Muv phenotype; 57% (n = 426) of *n1046* homozygous hermaphrodites were Muv, whereas 17% (n = 194) of *lin-34(n1046)*/+ heterozygotes were Muv. Many *n1046* hermaphrodites have two protrusions, one anterior to the vulva, and one posterior (Figure 3l). Both heterozygous and homozygous *n1046* males are wild type in phenotype as viewed with a dissecting microscope.

n1046 was mapped relative to *lin-3* using the *lin-3* allele *n1059*, which results in a larval lethal phenotype (see above). The phenotype of animals of genotype *lin-34(n1046)/nDf27* is equivalent to the phenotype of *lin-34(n1046)*/+ animals; thus, it can not be determined whether *nDf27* fails to complement *n1046*.

As detailed in MATERIALS AND METHODS, *n1046* is an amber mutation as it is suppressed by *sup-7*. The suppression of *n1046* by *sup-7* is recessive in that a single copy of the suppressor does not markedly reduce the penetrance of the mutation as a homozygote. Fifty-seven percent (n = 426) of hermaphrodites of genotype *lin-34(n1046) unc-22* were Muv, whereas 68% (n = 104) of hermaphrodites of genotype *lin-34(n1046) unc-22; sup-7 dpy-7*/+ + were Muv and 13% (n = 182) hermaphrodites of genotype *lin-34(n1046) unc-22; sup-7 dpy-7*

were Muv. However, *sup-7* may be a partially dominant suppressor of *lin-34(n1046)/+*, because, at 25°, 17% (n = 339) of hermaphrodites of genotype *lin-34(n1046) unc-22/+ +* were Muv, whereas 8% (n = 199) of hermaphrodites of genotype *lin-34(n1046) unc-22/+ +*; *sup-7 dpy-7/+ +* were Muv.

let-23(mn23,mn216,mn224,n1045) II: *Vulvaless*: *n1045* results in a cold-sensitive Vul phenotype with an associated cold-sensitive larval lethality. At 15°, 49% of *n1045* hermaphrodites arrest during larval development, 50% are Vul and 1% have a functional vulva (n = 219). At 20°, 51% of *n1045* hermaphrodites arrest during larval development, 14% are Vul or severely egg-laying defective (Egl) and 35% have a functional vulva (n = 763). At 25°, 28% of *n1045* hermaphrodites arrest during larval development, 2% are Vul or severely Egl and 70% have a functional vulva (n = 310). The *n1045* hermaphrodites that arrest during larval development do so with a rigid rod-like phenotype during what appears by size to be the L2 stage (Figure 1e). Males are phenotypically wild type as viewed with a dissecting microscope.

At 20° and 25° *n1045* hermaphrodites have two distinct phenotypes not seen in *n1045* hermaphrodites grown at 15°. First, some of these hermaphrodites are Egl but not Vul; these hermaphrodites do not lack a vulva but rather have an abnormal vulva and are able to release some eggs or larvae. Second, some egg-laying-competent hermaphrodites are Muv; these animals have a functional vulva and one or two ectopic supernumerary vulva-like structures (Table 4).

At 15°, the penetrance of the Vul defect in *n1045* hermaphrodites that pass through a dauer larval stage is equivalent to that of unstarved animals: 95% (n = 79) of adult hermaphrodites that have passed through a dauer larval stage are Vul, equivalent to the 98% (n = 111) of unstarved adult hermaphrodites that are Vul. However, the penetrance of the Vul phenotype markedly decreases in *n1045* hermaphrodites that do not pass through a dauer larval stage but have been starved before reaching adulthood; 47% (n = 287) of *n1045* hermaphrodites that have been starved before reaching adulthood are Vul.

n1045 was mapped to LGII between *vab-9* and *unc-4* by three-factor crosses (Table 2). Recently, a detailed genetic analysis of this region of LGII has identified many essential genes (SIGURDSON, SPANIER and HERMAN 1984). Using a series of deficiencies, C. SIGURDSON (personal communication) mapped *n1045* to the same region of LGII as *let-23* and subsequently determined that *n1045* failed to complement an allele of *let-23*. Hermaphrodites bearing any of the other three *let-23* alleles, *mn23*, *mn216* and *mn224* (HERMAN 1978; SIGURDSON, SPANIER and HERMAN 1984), arrest during larval development with the same phenotype that is seen among *n1045* hermaphrodites and fail to complement *n1045* for the Vul activity. Thus, *let-23* appears to have two activities, one that functions during early larval development and another that functions later during vulval development.

The phenotype caused by *n1045* is enhanced when *n1045* is in *trans* to other *let-23* alleles or to a deficiency of the locus (P. STERNBERG, personal communication). Thus, *n1045* probably does not totally eliminate *let-23* activity. Nonetheless, *n1045* is an amber mutation as it is suppressed by the amber suppressor

TABLE 10

Suppression by sup-7 *of the larval lethal defect of* let-23

let-23 genotype	Temperature	% hermaphrodites of given genotype that arrest during larval development		
		sup-7(+)/sup-7(+)	*sup-7/sup-7(+)*	*sup-7/sup-7*
n1045	15°	49 (n = 219)	2 (n = 919)	ND
	20°	51 (n = 763)	ND	0.5 (n = 206)
	22.5°	41 (n = 673)	ND	5 (n = 95)
n1045/Df	25°	78 (n = 85)	33 (n = 35)	ND

The penetrance of the larval lethal phenotype is presented as a percentage of the hermaphrodites of differing genotypes at different temperatures that arrested during larval development. Animals of differing genotypes and at different temperatures were obtained in the following ways. *n1045; sup-7/+*, 15°: 15 animals of genotype *let-23(n1045) unc-4; sup-7 dpy-7/+ +* were allowed to lay eggs for 1 day and the number of progeny that arrested as larvae was counted. *n1045; sup-7*, 20°: ten fertile animals of genotype *let-23(n1045) unc-4; sup-7 dpy-7* were allowed to lay eggs and the number of hermaphrodites that arrested as larvae was counted. *n1045; sup-7*, 22.5°: the degree of larval lethality was measured as described at 20°. *n1045/Df*, 25°: males of genotype *mnDf68 unc-4/C1 dpy-10 unc-52* were mated with hermaphrodites of genotype *let-23(n1045) unc-4; dpy-7*. The number of Unc non-Dpy hermaphrodites of genotype *let-23(n1045) unc-4/mnDf68 unc-4; dpy-7/+* and the number of wild-type hermaphrodites of genotype *let-23(n1045) unc-4/C1 dpy-10 unc-52; dpy-7/+* were counted. The number of wild-type hermaphrodites was much greater than the number of Unc non-Dpy hermaphrodites, and the ratio of the number of Unc non-Dpy hermaphrodites to the number of wild-type hermaphrodites was taken as the percentage of Unc hermaphrodites that did not arrest during larval development. *n1045/Df; sup-7/+*, 25°: males of genotype *mnDf68 unc-4/C1 dpy-10 unc-52* were mated with hermaphrodites of genotype *let-23(n1045) unc-4; sup-7 dpy-7*. The above protocol was followed to determine the degree of larval lethality. ND, not determined.

sup-7 (see MATERIALS AND METHODS). Based upon the phenotype observed using a dissecting microscope, *n1045* appears to be suppressed completely by two copies of *sup-7* but only partially by one copy (Tables 10 and 11).

MT300, ***n300*** *and* nT1(IV;V): MT300 hermaphrodites never form ventral protrusions or vulvae. Males have slightly rounded tails.

The isolation of the strain MT300 is described in MATERIALS AND METHODS. The single egg-laying-competent MT300 hermaphrodite that was identified was mated with wild-type males. The resulting F_1 males were mated with the two mapping strains. Fewer than ¼ Vul animals were seen among the F_2 progeny from these crosses. In addition *unc-5 IV* and *dpy-11 V* appeared to be genetically linked. These two observations suggested that there might be a chromosome abnormality in this strain, possibly a translocation between LGIV and LGV. The results of several experiments, detailed in Table 12, support this hypothesis. First, the translocation heterozygote segregates approximately $^{10}/_{16}$ dead eggs. This is the fraction of inviable progeny that would segregate from a hermaphrodite heterozygous for a reciprocal translocation if all aneuploid progeny do not survive. Second, mutations on LGIV and LGV appear to be tightly linked. Third, the translocation suppresses crossing over on the right arm of LGIV and on the left arm of LGV. Crossover suppression is not observed on the left arm of LGIV (between the markers *dpy-9* and *unc-17*)

TABLE 11

Suppression by sup-7 *of the Vulvaless defect of* let-23

		% adult hermaphrodites of given genotype that have a Vul phenotype		
let-23 genotype	Temperature	*sup-7(+)/sup-7(+)*	*sup-7/sup-7(+)*	*sup-7/sup-7*
n1045	15°	98 (n = 111)	[a]	ND
	20°	28 (n = 373)	8 (n = 97)	3 (n = 40)
n1045/Df	25°	100 (n = 20)	4 (n = 23)	ND

The penetrance of the Vul phenotype is presented as a percentage of adult hermaphrodites of differing genotypes at different temperatures that were Vulvaless. Animals of differing genotypes and at different temperatures were obtained in the following ways. *n1045; sup-7/+*, 15°: 15 animals of genotype *let-23(n1045) unc-4; sup-7 dpy-7/+ +* were allowed to lay eggs for 1 day. *n1045; sup-7/+*, 20°: *let-23(n1045); him-5* males were mated with hermaphrodites of genotype *let-23(n1045) unc-4; sup-7 dpy-7* and the fertile non-Unc progeny were scored for their ability to lay eggs. *n1045; sup-7*, 20°: 141 L4 hermaphrodites of genotype *let-23(n1045) unc-4; sup-7 dpy-7* were picked separately; of those that were fertile, the percentage that were egg-laying competent was measured. The remaining hermaphrodites were sterile as a consequence of the presence of *sup-7*; however, a vulval structure was also observed in these hermaphrodites with the dissecting microscope. *n1045/Df*, 25°: the penetrance of the Vulvaless defect in hermaphrodites of genotype *dpy-10 let-23(n1045)/mnDf68 unc-4* (P. STERNBERG, personal communication). *n1045/Df; sup-7/+*, 25°: males of genotype *mnDf68 unc-4/C1 dpy-10 unc-52* were mated with hermaphrodites of genotype *let-23(n1045) unc-4; sup-7 dpy-7*. If the Unc non-Dpy hermaphrodites of genotype *let-23(n1045) unc-4/mnDf68 unc-4; sup-7 dpy-7/+ +* were fertile, they were scored for the ability to lay eggs; if not, the presence of a vulval structure was ascertained either using Nomarski optics or with a dissecting microscope. ND, not determined.

[a] Many fertile, egg-laying-competent hermaphrodites of putative genotype *let-23(n1045) unc-4; sup-7 dpy-7/+ +* were observed among the progeny of hermaphrodites of genotype *let-23(n1045) unc-4; sup-7 dpy-7/+ +* grown at 15°, but their number was not ascertained.

and on the right arm of LGV (between the markers *dpy-11* and *unc-51*) (E. FERGUSON, unpublished results). This translocation was named *nT1(IV;V)* and is maintained in the balanced strains *nT1(IV)/unc-5; nT1(V)/dpy-11* or *nT1(IV)/unc-8(n491); nT1(V)/unc-60*. Vul hermaphrodites segregating from a homozygous *nT1(IV;V)* parent are much healthier than those segregating from a balanced heterozygote.

We have named the mutation in the strain MT300 that is responsible for the Vul phenotype *n300*. *n300* and the translocation have always cosegregated. All hermaphrodites heterozygous for *nT1(IV; V)*, *i.e.*, those hermaphrodites that segregate $^{10}/_{16}$ dead eggs and that exhibit pseudolinkage between LGIV and LGV, have in addition always segregated *n300*. We do not know whether the Vul phenotype is caused by the translocation, possibly by one or both of its breakpoints, or whether the Vul mutation is simply a secondary mutation present on the translocation. Complementation-screening experiments (GREENWALD and HORVITZ 1980) to induce a point mutation failing to complement the Vul phenotype of *n300* have not succeeded (E. FERGUSON, unpublished results), and, thus, we do not know whether *n300* defines a single gene that can mutate to result in a Vul phenotype.

Efficiency of male mating: Vulval cell lineages occur only in hermaphrodites. Since cells homologous to the vulval precursor cells generate male-specific

TABLE 12

Characterization of nT1(IV;V)

Dead eggs	Wild type	Linkage of LGIV and LGV[a] Dpy-11	Unc-5	Dpy-11 Unc-5	Vul
1049	400	0	0	75	50
66.6%	25.4%	0%	0%	4.8%	3.2%
		Crossover suppression of the right arm of LGIV[b]			
Dead eggs	Wild type	Dpy-4	Unc-17	Dpy-4 Unc-17	Vul
1181	469	0	0	120	62
64.4%	25.6%	0%	0%	6.6%	3.4%
		Crossover suppression of the left arm of LGV[c]			
Dead eggs	Wild type	Dpy-11	Unc-60	Dpy-11 Unc-60	Vul
1175	463	1	0	108	79
64.3%	25.4%	0.05%	0%	5.9%	4.1%

[a] Progeny counts from hermaphrodites of genotype *nT1(IV)/unc-5; nT1(V)/dpy-11*. Single hermaphrodites were allowed to lay eggs for 24 hr and then removed. Eggs that had not hatched after 24 hr were counted as "dead eggs."

[b] Progeny counts from hermaphrodites of genotype *nT1(IV)/unc-17 dpy-4; nT1(V)/+*.

[c] Progeny counts from hermaphrodites of genotype *nT1(IV)/+; nT1(V)/unc-60 dpy-11*.

structures required for mating, it was of interest to determine whether mutations that affect vulval development also have effects in males. The phenotypes of mutant males as viewed with a dissecting microscope are described above and in Table 3. Males also were tested for their abilities to mate, as described in MATERIALS AND METHODS. For each gene an allele of high penetrance was chosen. If a mutant was severely deficient in male mating ability, a mutant carrying a second allele of that gene (if available) was tested to decrease the likelihood that the effect was caused by a secondary mutation.

The results of these tests are presented in Table 13. The numbers of male progeny vary considerably among experiments and provide only an approximate indication of mating ability. Even so, one generalization is apparent: male mating ability is eliminated by most Muv mutations. Specifically, *lin-1, lin-13, lin-15, lin-17* and *lin-31* males do not mate, *lin-12(Muv)* homozygous males do not mate and *lin-34* males mate very poorly. However, *lin-8; lin-9* and *lin-18* males mate. Males carrying Vul mutations in any of six genes—*lin-4, lin-11, lin-25, lin-26, let-23* and *n300*—either do not mate or mate very poorly. Males carrying Vul mutations in any of seven other genes—*lin-2, lin-3, lin-7, lin-10, lin-12(Vul), lin-24, lin-33*—mate with an efficiency approximately equal to that of the wild type.

DISCUSSION

We have identified and characterized 95 mutants of the *C. elegans* hermaphrodite in which the vulval cell lineages are altered. These mutants have been isolated based on their displaying one of two phenotypic abnormalities in vulval

TABLE 13

Male-mating ability of Lin mutants

Genotype	No. of cross-progeny	% control
N2	1789	
him-5(e1467)	1894	
him-9(e1487)	1500	
Vul		
lin-2(e1309); him-5	1383	73
lin-3(e1417); him-5	1299	69
lin-4(e912); him-5	0	0
lin-7(e1413); him-5	2089	110
lin-10(1439); him-5	1181	62
lin-11(n382); him-5	0	0
lin-11(n389); him-5	2	0.1
lin-12(n302); him-5	845	45
lin-12(n379); him-5	872	46
lin-24(n432)	2586	146
lin-24(n1057)/+	1907	106
lin-25(e1446) him-5	0	0
lin-25(n545) him-5	0	0
lin-26(n156); him-5	0	0
lin-33(n1043)	1593	89
let-23(n1045); him-5	352	19
n300; him-9	0	0
Muv		
lin-1(e1777); him-5	0	0
lin-8(n111)	1016	57
lin-9(n112); him-5	1477	78
lin-8(n111); lin-9(n112); him-5	569	30
+ lin-12(n137)/unc-32 +; him-5	1899	100
lin-12(n137); him-5	0	0
lin-12(n427); him-5	0	0
lin-13(n387); him-5	0	0
him-5; lin-15(n309)	0	0
lin-15(n377)	2	0.1
lin-17(n671); him-5	0	0
him-5; lin-18(e620)	734	38
lin-31(n301); him-5	0	0
lin-31(e1750); him-5	0	0
lin-34(n1046); him-5	95	5

anatomy: Vulvaless (Vul) mutants lack a vulva, and Multivulva (Muv) mutants have one or more protrusions along the ventral midline. These mutants define 22 complementation groups, 15 of which are represented by multiple alleles. The phenotypes of most, but not all, of these mutants result from single-gene recessive mutations. The Vulvaless phenotype of one strain, MT300, is associated with a reciprocal translocation involving linkage groups IV and V. Thirteen mutations that result in partially dominant phenotypes have been assigned

to four complementation groups, *lin-12 III, lin-24 IV, lin-33 IV* and *lin-34 IV*. One allele of *lin-24* results in a Vulvaless phenotype when heterozygous with a wild-type allele but results in a wild-type phenotype when homozygous. Certain mutations in each of the genes *lin-9, lin-13* and *lin-15* result in maternal effects at low temperatures. The penetrance of the Vul phenotype resulting from mutations in three genes is reduced in hermaphrodites that have passed through a dauer larval stage. [A dauer larva is an alternate developmental stage that is entered as a consequence of starvation during the first larval stage (CASSADA and RUSSELL 1975).] The penetrance of the Vul phenotype resulting from mutations in three other genes is reduced in hermaphrodites that did not pass through a dauer larval stage but that have been starved before reaching adulthood. Ten mutations in seven genes have been shown to be amber mutations; however, as discussed below, four of these amber mutations may not result in the complete loss of function of their respective loci.

To understand how these 22 genes function in the development of the vulva, it is necessary to determine how the mutations that define these genes affect gene function, to observe the effects of these mutations on the vulval cell lineages and to examine the patterns of interaction among mutations in different genes. In this manuscript, we have analyzed how these mutations affect gene function. In addition, for some of the mutations that do not eliminate gene function, we have determined the phenotype that probably results from the complete absence of gene function.

We have used four criteria in attempting to determine the nature of these mutations and, in particular, to determine which of these mutations result in a loss of gene function: (1) the number of alleles of a given gene (the isolation of a large number of alleles suggests that the observed phenotype may be the result of the elimination of gene function); (2) the nature of the phenotype (dominant phenotypes usually do not result from either the loss or partial reduction of gene function; recessive phenotypes that are not the most extreme observed phenotype are likely to result from a reduction rather than an elimination of gene function); (3) the phenotype that results when a mutation is in *trans* to a deficiency of the locus (an enhancement of the phenotype of a recessive mutation in *trans* to a deficiency of the locus strongly suggests that the mutation does not completely eliminate gene function); (4) the existence of and phenotypes caused by amber mutations (amber mutations usually, but not always, result in the total lack of gene function; see below).

These four criteria suggest that existing mutations in six of the 22 genes result in the absence of gene function. Four genes, the Vul genes *lin-2* and *lin-7* and the Muv genes *lin-1* and *lin-31*, have 11 or more recessive alleles. We have identified amber alleles of three of these genes, *lin-1, lin-2* and *lin-7* (although the single amber allele of *lin-2* may not eliminate *lin-2* gene function; see below), and have determined that the severity of the effect of *lin-31* alleles is not enhanced by a deficiency of this locus. The phenotype resulting from mutations in the Vul gene *lin-10*, which has three alleles, also is not enhanced by a deficiency of the locus, and, thus, mutations in this gene may also result in the absence of gene activity. In addition, we have identified an amber allele

of the Muv gene *lin-18*. Although only two alleles of *lin-18* have been isolated, these mutations result in a subtle phenotype; thus, other alleles may have been missed in the screening process. Thus, the Vul phenotypes of *lin-2*, *lin-7* and *lin-10* and the Muv phenotypes of *lin-1*, *lin-18* and *lin-31* may be null phenotypes.

In contrast, it is likely that mutations in seven other genes (*lin-3*, *let-23*, *lin-26*, *lin-13*, *lin-15*, *lin-8*, *lin-9*) reflect a partial decrease in, but not a complete loss of, gene function. In particular, lethal alleles of the Vul genes *lin-3* and *let-23* have been identified. For both *let-23* (P. STERNBERG, personal communication) and *lin-3*, lethal alleles increase the penetrance of the Vul defect in *trans* to a Vul mutation, suggesting that these lethal alleles result in a further decrease, and possibly a total absence, of gene activity. In addition, the severity of the phenotypes resulting from alleles of three genes, the Vul gene *lin-26* and the Muv genes *lin-13* and *lin-15*, is greater when any of these alleles is in *trans* to an appropriate deficiency. In these cases, hermaphrodites of genotype *lin/Df* either arrest during larval development or have an increased incidence of sterility, suggesting that the null phenotypes of these three loci are lethality or sterility. The mutations *lin-8(n111)* and *lin-9(n112)*, which were isolated because together they result in a Muv phenotype, also may not be null alleles. Two other alleles of *lin-9* that will be described elsewhere result in a sterile phenotype and appear to be stronger alleles of the gene, suggesting that *lin-9(n112)* may only partially decrease *lin-9* gene activity. Although hermaphrodites of genotype *lin-8/Df* are phenotypically wild type, hermaphrodites of genotype *lin-8/Df; lin-9(n112)* have a much higher incidence of sterility than do hermaphrodites of genotype *lin-8; lin-9(n112)*, suggesting that the single allele of *lin-8* may not result in the null phenotype of this locus.

Although the null phenotypes of the other five genes with recessive mutations (the Vul genes *lin-4*, *lin-11*, *lin-25* and *n300* and the Muv gene *lin-17*) are not known, mutations in these genes seem likely to either decrease or eliminate gene activity. However, it is unlikely that the Muv and Vul mutations in four genes (*lin-12*, *lin-24*, *lin-33* and *lin-34*) that are represented only by dominant alleles decrease or eliminate the function of these genes. Dominant mutations usually result in the acquisition of a novel function by the gene product, in an increase in gene activity and/or in ectopic gene expression. The dominant Muv and Vul alleles of *lin-12* described in this paper already have been demonstrated to result in an increased activity and, possibly, in the ectopic expression of the *lin-12* gene; in addition, amber alleles of *lin-12* have been isolated and shown not to result in a Muv or Vul phenotype (GREENWALD, STERNBERG and HORVITZ 1983). The partially dominant Vul alleles of *lin-24* and *lin-33* cause vulval precursor cells to die (P. STERNBERG, personal communication). Hermaphrodites heterozygous for a deficiency of the *lin-24* locus are not Vul, suggesting that the phenotype observed in *lin-24* hermaphrodites does not result from a reduction in function of the *lin-24* locus but rather may result from either an increase in the amount of normal *lin-24* gene product or from the production of an altered *lin-24* gene product with a novel, cytotoxic function. A second putative allele of *lin-24*, *n1057*, results in a similar

Vul defect when heterozygous to a wild-type allele but results in a wild-type phenotype as a homozygote or in *trans* to a deficiency of the region. These observations suggest that the mutant phenotype of *n1057* is the result of an interaction between the wild-type and mutant *lin-24* gene products to generate a novel, cytotoxic activity.

In addition to *lin-12*, seven of the other 21 genes described in this study are represented by one or more amber alleles. As discussed above, amber mutations in the genes *lin-1*, *lin-7*, *lin-12* and *lin-18* probably result in the null phenotypes of these genes. However, amber mutations in the other four genes most likely do not result in null phenotypes. Specifically, the amber mutations *lin-2(e1453)* and *let-23(n1045)* appear to reduce, but not completely eliminate, gene function: many alleles of *lin-2* are of higher penetrance than *lin-2(e1453)*, and the severity of the Vul phenotype of *let-23(n1045)* is enhanced in *trans* both to other alleles of the gene and to a deficiency of the locus. Presumably, the amber fragments in these mutants retain some gene activity. Also, *lin-24(n1057)* is an amber allele, and, thus, the Vul phenotype of hermaphrodites of genotype *lin-24(n1057)/+* probably results from the interaction between the amber fragment of *lin-24(n1057)* and the wild-type *lin-24* gene product. Since the amber mutation *lin-34(n1046)* results in a partially dominant phenotype, it also probably does not cause a reduction or loss of gene function. Other amber mutations that do not result in total loss of gene function have been observed in both prokaryotes, *e.g.*, the first identified mutation in *E. coli* DNA polymerase (DE LUCIA and CAIRNS 1969; GROSS and GROSS 1969), and in *C. elegans*, *i.e.*, null mutations in the gene *tra-1* of *C. elegans* result in the transformation of *XX* animals to phenotypic males (HODGKIN and BRENNER 1977; J. HODGKIN, personal communication), and one of the eight amber alleles of this gene results in incomplete transformation and thus is likely to retain some gene activity (J. HODGKIN, personal communication).

The amber alleles of *lin-1*, *lin-2*, *lin-7* and *lin-18* were suppressed well by a single copy of one of the amber suppressors *sup-5* or *sup-7*, *i.e.*, *lin*(amber)/*lin*(amber); *sup*/+ animals were phenotypically wild type, suggesting that relatively little activity is needed from these genes to produce a wild-type phenotype. For three of these genes, *lin-1*, *lin-2* and *lin-7*, a further two-fold reduction in gene activity resulted in a partially mutant phenotype, as some hermaphrodites of genotype *lin*(amber)/*lin*(null, nonamber); *sup*/+ were visibly abnormal. *lin-18* hermaphrodites of similar genotype were phenotypically wild type. We can estimate the relative amount of *lin* gene product needed to result in a wild-type phenotype. In hermaphrodites homozygous for both *sup-7* and *unc-15(e1214)*, an amber allele of a gene that probably encodes paramyosin, approximately 40% of the wild-type level of paramyosin was restored (WATERSTON 1981). If the suppressed polypeptide products of *lin-1*, *lin-2* and *lin-7* function with an efficiency equal to that of the corresponding wild-type products, approximately 10% of the wild-type gene activity of these three genes is needed for the production of a wild-type phenotype. Similarly, less than 10% of the wild-type *lin-18* gene activity is needed for the production of a wild-type phenotype. [These estimates assume that both the amber and nonamber

alleles used in these experiments eliminate gene activity. However, the *lin-2* and *lin-18* alleles used may not totally eliminate gene activity (see RESULTS) and, hence, the estimates for these two genes of the amount of gene activity that is necessary for the production of a wild-type phenotype may be low.] Similar or higher thresholds have been found for a number of different gene-enzyme systems in *Drosophila melanogaster* (reviewed by O'BRIEN and MACINTYRE 1978), suggesting that *lin-1, lin-2, lin-7* and *lin-18* may encode products that function catalytically (as opposed to stoichiometrically; *e.g.*, see SNUSTAD 1968).

The suppression by amber suppressors of the mutations *lin-24(n1057)* and *lin-34(n1046)* is recessive; *i.e.*, the mutant phenotypes are suppressed only in hermaphrodites homozygous for the suppressor. If these mutations result in abnormally functioning gene products (as suggested above), then the dose-dependent suppression of these mutations is caused by either the necessity of the restoration of a stoichiometric amount of wild-type gene product or by the necessity of a reduction in the amount of abnormally functioning gene product.

One of our goals has been to identify all genes that affect the vulval cell lineages. So far we have identified 25 genes that can mutate to generate a Muv or Vul phenotype (Table 14). Twenty-two of these genes are described in this manuscript and three genes (*unc-83, unc-84* and *lin-14*) have been described elsewhere (HORVITZ and SULSTON 1980; SULSTON and HORVITZ 1981; AMBROS and HORVITZ 1984). The alleles of *lin-14* that result in a Vul phenotype are dominant and result in the overproduction and/or ectopic expression of *lin-14* gene activity (AMBROS and HORVITZ 1984). Some recessive mutations in *unc-83* and *unc-84* probably result in loss of gene function (W. FIXSEN, personal communication). The class of genes for which we were most likely to saturate is the set with null alleles that result in a Muv or Vul phenotype without also causing lethality or sterility. The eight genes (*lin-1, lin-2, lin-7, lin-10, lin-18, lin-31, unc-83, unc-84*) believed to be of this class are all represented by multiple alleles, six of them by ten or more alleles. It seems likely that we have identified most or all such genes; if so, there are very few genes of this class (Table 14). However, 12 other genes are defined by recessive mutations, which presumably result in loss or reduction of function. Seven of these genes (*lin-3, lin-8, lin-9, lin-13, lin-15, lin-26, let-23*) probably have lethal or sterile null phenotypes, and five other genes (*lin-4, lin-11, lin-17, lin-25, n300*) have unknown null phenotypes. We may also have identified most genes that by reduction of gene function can mutate to give a Muv or Vul phenotype (Table 14). The Muv or Vul alleles of these genes may be relatively infrequent mutations either that generally reduce (but not eliminate) gene function or that selectively reduce gene function in the vulval cells. The frequency with which we might expect to identify in a given gene mutations that result in a dominant phenotype—because of altered, increased or ectopic gene activity—is difficult to estimate. Nonetheless, we may have identified most genes that are able to mutate to a dominant Muv or Vul phenotype with frequencies comparable to the frequencies of dominant mutations in the five genes listed in Table 14.

TABLE 14

The extent of saturation for genes that can mutate to a fertile Muv or Vul phenotype

	No. of alleles of genes in each class							
	(1) Genes that were identified by recessive mutations and that have a viable null phenotype		(2) Genes that were identified by recessive mutations and that have a lethal or sterile null phenotype		(3) Genes that were identified by recessive mutations and that have an unknown null phenotype		(4) Genes that were identified by dominant mutations	
	lin-1	16	*lin-3*	2	*lin-4*	1	*lin-12*	7
	lin-2	13	*lin-8*	1	*lin-11*	4	*lin-24*	2
	lin-7	13	*lin-9*	1	*lin-17*	5	*lin-33*	2
	lin-10	3	*lin-13*	2	*lin-25*	2	*lin-34*	1
	lin-18	2	*lin-15*	5	*n300*	1	*lin-14*	2
	lin-31	11	*lin-26*	1				
	unc-83	10	*let-23*	1				
	unc-84	16						
Poisson estimate of no. of unidentified genes		0		2.5		1.0		0.3

The extent of saturation for genes that can mutate to a fertile Muv or Vul phenotype. The numbers of *unc-83* and *unc-84* alleles were obtained from W. FIXSEN (personal communication), and the number of dominant *lin-14* alleles was obtained from AMBROS and HORVITZ (1984). If a mutation in each gene can occur with equal likelihood (*i.e.*, if the probability of a gene's having a given number of alleles follows a binomial distribution), the Poisson function can be used to estimate the number of genes not yet identified. However, it is evident that some genes in each class have a greater number of alleles than would be expected if a mutation in every gene of that class could be recovered with equal frequency, prohibiting a formal application of the Poisson function to these data. Nonetheless, to obtain a very approximate estimate of the number of genes not yet identified, we have applied the Poisson function to these data. In an effort to reduce an overemphasis on genes with large numbers of alleles, for the last three classes of genes we calculated m, the average number of alleles per gene, according to MENEELY and HERMAN (1981), using the formula $f = (1 - e^{-m} - me^{-m})/(1 - e^{-m})$, where f is the fraction of identified genes represented by more than one allele.

Other classes of genes in addition to those we have identified may be involved in the control of the vulval cell lineages. We would not necessarily have identified genes that can mutate to generate sterile Muv or Vul animals, nor would we have identified genes with Muv or Vul mutations that display maternal effects. More generally, genes with redundant functions may be able to be identified only as a result of rare multiple mutations [or, in some cases, as a result of relatively rare dominant mutations, *e.g.*, see GREENWALD and HORVITZ (1980)]. Other genes involved in the control of the vulval lineages may not be able to mutate to give a Muv or Vul phenotype. Some such genes might be identified by mutations that act either to suppress or to enhance phenotypes caused by mutations known to affect vulval lineages. Extragenic suppressors of mutations in *lin-12* (E. FERGUSON, unpublished results) and of mutations in *lin-1* (K. EDWARDS, personal communication) and extragenic enhancers of mutations in *lin-8* or *lin-9* (E. FERGUSON, unpublished results) have been identified that define additional genes that may be involved in vulval development. We

hope that the identification and characterization of many of the genes responsible for vulval development will reveal aspects of the genetic and, ultimately, of the molecular specification of cell lineage in *C. elegans*.

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