EGG-LAYING DEFECTIVE MUTANTS OF THE NEMATODE *CAENORHABDITIS ELEGANS*

CAROL TRENT, NANCY TSUNG AND H. ROBERT HORVITZ

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

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ABSTRACT

We have isolated 145 fertile mutants of C. *elegans* that are defective in egg laying and have characterized 59 of them genetically, behaviorally and pharmacologically. These 59 mutants define 40 new genes called *egl*, for *egg-laying abnormal*. Most of the other mutants are defective in previously identified genes. The *egl* mutants differ with respect to the severity of their egg-laying defects and the presence of behavioral or morphological pleiotropies. We have defined four distinct categories of mutants based on their responses to the pharmacological agents serotonin and imipramine, which stimulate egg laying by wild-type hermaphrodites. These drugs test the functioning of the vulva, the vulval and uterine muscles and the hermaphrodite-specific neurons (HSNs), which innervate the vulval muscles. Mutants representing 14 *egl* genes fail to respond to serotonin and to imipramine and are likely to be defective in the functioning of the vulva or the vulval and uterine muscles. Four mutants (representing four different genes) lay eggs in response to serotonin but not to imipramine and appear to be egg-laying defective because of defects in the HSNs; three of these four were selected specifically for these drug responses. Mutants representing seven *egl* genes lay eggs in response to serotonin and to imipramine. One *egl* mutant responds to imipramine but not to serotonin. The remaining *egl* mutants show variable or intermediate responses to the drugs. Two of the HSN-defective mutants, *egl-1* and *her-1(n695)*, lack HSN cell bodies and are likely to be expressing the normally male-specific program of HSN cell death. Whereas *egl-1* animals appear to be defective specifically in HSN development, *her-1(n695)* animals exhibit multiple morphological pleiotropies, displaying partial transformation of the sexual phenotype of many cells and tissues. At least two of the *egl* mutants appear to be defective in the processing of environmental signals that modulate egg laying and may define new components of the neural circuitry that control egg laying.

THE genetic analysis of behavior has been a focus of recent studies of the fruit fly (e.g., BENZER 1967, 1971; HALL 1979; PAK 1979), the cricket (BENTLEY 1975) and the mouse (CAVINESS and RAKIC 1978; MULLEN and HERRUP 1979). Among mutants altered in a specific behavior should be animals with defects in the underlying neural circuitry. The analysis of such mutants may provide insight into how the components of the nervous system produce a behavior and how genes control the specification, formation and functioning of a neural circuit.

The free-living nematode *Caenorhabditis elegans* offers a number of advantages for the study of the relationship between genes and the nervous system (BRENNER 1973). The neuroanatomy of *C. elegans* is simple and invariant: the entire circuitry of the 302-cell nervous system of the adult hermaphrodite has been established from electron micrographs of serial sections (WARD *et al.* 1975; WARE *et al.* 1975; WHITE *et al.* 1976; ALBERTSON and THOMSON 1976; SULSTON, ALBERTSON and THOMSON 1980; J. WHITE, E. SOUTHGATE, N. THOMSON and S. BRENNER, personal communication). Thus, mutants with defects in the nervous system can be analyzed at the level of single, identified neurons. The complete cell lineage of *C. elegans* from the fertilized egg to the mature adult has been determined (SULSTON and HORVITZ 1977; KIMBLE and HIRSH 1979; SULSTON, ALBERTSON and THOMSON 1980; SULSTON *et al.* 1983), which permits a detailed characterization of mutants with behavioral defects that have a developmental basis. Genetic manipulation of *C. elegans* is straightforward and facilitated by its small size, ease of culture and short life cycle (BRENNER 1974; HERMAN and HORVITZ 1980). Reproduction by hermaphrodite self-fertilization allows the ready isolation of autosomal and sex-linked mutations as well as the maintenance of mutants with severe behavioral defects, since mating is not required for propagation. Reproduction by hermaphrodite-male mating also occurs, which makes genetic analysis possible. A number of behaviors have been studied genetically in *C. elegans*: locomotion (BRENNER 1974), chemotaxis (WARD 1973; DUSENBERY, SHERIDAN and RUSSELL 1975; LEWIS and HODGKIN 1977), male mating (HODGKIN 1982), osmotic avoidance (CULOTTI and RUSSELL 1978), thermotaxis (HEDGECOCK and RUSSELL 1975) and response to tactile stimulation (CHALFIE and SULSTON 1981).

This paper describes a genetic analysis of egg laying by the *C. elegans* hermaphrodite. The anatomical components of the egg-laying system have been defined using a combination of light and electron microscopy: the egg-laying system consists of an 18-cell vulva, 16 sex (vulval and uterine) muscle cells and 16 neurons (as defined by connectivity) (WHITE *et al.* 1976; SULSTON and HORVITZ 1977; J. WHITE and R. HORVITZ, unpublished observations). The neurons providing direct synaptic inputs to the sex muscles are the two hermaphrodite-specific neurons (HSNs) and the six ventral C neurons (VCs). Other neurons provide synaptic inputs indirectly, via the HSNs: five mechanosensory neurons (the microtubule cells: AVM, ALMs and PLMs; see CHALFIE and SULSTON 1981), two interneurons (BDUs) and a single neuron with apparent mechanosensory endings in the region of the vulva (PVT). These neurons have been selectively eliminated using either a laser microbeam or mutations; only the elimination of the two HSNs resulted in obvious defects in egg laying (R. HORVITZ and J. SULSTON, unpublished observations). Pharmacological, behavioral and histochemical studies have suggested that, in addition to those neurons that provide synaptic inputs, other neurons function in the egg-laying system and are likely to act via humoral input(s) (HORVITZ *et al.* 1982; TRENT 1982).

A number of mutants with defects in egg laying have been identified previously. Most of these mutants are abnormal in the development of the vulva (HORVITZ and SULSTON 1980) or in muscle structure and function (WATERSTON,

THOMSON and BRENNER 1980; GREENWALD and HORVITZ 1980). In an attempt to obtain mutants altered in the functioning or development of the neural components of the egg-laying system, we have isolated and characterized a new set of mutants defective in egg-laying.

MATERIALS AND METHODS

Strains and genetic nomenclature: *C. elegans* var. Bristol strain N2 was the wild-type parent of all strains used in this work. The genes and alleles used in mapping and other experiments are listed. N2 and most of the mutant strains have been described by BRENNER (1974) and by RIDDLE and SWANSON (1982). We have also used a number of dominant mutations isolated and mapped by J. PARK (personal communication): *egl-30(n715) I; unc-8(n491) IV; unc-102(494,n774) X.* *ced-3(n717) IV* was isolated and characterized by H. ELLIS (personal communication). *tra-2(e1875)* was characterized by J. HODGKIN (personal communication). The dominant LGII crossover suppressor *C1 dpy-10(e128) unc-52(e444)* was decribed by HERMAN (1978).

LGI: lin-6(e1466); unc-11(e47); dpy-5(e61); daf-8(e1393); unc-13(e1091); fer-1(hc1); unc-29(e1072); unc-75(e950); unc-59(e1005); unc-54(e190).

LGII: cat-2(e1112); unc-85(e1414); dpy-10(e128); tra-2(n196); rol-6(e187); unc-4(e120); unc-53(e404,n152); rol-1(e91); lin-7(e974); daf-5(e1386); unc-52(e444).

LGIII: daf-7(e1372); dpy-17(e164); daf-4(e1364); unc-86(e1416); unc-32(e189); unc-69(e587); dpy-18(e364).

LGIV: dpy-9(e12); daf-1(m40); unc-17(e245); dpy-13(e184); unc-8(e49,n491); daf-14(m77); dpy-20(e1362); unc-30(e191); ced-3(n717); dpy-4(e1166).

LGV: unc-60(e677); dpy-11(e224); unc-42(e270); sma-1(e30); him-5(e1467,e1490); unc-76(e911); dpy-21(e459); unc-51(e369).

LGX: unc-102(e1598,n494,n774); dpy-3(e27); daf-3(e1376); lon-2(e678); mec-2(e75); dpy-7(e1324); unc-18(e81); unc-9(e101); unc-3(e151).

This paper conforms to the standardized nomenclature for *C. elegans* genetics (HORVITZ *et al.* 1979).

Sources of egg-laying defective mutants: All mutants were isolated after mutagenesis of N2 with ethyl methanesulfonate (BRENNER 1974). Methods for identifying egg-laying-defective mutants have been described by HORVITZ and SULSTON (1980). All but ten of the egg-laying-defective mutants isolated in this study were obtained by screening the F_2 progeny of mutagenized hermaphrodites grown at 25° for "bags of worms" in which the progeny had hatched internally or for hermaphrodites bloated with late-stage eggs. The following mutants with dominant defects in egg-laying were picked from the F_1 generation of mutagenized hermaphrodites: *egl-36(n728)* was isolated by J. PARK; *her-1(n695)* by E. FERGUSON and *egl-2(n693)* by H. ELLIS. *egl-37(n742)* was reisolated from the strain MT1142, which was obtained from J. LEWIS and also contains the levamisole-resistant mutation *lev-1(x22)* (LEWIS *et al.* 1980a).

egl-1(n487), egl-5(n486) and *egl-10(n692)* were isolated using protocols designed specifically to identify mutants with lesions in the HSNs. Animals in which the HSNs have been eliminated by laser ablation are egg-laying defective but release eggs when treated with serotonin; they do not release eggs when treated with imipramine (R. HORVITZ and J. SULSTON, unpublished results). Wild-type hermaphrodites lay eggs in response to both drugs. *egl-1(n487)* and *egl-10(n692)* were isolated using the following protocol. Young adult F_2 progeny of mutagenized N2 hermaphrodites were picked at random and placed individually in microtiter wells containing 0.5 mg/ml of imipramine in M-9 buffer. (M-9 buffer is defined by BRENNER 1974.) After 90 min, serotonin was added (to a final concentration of 3 mg/ml) to wells with animals that did not lay eggs in response to imipramine. Animals that laid eggs in response to serotonin were then picked individually onto Petri dishes and their progeny scored for egg laying. If egg-laying defective, these progeny were tested with serotonin and imipramine; plates with only wild-type animals were discarded. Some egg-laying defective progeny failed to show the expected drug responses but, nonetheless, were saved as egg-laying defective mutants. [*lin-17(n698), egl-8(n488)* and *egl-35(n694)* were obtained in this way. *lin-17* animals are resistant to both drugs. *egl-35(n694)* animals are sensitive to both drugs, and *egl-8(n488)* animals are variably responsive to imipramine and resistant to serotonin.] *egl-5(n486)* was isolated

using a different scheme. F_2 progeny of mutagenized N2 hermaphrodites were washed from Petri dishes with M-9 buffer and resuspended in a solution of 0.5 mg/ml of imipramine in M-9 buffer. After 90 min, the adults were separated from eggs by pelleting through 20% sucrose (5 min at 180 × g) and then resuspended in 3 mg/ml of serotonin. The eggs released during the serotonin treatment were separated from the adults (as in the previous step) and then transferred to Petri dishes. Approximately 3 days later, the animals were scored for egg laying; bloated hermaphrodites (*e.g., n486*) were saved and wild-type animals discarded.

A number of mutants defective in egg laying have been identified previously: (1) muscle mutants, which show abnormal somatic muscle structure and are presumed to be abnormal in vulval muscle structure as well: *unc-54* (EPSTEIN, WATERSTON and BRENNER 1974); *unc-93* (GREENWALD and HORVITZ 1980); *unc-15* (WATERSTON, FISHPOOL and BRENNER 1977); *unc-22, unc-52, unc-60, unc-82* and *unc-87* (WATERSTON, THOMSON and BRENNER 1980), (2) cell lineage mutants, most of which are defective in the development of the vulva: *unc-59, unc-83, unc-84, unc-85, unc-86, lin-1, lin-2, lin-3, lin-4* and *lin-7* (HORVITZ and SULSTON 1980); *lin-10, lin-12, lin-14, lin-15, lin-17, lin-24, lin-28, lin-31* and *sup-17* (E. FERGUSON, P. STERNBERG, V. AMBROS and R. HORVITZ, unpublished results), and (3) dauer-constitutive mutants: *daf-1, daf-4, daf-7, daf-8* and *daf-14* (RIDDLE, SWANSON and ALBERT 1981; D. RIDDLE, personal communication). We have examined the egg-laying phenotypes of mutants defective in many other known *C. elegans* genes and have found that the following mutants are abnormal in egg laying: *unc-6, unc-40, unc-45, unc-51, unc-53, unc-98, tra-2(e1875)* and heterozygotes for other *tra-2* alleles. Mutations in a number of genes confer relatively low penetrance egg-laying defects and, with the exception of the *tra-2* alleles, are not included in this list.

Some of the mutations affecting egg laying that we have isolated are new alleles of previously identified genes: *unc-40(n324,n473), unc-53(n152,n166,n569), unc-54(n325,n326,n327,n398), unc-59(n391), unc-83(n159,n320,n331,n368,n370), unc-84(n296,n321,n322,n323,n369,n399), unc-85(n319,-n471), unc-93(n200), lin-1(n383,n430,n431), lin-2(n308,n385,n673,n760,n763), lin-7(n308,n385,n673,-n760,n763), lin-31(n301,n428,n762), lin-10(n299), lin-12(n302,n676), lin-14(n530,n536), lin-17(n671,n698), daf-1(n690), daf-7(n696)* and *tra-2(n196)*. Nine mutations affecting vulval development defined four new *lin* genes (E. FERGUSON and V. AMBROS, personal communication): *lin-11(n382,n389,n566,n672), lin-25(e1446,n545), lin-26(n156), lin-29(n333,n836).*

Genetic methods: General techniques for the culture and genetic analysis of *C. elegans* have been described by BRENNER (1974). Most of the mapping and complementation tests were performed at 25°.

For complementation testing, heterozygous or hemizygous males of one egg-laying defective strain were crossed with hermaphrodites of a second egg-laying defective strain containing a marker that allowed cross-progeny to be distinguished from self-progeny. The egg-laying phenotypes of the cross-progeny hermaphrodites were then scored.

Mutations were mapped to linkage groups using two triply marked tester strains: *dpy-5 I; bli-2 II; unc-32 III* and *unc-5 IV; dpy-11 V; lon-2* X. *Trans* heterozygotes for the mutation and the markers of each tester strain were constructed, and, from their progeny, egg-laying defective hermaphrodites (Egl nonmarker) were picked and scored for segregation of the mapping markers. The frequency of animals segregating a particular marker is $2p/(1 + p)$, or ⅔ for unlinked markers and $2p$ for closely linked markers (p = frequency of recombination). The dominant mutations *egl-23(n601)* and *egl-36(n728)* were mapped to linkage groups by constructing *trans* heterozygotes with dominant or recessive markers on each linkage group and scoring their progeny for wild-type animals. If a recessive marker was used, the frequency of wild-type animals was $p/2 - p^2/4$, or 3⁄16 for unlinked markers and $p/2$ for closely linked markers; if a dominant marker was used, the frequency of wild-type animals was $p^2/4$.

The mutations were mapped more precisely using one or more of the following protocols. Heterozygotes of genotype *ab/c* were constructed and from their progeny: (1) A non-B and/or B non-A recombinants were picked and scored for segregation of C progeny; this protocol is a standard three-factor cross (BRENNER 1974). (Animals of genotype *a/a* are of phenotype A, etc.). (2) C hermaphrodites were picked and scored for segregation of A, B, or AB progeny. (3) Wild-type (non-A non-B non-C, where *a* or *b* is a dominant marker) recombinants were picked and scored for segregation of B (if *a* is dominant) or A (if *b* is dominant). Protocols 1, 2 and 3 are discussed in more detail in the legends to Tables 2, 3 and 4.

Almost all mutations were mapped using protocols 1, 2 and/or 3. Linkage of *egl-26(n481)* to *cat-*

2 was shown by a two-factor cross: zero of 14 Egl animals picked from the progeny of *egl-26* +/+ *cat-2* heterozygotes segregated Cat-2 progeny. Formaldehyde-induced fluorescence histochemistry was used to score the Cat-2 phenotype (SULSTON, DEW and BRENNER 1975). The map position of *egl-39(n730)* was established using a *cis* three-factor cross. Unc non-Dpy and Dpy non-Unc recombinants segregating from the heterozygote *dpy-17 unc-32 egl-39*/+ + + were picked and their progeny scored for segregation of Unc Egl or Dpy Egl progeny, respectively: zero of three Dpy non-Unc recombinants segregated Dpy Egl (non-Unc) animals; one of one Unc non-Dpy recombinant segregated Unc Egl (non-Dpy) animals. The *egl-15* mutation failed to complement the deficiency *nDf19*, isolated and characterized by V. AMBROS (personal communication), which extends from *unc-58* on the left to *sma-5* on the right.

egl; daf-3 double mutants were generated as follows. Heterozygotes of genotype *egl*/+; *unc-102(n774) dpy-3*/*daf-3* were constructed by crossing *daf-3* males with *egl; unc-102(n774) dpy-3* hermaphrodites. From the progeny of these heterozygotes, Egl Unc non-Dpy animals [genotype *egl; unc-102(n774) dpy-3*/*daf-3*] were picked. From their progeny, non-Unc animals (genotype *egl; daf-3*) were picked.

egl; daf-5 double mutants were constructed as follows. Heterozygotes of genotype *egl*/+; *C1 dpy-10 unc-52*/*daf-5* were generated by crossing *daf-5* males with *egl; C1 dpy-10 unc-52*/*rol-6* hermaphrodites. From the progeny of these heterozygotes Egl non-Dpy non-Unc animals were picked. From the progeny of these *egl; C1 dpy-10 unc-52*/*daf-5* hermaphrodites, non-Dpy non-Unc animals were picked. Hermaphrodites that did not segregate Dpy Unc animals were of genotype *egl; daf-5*.

Pharmacological tests: A number of pharmacological agents stimulate the release of eggs when applied exogenously to wild-type hermaphrodites (HORVITZ *et al.* 1982; R. HORVITZ and C. TRENT, unpublished observations). For the pharmacological characterization of the egg-laying-defective mutants, we have used four drugs that reproducibly stimulate egg laying by wild-type hermaphrodites: serotonin, imipramine, levamisole and phentolamine. The sites of action of these drugs in the *C. elegans* egg-laying system are unknown. Serotonin (5-hydroxytryptamine) is a neurotransmitter found widely in both vertebrates and invertebrates (GOODMAN and GILMAN 1975); *C. elegans* appears to have only two serotonergic neurons, the pharyngeal neurosecretory motor neurons (HORVITZ *et al.* 1982). Imipramine (a tricyclic antidepressant) is believed to affect biogenic amine metabolism: it blocks the uptake of norepinephrine and serotonin by adrenergic and tryptaminergic nerve terminals, respectively (GOODMAN and GILMAN 1975). Levamisole acts as a cholinergic agonist in *C. elegans* (BRENNER 1974; LEWIS *et al.* 1980b). Phentolamine blocks α-adrenergic receptors in vertebrates (GOODMAN and GILMAN 1975) and blocks octopaminergic receptors in the locust (EVANS and O'SHEA 1978).

To test for sensitivity to a particular drug, hermaphrodites were placed individually in microtiter wells containing a solution of the drug dissolved in M-9 buffer, and, at various times after transfer, the number of eggs in each well was counted. Final counts were taken at 60 min for serotonin, levamisole and phentolamine and at 90 min for imipramine. For each mutant, seven to ten hermaphrodites were tested individually per drug per experiment. Mutants were assigned to one of five categories based on their responses to serotonin and to imipramine (see RESULTS). To assay the reliability of these assignments, we tested the responses to serotonin and to imipramine multiple times for the wild type and for one mutant from each of four of the five categories: *egl-23* (category A), *egl-1* (B), *egl-40* (C) and *egl-2* (D). (Category E consists of those mutants with variable or intermediate responses.) For these five strains, repeated determinations gave very similar results. Fifteen other mutants (again representing the various categories) were tested at least twice, and, for 12 of these mutants, the results were consistent, *i.e.*, the category to which the mutant would be assigned was the same for the two (or more) determinations. For *egl-4*, *egl-9* and *egl-37* hermaphrodites, separate determinations gave different results. For example, in each of two experiments, some *egl-37* hermaphrodites responded well and others responded poorly to serotonin and to imipramine, indicating an assignment to category E; in a third experiment, all animals responded reasonably well to both drugs and, based on this determination, an assignment to category C would have been made. All other mutants were tested once. Based on the general reproducibility of this assay and since 14 of the 38 mutants in categories A–D have been tested at least twice, it seems reasonable to expect that most mutants in these categories have been assigned appropriately.

In most cases, young unbloated or slightly bloated (a few eggs retained) hermaphrodites were tested. For most mutations with incomplete penetrance, moderately or very bloated animals were

used. For six mutations, representing the various categories of drug responses, both unbloated and moderately or very bloated animals were tested. In general, bloated animals laid more eggs than unbloated animals, but the overall nature of the response (*i.e.*, sensitive or resistant to the drug) was generally the same in both cases. The observation that bloated animals may lay more eggs than unbloated animals should not affect the validity of the categorization of those mutants tested as bloated animals; such mutants (*e.g.*, *egl-24* and *egl-33*) were generally resistant or weakly responsive to the drugs (*i.e.*, in category A), and, presumably, unbloated animals would have been at least as resistant to the drugs.

The concentrations of the drugs used were 5 mg/ml for serotonin, 0.75 mg/ml for imipramine, 0.1 mg/ml for levamisole and 10 mg/ml for phentolamine. The high concentrations needed to elicit a response probably reflect the general impermeability of *C. elegans* (BRENNER 1974; SULSTON, DEW and BRENNER 1975; LEWIS *et al.* 1980b). Serotonin, imipramine and levamisole were obtained from Sigma Chemical Company. Phentolamine was a gift from CIBA Pharmaceutical Company.

Mating tests: Tests to determine the efficiency of male mating were performed as described by HODGKIN, HORVITZ and BRENNER (1979). Six L4 males and six L4 *dpy-11* hermaphrodites were placed on a Petri dish seeded with a small (about 1 cm) circle of *Escherichia coli* and about 24 hr later the males were removed. Hermaphrodites were transferred to fresh dishes each day, and total cross-progeny were counted. *egl-5* and *egl-27* males did not mate in this test or in other tests in which 20–30 L4 males were placed with 6 L4 *dpy-11* hermaphrodites and the males removed about 48 hr later. *egl-2* and *egl-5* males from double mutant strains containing the *egl* mutation and *him-5(e1467)* were used for the mating assays. For *egl-27*, the strain *egl-27; him-5(e1490)*, provided by J. HODGKIN, was used.

Microscopy: The microscopic techniques used in this study have been described: Nomarski differential interference contrast optics for the observation of living animals (SULSTON and HORVITZ 1977) and polarized light microscopy for the observation of muscle structure (EPSTEIN, WATERSTON and BRENNER 1974).

RESULTS

Isolation and preliminary characterization of egg-laying-defective mutants: We have isolated 145 new mutants defective in egg laying. These mutants are fertile but show abnormalities in the release of progeny. Most of these mutants were identified by screening the F_2 progeny of mutagenized wild-type hermaphrodites for animals bloated with late-stage eggs or for bags of worms in which the progeny had hatched internally. Three mutants were isolated using protocols designed specifically to identify mutants defective in the development or functioning of the HSN neurons.

Preliminary characterization of these mutants involved examining their phenotypes for similarities to those of mutants defective in previously defined genes, complementation testing and, in some cases, mapping to a linkage group. Mutants defective in many of the classes of genes known to affect egg laying have distinctive phenotypes: (1) most mutants defective in muscle structure (such as *unc-54*; BRENNER 1974; EPSTEIN, WATERSTON and BRENNER 1974) are paralyzed or exhibit slow locomotion; (2) vulvaless mutants (such as *lin-7*; HORVITZ and SULSTON 1980) release no eggs or larvae and form bags of worms; many show incomplete penetrance; (3) dauer-constitutive mutants (such as *daf-14*; RIDDLE, SWANSON and ALBERT 1981) form dauer larvae even in the presence of food (dauer larvae are alternative third-stage larvae normally formed in response to a limited food supply).

By complementation analysis, 52 mutations affecting egg laying were found to be alleles of 18 previously identified *lin*, *unc* and *daf* genes (see MATERIALS

AND METHODS for a list of these alleles and a brief description of and references to these genes). One egg-laying defective mutant proved to be a *tra-2* heterozygote. [XX homozygotes of *tra-2* alleles are phenotypically male (HODGKIN and BRENNER 1977), and heterozygotes are hermaphrodites that exhibit a low penetrance egg-laying defect.] Nine mutants defined four new *lin* genes. For the purposes of this study, most of these mutants, as well as 25 others (nine with abnormal muscle structure, 12 with abnormal vulvae and four similar and presumably allelic to *unc-6* X), were not characterized further. The remaining 59 mutants were characterized in detail genetically, behaviorally and pharmacologically.

Mapping and complementation analysis of mutations affecting egg laying: Each mutation was mapped to a linkage group, and complementation tests were performed among recessive and weakly semidominant mutations [such as *egl-12(n602)* and *egl-10(n692)*] located on the same linkage group. One allele of each complementation group defined by the recessive and weakly semidominant mutations, various semidominant mutations [*egl-6(n592), egl-7(n575), egl-30(n686)* and *egl-40(n606)*] and all dominant mutations were mapped more precisely by three-factor crosses using the protocols described in MATERIALS AND METHODS and in the legends to Tables 2, 3 and 4. The data are summarized in these tables, and the map positions are shown in Figure 1.

These mutations define 40 new genes (see Table 1), designated *egl* (*egg-laying abnormal*), and include a novel allele of *her-1*. Four *egl* genes are defined by dominant alleles: *egl-1, egl-2, egl-23* and *egl-36*. The map position of *egl-23* clearly distinguishes it from other *egl* genes. The alleles of the other three (*egl-1, egl-2* and *egl-36*) were separated by recombination from nearby *egl* genes (see Table 4). The map positions of the semidominant mutations *egl-7(n575)* and *egl-30(n686)* distinguish them from other *egl* genes. The semidominant mutations *egl-30(n686)* and *egl-30(n715)* appear to be allelic based on data of J. PARK (personal communication). The semidominant mutations *n592* and *n606* appear to complement nearby genes affecting egg laying and have been tentatively assigned as *egl-6* and *egl-40*, respectively.

Egg-laying phenotypes: In the laboratory, *C. elegans* is maintained on a lawn of *E. coli*, which serves as its food supply. Under these conditions at 20° or 25° a young adult hermaphrodite lays as many as eight to nine eggs per hour (BYERLY, CASSADA and RUSSELL 1976). Eggs are normally laid about 3 hr (at 20°) after fertilization at approximately the 30-cell stage (HIRSH, OPPENHEIM and KLASS 1976). Morphogenesis begins at about 6 hr, and hatching occurs at about 14 hr after fertilization (SULSTON *et al.* 1983). The various stages of embryo morphogenesis (VON EHRENSTEIN and SCHIERENBERG 1980), *e.g.*, lima bean, comma and pretzel are easily identified with the dissecting microscope.

We have characterized the egg-laying behavior of mutants defective in each *egl* gene and in some of the previously defined genes affecting egg laying. Individual hermaphrodites were studied to determine: (1) what fraction of progeny the animal released, *i.e.*, most, some or none; (2) the earliest stage of progeny released (see Figure 2): early egg (to the beginning of morphogenesis, *i.e.*, to the lima bean stage), late egg (lima bean through pretzel stage) or larva;

TABLE 1

Egg-laying defective mutants

Gene	LG	Alleles	Comments
egl-1	V	*n487dm*	Males mate; HSN cell bodies absent
egl-2	V	*n693dm*	Males mate poorly; weak kinker
egl-3	V	*n150ts,n588sd** *n589ts,n729*	Coiler
egl-4	IV	*n478,n477,n479ts* *n579,n612ts*	
egl-5	III	*n486*	Males do not mate; coiler; HSN cell bodies absent or displaced
egl-6	X	*n592sd*	Males mate; weak kinker
egl-7	III	*n575ts*,sd*	
egl-8	V	*n488*	
egl-9	V	*n586ts,n571*	
egl-10	V	*n692sd*,ts** *n480ts*	Males mate; sluggish and weak kinker
egl-11	V	*n587ts**	
egl-12	V	*n602sd*,n599*	
egl-13	X	*n483,e1447*	Males mate[a]
egl-14	X	*n549*	Males mate poorly
egl-15	X	*n484*	Males mate; sex muscle defects
egl-16	X	*n485*	Males mate[a]; vulval abnormalities
egl-17	X	*e1313*	Males mate
egl-18	IV	*n162,n474,n475*	Vulval abnormalities
egl-19	IV	*n582*	Slow and floppy; long
egl-20	IV	*n585ts**	
egl-21	IV	*n611ts*,n476,n576*	Coiler
egl-22	IV	*n422ts*,n577ts*	Limp paralyzed; ts for Egl
egl-23	IV	*n601dm*	Sluggish
egl-24	III	*n572*	
egl-25	III	*n573*	Tail morphology variably abnormal
egl-26	II	*n481*	Abnormal vulva
egl-27	II	*n170*	Males do not mate
egl-28	II	*n570ts**	
egl-29	II	*n482*	Abnormal vulva
egl-30	I	*n686sd,n715sd*	*n686* slow, *n715* paralyzed
egl-31	I	*n472*	Sex muscle defects; backward Unc
egl-32	I	*n155ts**	Males mate
egl-33	I	*n151ts*	Kinker
egl-34	I	*n171,e1452*	
egl-35	III	*n694ts*	
egl-36	X	*n728dm*	Males mate
egl-37	II	*n742ts**	
egl-38	IV	*n578*	Very sluggish; abnormal vulva
egl-39	III	*n730ts*	Sluggish; weak coiler
egl-40	IV	*n606ts,sd*	Males mate
her-1	V	*n695sd,ts**	HSN cell bodies absent; XX animals variably transformed into abnormal males

For genes with multiple alleles, the canonical allele (i.e., the allele used for mapping and for the behavioral and pharmacological characterizations) is listed first. The mutation *egl-30(n715)* was isolated and genetically characterized by J. PARK (personal communication). Male mating was not systematically examined and, for most mutants tested, was not determined quantitatively. For mutations exhibiting incomplete penetrance, it is not clear whether phenotypically mutant males are capable of mating (i.e., mating ability may result from the presence of phenotypically wild-type males). The abnormalities in locomotion are described using the categories of HODGKIN (1982). Abbreviations used are: LG, linkage group; *dm*, dominant; *sd*, semidominant; *sd**, weakly semidominant; *ts*, temperature sensitive (non-Egl or very weakly Egl at 15°); *ts**, partially temperature sensitive.

[a] These mutants show incomplete penetrance for their egg-laying defects.

TABLE 2

Standard three-factor crosses (protocol 1)

Genotype of heterozygote	Phenotype of selected recombinant	Genotype of selected recombinant (with respect to *trans* marker)
+ *egl-1* +/*dpy-11* + *unc-76*	Dpy	11/12 *egl*/+
	Unc	1/10 *egl*/+
+ + *egl-3*/*dpy-11 unc-42* +	Unc	0/23 *egl*/+
egl-3 + +/+ *unc-42 sma-1*	Unc	0/23 *egl*/+
+ *egl-4* +/*dpy-9* + *unc-17*	Dpy	5/12 (dpy) *egl*/(dpy) + (unc)
		4/12 (dpy) +/(dpy) + (unc)
		2/12 (dpy) *egl*/(dpy) +
		1/12 (dpy) *egl*/(dpy) *egl*
+ *egl-6* +/*lon-2* + *unc-18*	Lon	8/20 *egl*/+
lon-2 + *egl-6*/ + *mec-2* +	Lon	1/3 *mec*/+
	Egl	1/1 *mec*/+
+ *egl-9* +/*unc-42* + *sma-1*	Unc	5/13 *egl*/+
lon-2 + *egl-13*/+ *mec-2* +	Egl	1/4 *mec*/+
lon-2 + *egl-14*/+ *mec-2* +	Lon	2/6 *mec*/+
	Egl	5/8 *mec*/+
+ *egl-15* +/*dpy-7* + *unc-9*	Unc	5/11 *egl*/+
+ *egl-19* +/*unc-8(e49)* + *dpy-20*	Unc	2/11 *egl*/+
+ *egl-20* +/*dpy-13* + *unc-30*	Dpy	9/13 *egl*/+
	Unc	3/14 *egl*/+
+ *egl-21* +/*unc-8(e49)* + *dpy-20*	Unc	5/8 *egl*/+
+ *egl-23* +/*unc-30* + *dpy-4*	Unc	5/11 *egl*/+
	Dpy	11/17 *egl*/+
+ *egl-28* +/*dpy-10* + *unc-4*	Unc	1/12 *egl*/+
+ *egl-29* +/*dpy-10* + *rol-1*	Rol	3/10 *egl*/+
+ + *egl-29*/*dpy-10 unc-4* +	Dpy	6/6 *egl*/+
	Unc	0/10 *egl*/+
+ *egl-32* +/*dpy-5* + *unc-75*	Dpy	4/12 *egl*/+
	Unc	3/12 *egl*/*egl*, 6/12 *egl*/+
+ *egl-33* +/*dpy-5* + *unc-75*	Dpy	4/12 *egl*/+
	Unc	5/12 *egl*/+
+ *egl-36* +/*dpy-7* + *unc-9*	Unc	3/11 *egl* (unc)/(dpy) + (unc)
		7/11 + (unc)/(dpy) + (unc)
		1/11 *egl* (unc)/+ (unc)
+ *egl-37* +/*dpy-10* + *unc-4*	Unc	2/9 *egl*/+
+ *egl-38* +/*unc-8(e49)* + *dpy-20*	Unc	5/10 *egl*/+
	Dpy	4/6 *egl*/+
+ *egl-40* +/*unc-8(e49)* + *dpy-20*	Unc	5/10 *egl*/+
+ *her-1(n695)* +/*dpy-11* + *unc-42*	Unc	1/11 *egl*/+

Heterozygotes of genotype *ab*/*c* were constructed and, from their progeny, A non-B and/or B non-A recombinants were picked and scored for segregation of the *trans* marker. The frequency of recombinants of each class carrying the *trans* marker *c* reflects the relative position of *c* with respect to *a* and *b*. For example, (1) 11 of 12 Dpy and one of ten Unc recombinants from *dpy-11* + *unc-76*/+ *egl-1* + heterozygotes segregated Egl-1 progeny, indicating that *egl-1* is between *dpy-11* and *unc-76* and closer to the latter; and (2) zero of ten Unc and six of six Dpy from *dpy-10 unc-4* +/+ + *egl-29* heterozygotes segregated Egl-29 progeny indicating that *egl-29* is either to the right of or to the left of and close to *unc-4*.

TABLE 3

Three-factor crosses (protocol 2)

Genotype of heterozygote	Genotype of Egl progeny (with respect to *trans* markers)	Estimated recombination frequency (%) 100p	
		L	R
+ *egl-4* +/*dpy-9* + *unc-17*	4/16 *dpy*/+, 4/16 *unc*/+	13	13
+ + *egl-5*/*dpy-17 unc-32* +	3/50 *dpy*/+	3	<1
egl-7 + +/+ *dpy-17 unc-32*	1/34 *dpy unc*/+ +, 3/34 *unc*/+	1	6
+ *egl-9* +/*unc-42* + *sma-1*	1/51 *sma*/+	<1	1
+ + *egl-10*/*unc-42 sma-1* +	1/39 *unc sma*/+ +, 2/39 *unc*/+	4	1
+ *egl-10* +/*dpy-11* + *unc-76*	4/44 *dpy*/+, 3/44 *unc*/+	5	3
egl-11 + +/+ *dpy-11 unc-42*	1/49 *unc*/+	<1	1
+ *egl-12* +/*dpy-21* + *unc-51*	1/24 *dpy*/+, 2/24 *unc*/+	2	4
egl-13 + +/+ *dpy-7 unc-9*	1/38 *dpy unc*/+ +, 9/38 *unc*/+	1	13
+ *egl-14* +/*lon-2* + *unc-18*	6/50 *lon*/+, 3/50 *unc*/+	6	3
+ *egl-15* +/*dpy-7* + *unc-9*	3/24 *dpy*/+, 3/24 *unc*/+	6	6
+ *egl-16* +/*dpy-7* + *unc-3*	16/45 *dpy*/+, 2/45 *unc*/+	18	2
egl-17 + +/+ *unc-102 dpy-3*	2/48 *dpy*/+	<1	2
+ *egl-18* +/*dpy-9* + *unc-17*	2/10 *dpy*/+, 2/10 *unc*/+	10	10
+ *egl-20* +/*dpy-13* + *unc-30*	1/47 *dpy*/*unc*, 9/47 *dpy*/+, 5/47 *unc*/+	11	6
egl-22 + +/+ *unc-30 dpy-4*	1/50 *unc dpy*/+ +, 1/50 *dpy*/+	1	2
+ *egl-23* +/*unc-17* + *dpy-4*	5/12 *unc*/+	21	<4
+ *egl-24* +/*dpy-17* + *unc-32*	2/30 *dpy*/+, 2/30 *unc*/+	3	3
+ *egl-25* +/*dpy-17* + *unc-32*	1/47 *dpy*/*unc*, 2/47 *unc*/+	1	3
egl-26 + +/+ *dpy-10 unc-4*	23/47 *dpy unc*/+ +, 1/47 *unc*/+	24	26
egl-27 + +/+ *dpy-10 unc-4*	4/50 *unc*/+	<1	4
egl-30 + +/+ *lin-6 dpy-5*	6/47 *lin dpy*/+ +, 4/47 *dpy*/+	6	11
+ + egl-31/*dpy-5 unc-13* +	2/54 *dpy unc*/+ +, 2/54 *dpy*/+	4	2
egl-31 + +/+ *fer-1 unc-75*	10/58 *unc*/+	<1	9
+ *egl-32* +/*dpy-5* + *unc-75*	2/24 *dpy*/+, 5/24 *unc*/+	4	10
egl-34 + +/+ *unc-11 dpy-5*	6/154 *dpy*/+	<1	2
+ + *egl-35*/*unc-69 dpy 18* +	2/15 *unc dpy*/+ +, 1/15 *unc*/+	10	7
+ *egl-37* +/*dpy-10* + *unc-4*	2/36 *dpy*/+	3	<2
+ + *egl-39*/*dpy-17 unc-32* +	1/24 *dpy unc*/+ +	>2	2
+ *egl-40* +/*unc-8(e49)* + *dpy-20*	1/34 *unc*/+	1	<1

Heterozygotes of genotype *ab/egl* were constructed and from their progeny Egl hermaphrodites were picked and scored for segregation of A, B or AB progeny. (For dominant mutations, only the progeny of *egl/egl* hermaphrodites were scored.) The frequencies of A, B and AB progeny reflect the position of *egl* relative to *a* and *b* and the recombination distance between *a* or *b* and *egl*. The frequency of *egl* hermaphrodites heterozygous for *a* or *b* is $2p/(1 + p) \simeq 2p$ for small p (p = frequency of recombination). The column L indicates an approximate estimate of the percent recombination between *egl* and the left marker *a*, and R indicates an approximate estimate of the percent recombination between *egl* and the right marker *b*. For example, (1) from the heterozygote *lon-2* + *unc-18*/+ *egl-14* +, six of 50 Egl-14 animals segregated Lon progeny and three of 50 segregated Unc progeny, indicating that *egl-14* is between *lon-2* and *unc-18* and approximately 6% right of *lon-2* and 3% left of *unc-18*; and (2) from the heterozygote *unc-42 sma-1* +/+ + *egl-10*, one of 39 Egl-10 hermaphrodites segregated Unc Sma progeny and two of 39 segregated Unc progeny, indicating the *egl-10* is right of both *unc-42* and *sma-1* and is approximately 2.6% from the *unc-42* and 1.3% from *sma-1*.

TABLE 4

Three-factor crosses (protocol 3)

Genotype of heterozygote	No. of wild type/total progeny	Recombination frequency (%) (100p)	Genotype of wild-type recombinants (with respect to recessive markers)
dpy-11 + egl-1/+ sma-1 +*	ND		6/6 *dpy/sma*
egl-2 + dpy-11/+ unc-60 +*	2/533	0.8	2/2 *dpy/unc*
+ egl-2 dpy-11/egl-8 + +*	1/1200	0.2	1/1 *+/egl-8*
egl-10 + +/+ egl-1 unc-76*	2/215	2	2/2 *+/egl-10*
*+ egl-10 +/unc-42 + egl-1**	ND		2/2 *unc/egl-10*
egl-17 + +/+ unc-102(e1598) dpy-3*	ND		16/17 *+/egl* 1/17 *+/egl dpy*
+ + egl-22/unc-8(n491) dpy-20 +*	ND		11/16 *dpy/egl* 5/16 *+/egl*
egl-36 + unc-9/+ egl-15 +*	3/876	0.7	3/3 *unc/egl-15*
dpy-7 egl-36 +/+ + egl-15*	ND		2/2 *+/egl-15*

Heterozygotes of genotype *ab/c* (where *a* or *b* is a dominant marker) were constructed and, from their progeny, wild-type hermaphrodites were picked and scored for segregation of B (if *a* is dominant) or A (if *b* is dominant). The genotypes of the wild-type recombinants indicate the position of the dominant marker with respect to the recessive markers. The recombination frequency (p) between the dominant marker and the recessive marker *c* is defined by the frequency of wild-type recombinants among the total progeny, which is $p/2 - p^2/4 \simeq p/2$ for small p. For example, (1) six of six wild-type recombinants from *dpy-11 + egl-1*/+ sma-1 +* segregated Dpy hermaphrodites, indicating that *egl-1* is to the right of *sma-1* or to the far left of *dpy-11*; since other map data (see Table 3) place *egl-1* to the right of *dpy-11*, the order of these genes must be *dpy-11 sma-1 egl-1*; (2) from the heterozygote *unc-8(n491)* dpy-20 +/+ + egl-22*, five of 16 wild-type recombinants segregated Dpy progeny, indicating that *egl-22* must be to the right of *dpy-20*; and (3) three of a total of 876 progeny from *egl-36* + unc-9/+ egl-15 +* heterozygotes were wild-type recombinants and three of three of these wild types segregated Unc progeny, indicating that *egl-36* is approximately 0.7% left of *egl-15*. The three-factor cross with *egl-17* was performed by J. PARK (personal communication). *, dominant mutation; ND, not determined.

and (3) whether the animal became bloated with late-stage eggs. These data are summarized in section 2 of Table 5.

The egg-laying behaviors of individual hermaphrodites fell into five general categories: (1) *WT* (wild type), all progeny released and many early-stage eggs observed on the plate; (2) *M/E* (most/early), all or most progeny released and a few early-stage eggs (and many late-stage eggs) observed on the plate; (3) *M/L* (most/late), all or most progeny released and progeny release delayed, *i.e.*, the earliest stage observed was lima bean or later; (4) *S/L* (some/late), some progeny released, the animal formed a bag of worms, and progeny release delayed, *i.e.*, the earliest stage observed was lima bean or later; (5) *N* (none), no progeny released and the animal formed a bag of worms. Animals in category 1 were wild type in egg-laying behavior: they released early-stage eggs and never appeared bloated. Such animals reflect the incomplete penetrance of some *egl* mutations. Animals in categories 2–5 were abnormal in egg-laying behavior and generally appeared moderately or very bloated with late-stage eggs. Animals in categories 2 and 3 became bloated only transiently. Within categories 3 and 4, animals differed with respect to the earliest stage of progeny released (*i.e.*, the

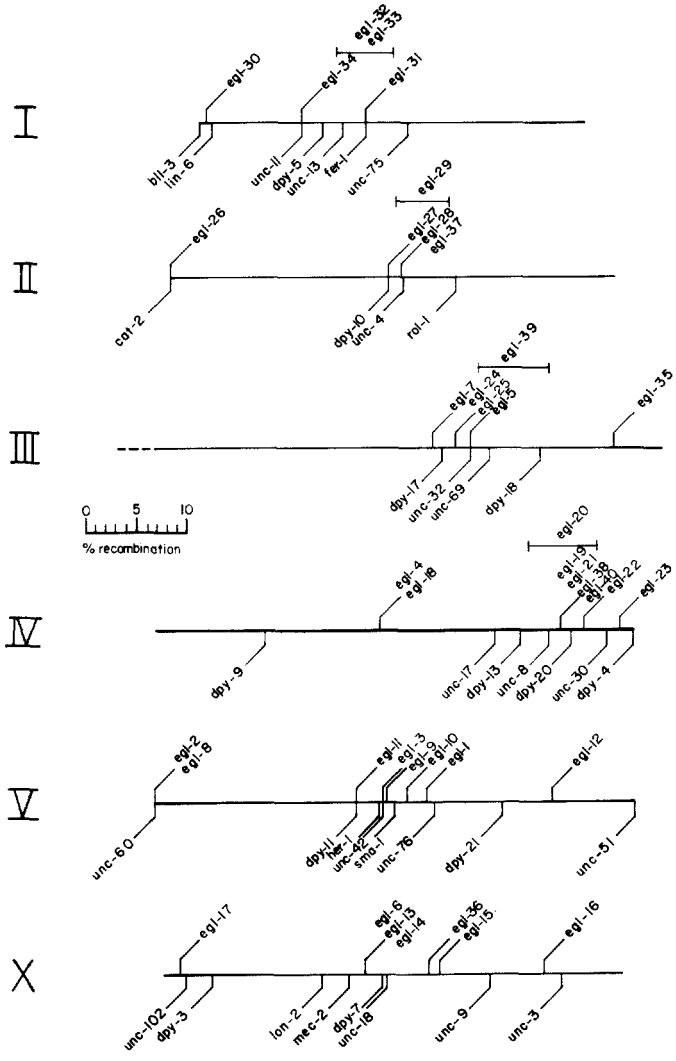

FIGURE 1.—Partial genetic map of *C. elegans* showing the positions of the *egl* genes (indicated above the line of the linkage group) and markers used for mapping (indicated below the line). With the exception of LGIII, the known extent of each linkage group is shown. The map positions of the *egl* genes are based on the data presented in Tables 2, 3 and 4 and in MATERIALS AND METHODS. Experiments by J. PARK (personal communication) have shown that *egl-30* maps to the right of *bli-3*.

earliest stage observed on the plate): some animals released lima bean stage or later, others released only pretzel-stage eggs or later and still others appeared to release only larvae.

The mutants showed striking overall differences in their defects in egg laying. Some mutants, such as *egl-38(n578)*, rarely released any progeny; other mutants, such as *egl-1*, released most of their progeny but at an abnormally late stage of (progeny) development. Still other mutants, such as *egl-7*, showed an interme-

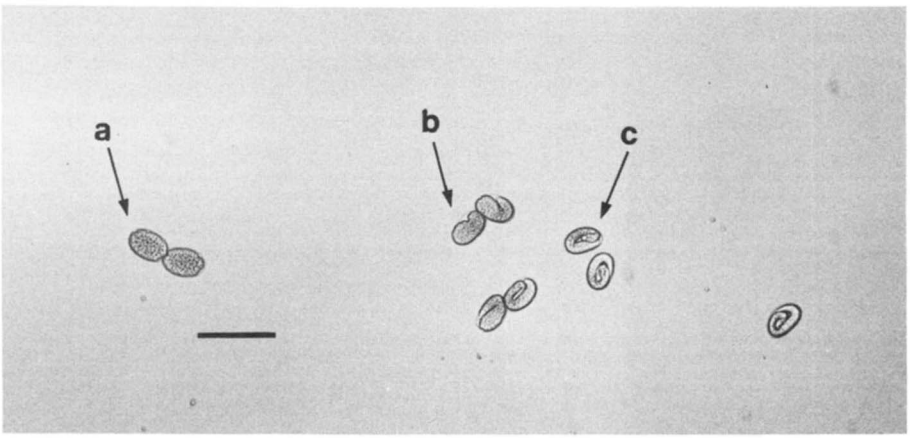

FIGURE 2.—Bright-field photomicrograph of selected stages of *C. elegans* embryogenesis. a, early egg (premorphogenetic), b, lima bean-stage egg, and c, pretzel-stage egg. Advanced stages, *i.e.*, lima bean through pretzel, are referred to as late eggs in the text. See VON EHRENSTEIN and SCHIERENBERG (1980) for a description of the various stages of embryo morphogenesis. Bar = 0.1 mm.

diate defect, releasing only some progeny. Figure 3 illustrates the phenotypes (from young to old adult) of wild-type, *egl-1* and *egl-38* hermaphrodites.

This characterization of individual hermaphrodites helps to reveal both the penetrance and expressivity of each mutation. As the data in Table 5 indicate, several mutants (such as *egl-29*) showed incomplete penetrance, and many mutants (such as *egl-5, egl-18, egl-25* and *egl-36*) exhibited variable expressivity with respect to one or more of the parameters used to describe egg-laying behavior. Some mutants, such as *egl-18* and *egl-25*, showed variation in the fraction of progeny released (ranging from none to most) and in the extent to which the animals appeared bloated (few eggs retained to many eggs retained). Other mutants, such as *egl-5* and *egl-36*, showed variation with respect to the fraction of progeny released (some/most or none/some, respectively) but did not show obvious variation with respect to bloating (all hermaphrodites became very bloated). In addition, for many of the mutants that released progeny, individuals varied with respect to the earliest stage of progeny observed on the plate (data not shown).

Other behavioral or morphological abnormalities: The egg-laying-defective mutants were examined for abnormalities in morphology and in other behaviors, such as locomotion. In some cases, the ability of males to mate was determined. Such pleiotropies are noted in Table 1. Many of the mutants are uncoordinated in locomotion.

Responses to pharmacological agents: The pharmacological agents serotonin and imipramine stimulate the release of eggs when applied exogenously to wild-type hermaphrodites (HORVITZ *et al.* 1982; also see Table 5). Vulvaless mutants, such as *lin-7*, and animals in which the vulval and uterine muscles have been eliminated using a laser microbeam (as well as muscle mutants such as *unc-54*) fail to respond to both drugs (R. HORVITZ and J. SULSTON, unpublished observations; also see Table 5). Hermaphrodites in which the HSN neurons have

TABLE 5

Characterization of egg-laying defective mutants

Gene	1. Responses to drugs: Category of response	Serotonin: +	Serotonin: (+)	Serotonin: (−)	Serotonin: −	Imipramine: +	Imipramine: (+)	Imipramine: (−)	Imipramine: −	Comments	2. Egg-laying behavior: 1 WT	2 M/E	3 M/L	4 S/L	5 N	Comments
Wild type		338	55	22	25	356	51	15	5		20					
egl-23	A			1	49			1	49	*nb/bl*				9	1	Larvae; few pretzel-stage eggs
egl-38	A			1	9				10	*nb/bl*					10	Occasionally lays eggs
egl-36	A			1	8				10	*bl*				5	5	
egl-26	A				10			1	9	*bl*	3			1	6	
lin-7	A				10				10	*bl*					10	
egl-30(n715)	A				10				10	*bl*					10	
unc-54	A				8				10	*bl*				6	4	Larvae only
egl-15	A				10				10			1	3	4	12	
egl-31	A				10				10				2	6	2	Larvae or pretzel-stage eggs; exploded animals
unc-53(n152)	A			1	7				7	*nb/bl*			2	7	1	Larvae; few pretzel-stage eggs
egl-17	A	1			9			2	8				4	11	5	
egl-29	A			3	7			6	4		2				8	
egl-33	A	1	1	1	6				9	*bl*, 25°			3	3	4	25°, exploded animals
egl-25	A	1	1	1	12	2	2	3	10	*bl*	1		4	8	4	variably bloated
egl-24	A		1	3	6	1	2	2	4	*bl*	4		4	1	1	
egl-16	A		3	2	5		1	4	5		1	2	1	1	4	Variably bloated; exploded animals
egl-13	A		1	1	8	1	3		5	*bl*	3			1	6	
unc-85	A				10	1	2	2	5		3		2	4	1	
unc-59	A	1	1	1	7		2	3	4	*bl*			1		6	
egl-1	B	42	4	2	2	1	3	5	41				10			
egl-5	B	19	1				1	3	16				9	11		
unc-86	B	10						2	8				7	3		
egl-10(n692)	B/C	28	1	0	1	9	5	2	15				10			
n695	B/A	14		2	5		3	3	28				18	7	3	

TABLE 5 (continued)

	1. Responses to drugs										2. Egg-laying behavior					
	Category of response	Serotonin:				Imipramine:				Comments	1 WT	2 M/E	3 M/L	4 S/L	5 N	Comments
Gene		+	(+)	(−)	−	+	(+)	(−)	−							
Wild type		338	55	22	25	356	51	15	5		20					
egl-40	C	37	9	2	1	33	13	4		25°			10			25°
egl-39	C	19				15	2			25°			7	3		25°
egl-7	C	17	2	1		18	2			25°			4	15[a]		25°
egl-22	C	17	2	1		14	3	2		25°		1	9			25°, variably bloated
egl-3	C	15	6	5	3	13	6	7	3	25°			2	8		25°
egl-35	C	6	4			4	4	2				1	9			
egl-10(n480)	C	10				7	3			25°		5	5			
egl-30(n715)/+	C	8	1	1		9	1			*nb*/bl			10			
daf-14	C	13	4	1		11	6	1					10			
daf-8	C	8	1	1		9	1						10			
daf-7	C	6	2	1	1	6	3						9	1		
daf-1	C	5	1	3	1	8		2					8		1	
egl-2	D				46	49	6	2	1					9	1	
egl-19	D?				20	3	9	5	3		1		7	2		
egl-20	E	4	1	2	3	3	3	1	2	25°			7	3		25°
egl-18	E		1	3	6	2	2	1	5	*bl*		2	5	2	1	Variably bloated
egl-34	E	1	1	1	7	3		2	5	*bl*	1		5	2	2	Variably bloated
egl-14	E	4		4	2	3	3	1	3			1	8	1		
egl-30(n686)	E	3	1	2	2	4		1	3				9	1		
egl-12	E		1	7	1	5	2	2	1				9			
egl-6	E	4	3	1	2	3	2	2	3				9	1		
egl-27	E	4	3	2	1	2	4	3	1				7	3		Variably bloated
egl-21	E	7	4	2	6	2	1	7	10	25°		1	9			25°
egl-11	E	4	1	1	4	4			6	25°			10			25°
egl-32	E	1	1	3	5	3	2	1	4	25°			2	8		25°

TABLE 5 (*continued*)

Gene	1. Responses to drugs: Category of response	Serotonin: +	Serotonin: (+)	Serotonin: (−)	Serotonin: −	Imipramine: +	Imipramine: (+)	Imipramine: (−)	Imipramine: −	Comments	2. Egg-laying behavior: 1 WT	2 M/E	3 M/L	4 S/L	5 N	Comments
Wild type		338	55	22	25	356	51	15	5		20					
egl-8	E			6	14	3	5	9	2			1	8	1		Variably bloated
egl-28	E	3	1	1	5	5	4		1	25°		3	6	1		25°
egl-4	E	16	6	9	9	15	3	10	13				10			
egl-9	E	12	6	5	5	5	9	2	13	25°			10			25°
egl-37	E	8	7	7	10	10	6	8	6	25°			7	3		25°
daf-4	E		2	4	14	6	7	2	3							

1. Responses to drugs: The responses of individual hermaphrodites to serotonin and imipramine were determined as described in MATERIALS AND METHODS. For each mutant, seven to ten animals were tested per drug per experiment. Multiple experiments were performed on the wild-type and on selected mutants. Each column indicates the number of animals with the following responses: +, ≥ eight eggs laid; (+), four to seven eggs laid; (−), one to three eggs laid; −, no eggs laid. Hermaphrodites were grown and tested at 20° unless otherwise indicated. Most temperature-sensitive mutants were grown at 25° and tested at 22–25°. Wild-type hermaphrodites grown and tested at 25° gave similar drug responses to those grown and tested at 20°. Tests were performed on young nonbloated or slightly bloated (a few eggs retained) adults unless indicated under comments: *bl*, moderately or very bloated animals tested; *nb/bl*, both nonbloated and bloated animals tested. For wild-type and all mutant strains, egg laying in the absence of drugs (*i.e.*, in the M-9 buffer control) was very low.

2. Egg-laying behavior: For each mutant, eight to ten L4 hermaphrodites were tested per experiment. Each hermaphrodite was placed individually on a Petri dish containing a lawn of *E. coli*, and the following were observed twice (or, in a few cases, once) a day for a period of 2–4 days: (1) whether the animal had released any progeny; (2) the earliest stage of progeny on the plate; (3) the phenotype of the hermaphrodite: unbloated (not retaining eggs or all progeny already released), bloated [retaining late (lima bean through pretzel stage) eggs], or bag of worms (progeny hatched internally). For some mutants, egg-laying behavior was studied twice, and the results were combined. Each column indicates the number of animals that showed a particular egg-laying behavior (categories 1–5 are defined in the text). Except where indicated, all animals in categories 2–5 became moderately or very bloated. Also indicated under comments are mutants that released only larvae or mostly larvae and a few pretzel-stage eggs. Hermaphrodites were grown and observed at 20° unless indicated.

[a] Two animals laid a few early eggs. "Exploded animals" refers to adult hermaphrodites that appear to have herniated at the vulva.

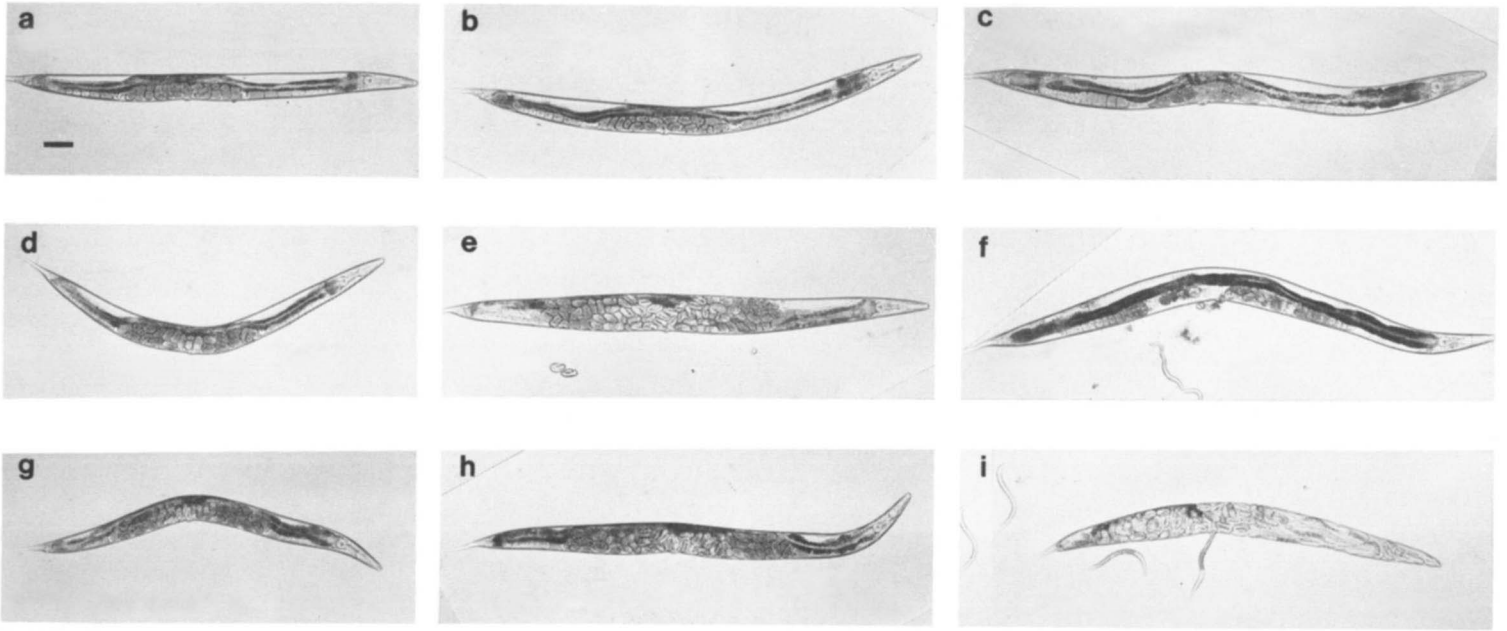

FIGURE 3.—Bright-field photomicrographs of wild-type (a–c), *egl-1* (d–f) and *egl-38* (g–i) hermaphrodites. Each series represents the phenotypes observed with increasing age. For both *egl* mutants, young adults (d and g) are somewhat bloated; they contain more eggs and eggs at later stages of development than the wild type (a). Older *egl-1* and *egl-38* animals (e and h) are very bloated with late eggs. In contrast to *egl-38* hermaphrodites, which form bags of worms (i), *egl-1* hermaphrodites eventually release most of their progeny (f). Bar = 0.1 mm.

been ablated with a laser microbeam (and the HSN-defective mutant *unc-86*; SULSTON and HORVITZ 1981) lay eggs in the presence of serotonin but not in the presence of imipramine (R. HORVITZ and J. SULSTON, unpublished observations; also see Table 5). To test the functioning of the vulva, the vulval and uterine muscles and the HSNs in the mutants defective in egg-laying, we have determined the drug responses of mutants defective in each *egl* gene and in some of the previously defined genes affecting egg laying.

The responses of these mutants to serotonin and imipramine are presented in section 1 of Table 5. These drug responses define four distinct categories of mutants: A, resistant to serotonin and to imipramine; B, sensitive to serotonin and resistant to imipramine; C, sensitive to serotonin and to imipramine; D, resistant to serotonin and sensitive to imipramine. A fifth category, E, includes all mutants that did not clearly belong in one of the categories A–D, *i.e.*, had variable or intermediate responses to the drugs.

Each mutant was also tested with the pharmacological agents levamisole and phentolamine, which stimulate egg laying by wild-type hermaphrodites. Mutants in categories A and B failed to lay eggs in response to levamisole and to phentolamine (data not shown). Within the other categories, the mutants differed in their responses to these drugs; often the results were ambiguous, especially with respect to phentolamine.

Category A: mutants resistant to serotonin and to imipramine: Mutants representing 14 *egl* genes, as well as mutants defective in *lin-7* (vulvaless), *unc-53*, *unc-54* (myosin deficient), *unc-59* and *unc-85*, are included in this category. Some of these mutants, such as *egl-23* and *egl-38*, showed virtually no response to serotonin and imipramine, whereas other mutants, such as *egl-16* and *unc-59*, showed a somewhat variable, but generally poor, response to these drugs. Mutants in the latter group exhibited variable expressivity of the egg-laying defect. Almost all of the *egl* mutants with severe egg-laying defects (*i.e.*, those that release few or no progeny and turn into bags of worms), such as *egl-23* and *egl-38*, are in this category.

Since animals with lesions in the vulval and uterine muscles or the vulva are known to be resistant to all drugs tested, some *egl* mutants with these drug responses are likely to have lost the function of one (or more) of these components. Consistent with this prediction, preliminary examination of some of these mutants has shown that *egl-15* and *egl-31* animals have defects in the development of the vulval and uterine muscles (P. STERNBERG, personal communication) and that *egl-16*, *egl-26*, *egl-29* and *egl-38* animals are abnormal in vulval morphology.

Included in category A is the *egl-30(n715)* mutant, which is paralyzed and never releases progeny; *egl-30(n715)* is semidominant for these phenotypes (*egl-30(n715)/+* animals are described in category C). *egl-30(n686)* animals, listed in category E, exhibit a less severe phenotype. *unc-59* and *unc-85* mutants, which are defective in cell lineage, have HSN and ventral cord abnormalities; these mutants are uncoordinated when they attempt to move backward (SULSTON and HORVITZ 1981).

Category B: mutants sensitive to serotonin and resistant to imipramine: egl-1,

egl-5 and *egl-10* mutants, as well as *her-1(n695)* and *unc-86* mutants, fall into this category. The *egl-1*, *egl-5* and *egl-10(n692)* mutants were isolated using protocols designed to identify animals with these drug specificities (see MATERIALS AND METHODS). Since *unc-86* animals (which are known to be HSN defective; SULSTON and HORVITZ 1981) and animals in which the HSNs have been ablated with a laser microbeam respond to serotonin but not to imipramine, our egg-laying defective mutants with these drug responses are candidates for loss of function of the HSN neurons. These mutants were examined by light microscopy using Nomarski optics for the presence of the HSN cell bodies. In addition, they were examined for possible pleiotropies with respect to the male nervous system: the efficiency of male mating was determined quantitatively.

The *egl-1* gene is defined by the dominant mutation *n487*. In *egl-1* L1 hermaphrodites the HSN cell bodies are absent. In a double mutant with *ced-3*, which causes the survival of cells that normally undergo programmed cell death (HORVITZ, ELLIS and STERNBERG 1982; H. ELLIS, personal communication), the HSN cell bodies are present (H. ELLIS, personal communication), and the egg-laying defect is suppressed. This result indicates that the lack of HSNs in *egl-1* hermaphrodites results from cell death. *egl-1* hermaphrodites release late eggs and/or larvae. The animals become transiently very bloated with late eggs but eventually release most of their progeny (see Figure 3). *egl-1* homozygous males are phenotypically wild type when viewed with the dissecting microscope and, in mating assays, produced about the same number of cross-progeny as did wild-type males.

n695 is incompletely dominant (some *n695*/+ heterozygotes show a mutant phenotype) and weakly temperature sensitive. At both 20° and 25°, the phenotype of *n695* animals ranges from egg-laying defective hermaphrodites (bloated with late-stage eggs but otherwise normal as seen with the dissecting microscope) to animals that have a male body shape and size and an abnormal male-like tail. Intersex animals (fertile hermaphrodites with abnormal male-like tails) are also observed. Wild-type XX animals are hermaphrodites, and X0 animals are males. Genetic experiments have demonstrated that these *n695* intersex and abnormal male animals are XX in genotype (TRENT 1982); thus, *n695* is a transformer mutation (HODGKIN and BRENNER 1977). *n695* appears to be an allele of the gene *her-1* (TRENT 1982): closely linked, *cis*-dominant suppressors of *n695* fail to complement previously defined recessive alleles of *her-1*, which cause X0 animals to be phenotypic hermaphrodites (HODGKIN 1980). *her-1(n695)*X0 animals are phenotypically wild-type males when viewed with the dissecting microscope and, in mating assays, produced about the same number of cross-progeny as did wild-type males.

The HSN cell bodies are absent in *her-1(n695)* hermaphrodites (H. ELLIS, personal communication). With respect to drug responses, there are two classes of fertile egg-laying defective *her-1(n695)* hermaphrodites: (1) sensitive to serotonin but not to imipramine, and (2) resistant to both drugs. Presumably, animals in the latter class have abnormalities in the vulva and/or in the sex muscles in addition to the HSN defect. As in *egl-1* hermaphrodites, the lack of HSNs in *her-1(n695)* animals appears to result from cell death: in *ced-3; her-1(n695)*

animals the HSN cell bodies are present (H. ELLIS, personal communication). However, in contrast to the *ced-3; egl-1* hermaphrodites, not all *ced-3; her-1(n695)* animals are suppressed for the egg-laying defect. Furthermore, bloated *ced-3; her-1(n695)* hermaphrodites (*i.e.*, animals in which the egg-laying defect is not suppressed) fail to respond to serotonin. These observations are consistent with the hypothesis that some *her-1(n695)* animals have multiple defects in the egg-laying system, affecting the vulva and/or the sex muscles as well as the HSNs, and that restoring HSN function to those animals is not sufficient to restore egg-laying ability. The variable severity of the egg-laying defect (some animals release no progeny and others release many) is also consistent with variable abnormalities in multiple components of the egg-laying system.

In addition to the egg-laying defect, *egl-5(n486)* animals have other obvious behavioral abnormalities: (1) animals tend to assume coiled postures, move forward fairly well when the Petri dish is tapped, but tend to coil when moving backward; and (2) males do not mate. Three additional *egl-5* mutations, recently identified by C. DESAI (personal communication), confer similar egg-laying defective, uncoordinated phenotypes. The HSN cell bodies are absent from their normal positions in *egl-5(n486)* animals. Double mutant *egl-5(n486); ced-3* animals are phenotypically similar to *egl-5(n486)* animals: in contrast to *ced-3; egl-1* and *ced-3; her-1(n695)* animals, the HSN cell bodies are not restored in the *egl-5(n486); ced-3* double mutant, and the egg-laying defect is not suppressed. *egl-5(n486)* males have gross morphological abnormalities of the tail.

egl-10(n692) animals fall into two classes: sensitive to serotonin but not to imipramine and sensitive to both drugs. A recently isolated *egl-10* allele, *n944*, results in drug responses similar to those of *egl-10(n692)* animals (C. DESAI, personal communication). Both *egl-10* mutants are slightly uncoordinated (they appear somewhat sluggish). *n692* and *n944* fail to complement the mutation *egl-10(n480)*. *egl-10(n480)* animals respond well to both serotonin and imipramine (see category C). The HSN cell bodies are present in *egl-10(n692)* animals. *egl-10(n692)* males appear phenotypically wild type when viewed with the dissecting microscope and in mating assays produced about 60% of the number of cross-progeny as did wild-type males.

Category C: mutants sensitive to serotonin and to imipramine: This category includes mutants defective in seven *egl* genes and four dauer-constitutive (*daf*) genes and *egl-30(n715)/+* heterozygotes. All four *daf* genes have temperature-sensitive (*ts*) alleles: at 25°, dauer larvae are formed even in the presence of a food supply (RIDDLE, SWANSON and ALBERT 1981), and those animals that do not become dauer larvae become egg-laying defective adults; at 15° few dauer larvae are formed, and (with the exception of *daf-1*) the animals develop into egg-laying defective adults. At least some alleles of all of the seven *egl* genes in this category are also temperature sensitive.

Since dauer-constitutive mutants respond to serotonin and to imipramine, some *egl* mutants with these drug responses may be similar in other ways to the *daf* mutants. RIDDLE, SWANSON and ALBERT (1981) have shown that dauer larva formation is suppressed in certain double mutants containing both dauer-constitutive and dauer-defective mutations (dauer-defective mutants fail to form

dauer larvae in the absence of a food supply). In addition, we have observed that the dauer-defective mutation *daf-3(e1376)* suppresses the egg-laying defect of (as well as dauer formation by) the dauer-constitutive mutants *daf-7* and *daf-14, i.e., daf-7; daf-3* and *daf-14; daf-3* strains are neither egg-laying defective nor dauer constitutive. This observation suggests that both phenotypes of the dauer-constitutive mutants result from the same physiological lesion. If an *egl* mutant were defective in the same component(s) or pathway(s) as the dauer-constitutive mutants, then the Egl phenotype might be suppressed by certain dauer-defective mutations. Double mutants were constructed between *daf-3*, which suppresses most known dauer-constitutive mutants (RIDDLE, SWANSON and ALBERT 1981; D. RIDDLE, personal communication), and four *egl* mutations from category C: *egl-3, egl-7, egl-35* and *egl-40*. None of these category C *egl* mutations was suppressed by *daf-3*. However, as described under category E, the egg-laying defects of mutants in two category E *egl* genes were suppressed by *daf-3*.

egl-10(n480) animals differ phenotypically from *egl-10(n692)* animals, which are in category B: *n480* animals have a less severe egg-laying defect and are not uncoordinated. *egl-30(n715)/+* heterozygotes release all of their progeny as late eggs and exhibit slow locomotion.

Category D: mutant(s) resistant to serotonin and sensitive to imipramine: This category is defined by the dominant mutant *egl-2*. *egl-2* animals appear to be hypersensitive to imipramine: bloated animals respond more quickly than do wild-type animals or bloated animals of other *egl* genotypes (data not shown). Both bloated and unbloated *egl-2* animals are sensitive to imipramine, resistant to serotonin and variably responsive to phentolamine; unbloated animals fail to respond to levamisole, whereas bloated animals respond well (data not shown). *egl-2* animals are slightly uncoordinated. Homozygous males mate with low efficiency and have variable morphological abnormalities of the tail.

A second mutant, *egl-19*, has been tentatively placed in this category. In contrast to *egl-2* animals, *egl-19* animals respond only weakly to imipramine.

Category E: variable or intermediate response to serotonin and to imipramine: Mutants defective in 16 *egl* genes, as well as a *daf-4* (dauer-constitutive) mutant, are included in this category. For some of these mutants the variable drug responses may reflect variation among individuals in the severity of the egg-laying defect. For example, the *egl-18* and *egl-27* mutations are clearly of variable expressivity: the degree of bloating varies and/or the fraction of progeny released ranges from none to most. However, most mutants in this category do not show variable expressivity for their egg-laying defects. Alleles of eight *egl* genes in this category and of *daf-4* are temperature sensitive. *egl-21* has three alleles; one allele (*n476*) fails to complement *daf-14* for the Egl and Daf phenotypes. It is possible that the *egl-21(n476)* strain carries two mutations, one in *egl-21* and one in *daf-14*.

Double mutants were constructed between *daf-3* and *egl-4(n478), egl-9, egl-12, egl-14, egl-20, egl-27, egl-28* and *egl-32* (see earlier discussion concerning category C mutants). Mutations in two genes, *egl-4* and *egl-32*, were suppressed by *daf-3*. *egl-4(n478)* was essentially fully suppressed at 20° and 15° and partially sup-

pressed at 25°. [*egl-4(n478)* animals are egg-laying defective at all three temperatures.] A second allele of this gene, n579, is also suppressed by *daf-3*. The egg-laying defect of *egl-32* animals is fully suppressed at 20° and 15° and partially suppressed at 25° in the *egl-32; daf-3* double mutant. (*egl-32* is weakly temperature sensitive: the egg-laying defect at 15° and 20° is less severe than at 25°.) In addition, the egg-laying defect of *egl-32* animals is also suppressed by the dauer-defective mutation *daf-5*: at 20° and 15° the double mutant *egl-32; daf-5* exhibits essentially wild-type egg-laying behavior. [The egg-laying defect of *egl-4(n478)* animals is only weakly suppressed by *daf-5*.] Thus, the phenotypes of the double mutants *egl-4; daf-3*, *egl-32; daf-3* and *egl-32; daf-5* suggest that these *egl* mutations and the dauer-constitutive mutations disrupt the functioning of the egg-laying system in similar ways.

DISCUSSION

We have isolated 145 mutants defective in egg laying and have characterized 59 of these genetically, behaviorally and pharmacologically. These 59 mutants have defined 40 new genes, which we have named *egl*, for egg-laying defective. Most of the other mutants are defective in previously identified genes. The total number of genes with alleles that affect egg laying is now 83. Since multiple alleles exist for only 12 of the 40 *egl* genes, it is clear that other such genes remain to be identified. The *egl* genes are found on all six linkage groups and are distributed on the genetic map similarly to other genes that have been identified (RIDDLE and SWANSON 1982).

We have defined four distinct categories of egg-laying-defective mutants based on their responses to the pharmacological agents serotonin and imipramine, which stimulate egg laying by wild-type hermaphrodites. These drugs test the functioning of the vulva, the sex muscles and the HSN neurons. Mutants in category A show either no response or a generally poor response to serotonin and to imipramine. Those mutants that show no response to these drugs generally exhibit severe defects in egg laying: they release few or no progeny and form bags of worms (in which the progeny have hatched internally). Most mutants with such severe defects in egg laying are in category A. The *egl* mutants that show a variable but generally poor response to the drugs are of variable expressivity with respect to the severity of their egg-laying defects, ranging from animals that release most of their progeny to animals that release none. Mutants in category B are stimulated to lay eggs by serotonin but not by imipramine. The mutants in category B generally exhibit weaker egg-laying-defective phenotypes than do mutants in category A: animals release some or most of their progeny but at an abnormally late stage of (progeny) development. Mutants in category C respond to serotonin and to imipramine; the egg-laying-defective phenotypes of these mutants are similar to those of mutants in category B. Category D is defined by the *egl-2* mutant, which does not respond to serotonin but responds well (and may be hypersensitive) to imipramine. *egl-2* animals show relatively severe defects in egg laying: they lay some (or occasionally no) progeny and form bags of worms. Those mutants that do not clearly belong in one of the categories A–D, such as mutants that exhibit

intermediate or variable responses to serotonin and imipramine, constitute a fifth category, E. A few category E mutants show obvious variable expressivity of the egg-laying defect; most mutants in category E show egg-laying-defective phenotypes similar to those of mutants in categories B and C.

Based on the drug responses of animals with defined lesions in the egg-laying system, predictions about the nature of the defects can be made for *egl* mutants in categories A and B. Vulvaless mutants and wild-type hermaphrodites in which the vulval and uterine muscles have been eliminated with a laser microbeam fail to respond to serotonin and to imipramine. Therefore, some mutants in category A may have lost the functioning of the vulva or of the sex muscles. Consistent with this prediction, preliminary characterization of some of the mutants in this category has shown that two have defects in sex muscle development and four have abnormal vulval morphology. Animals in which the HSNs have been ablated with a laser microbeam as well as *unc-86* animals, which are HSN defective (SULSTON and HORVITZ 1981), respond to serotonin but not to imipramine; therefore, mutants in category B are likely to have lost HSN function. Consistent with this prediction, three of four have obvious HSN abnormalities: the HSN cell bodies are absent in *egl-1* and *her-1(n695)* animals and are absent at least from their normal positions in *egl-5* animals.

The drug responses of mutants in categories C, D and E do not correspond to the responses of animals with lesions produced by elimination of any of the known components (neurons, sex muscles or vulva) of the egg-laying system (R. HORVITZ and J. SULSTON, unpublished observations). Therefore, the phenotypes of mutants in these categories may result from (1) loss of function (or altered function) of an unknown component of the egg-laying system, (2) altered function of a known component, or (3) partial or variable loss of function of a known component. For example, as we discuss later, some of the mutants in these categories (*e.g., egl-4, egl-32* and the dauer-constitutive mutants) may have lesions in as yet undefined neural components of the egg-laying system involved in modulation of the rate of egg laying in response to the presence of bacteria. The unusual phenotype of *egl-2* animals (category D) could result from altered function of a known component of the egg-laying system (*e.g.*, an alteration in the sex muscles resulting in a hypersensitivity or loss of sensitivity to a particular drug or neurotransmitter). Alternatively, the *egl-2* mutant may define a new component of the egg-laying system. It is unlikely that the phenotype of *egl-2* animals results from a partial loss of function of a known component: partial sex muscle or vulva function would probably result in responses ranging from weak to wild-type for both drugs, and partial HSN function would probably result in weak to wild-type responses to imipramine and a wild-type response to serotonin. In contrast, the phenotypes of some of the mutants in categories C and E may well result from partial loss of function of the vulva or the vulval and uterine muscles: mutants in these categories have variable or wild-type responses to serotonin and imipramine, and the egg-laying defects of these mutants are generally less severe than those of mutants in category A. For example, *egl-30(n715)*, which is in category A, is paralyzed and essentially never releases any progeny (and may have defects in the functioning of the body wall

and sex muscles); animals carrying a different allele of this gene, *egl-30(n686)*, as well as *egl-30(n715)/+* heterozygotes, show less severe defects in locomotion and in egg laying and are in category E and category C, respectively. Other mutants in categories C and E may result from partial loss of HSN function. For example, *egl-10(n480)* animals show a category C response, but individual *egl-10(n692)* animals show either a category B response or a category C response; furthermore, C. DESAI (personal communication) has recently isolated new alleles of *egl-10*, one of which *(n953)* results in animals with a category B response. It is possible that the phenotypes of *egl-10(n480)* animals and of category C *egl-10(n692)* animals result from partial loss of HSN function, whereas the phenotypes of *egl-10(n953)* animals and category B *egl-10(n692)* animals result from complete loss of (or at least a more severe reduction in) HSN function.

The morphological and behavioral pleiotropic effects of a mutation can indicate the specificity with which a gene functions. For example, are there genes that are necessary only for the development or functioning of a single neuron or type of neuron involved in egg laying? Similarly, are there genes that are necessary for the development or functioning of neurons involved in egg laying as well as for the development or functioning of specific other neurons involved in other behaviors? Furthermore, pleiotropic effects that result from a single defect in the nervous system can be used to define the specificity with which particular neurons function: some neurons may function only in egg laying (e.g., the HSNs), but, as we discuss later, others may have multiple roles and control other behaviors or aspects of development (such as dauer larva formation).

A common pleiotropic effect of the *egl* mutations is uncoordination: mutations in 13 of the 40 *egl* genes also result in defects in locomotion. Furthermore, mutations in 19 of the 94 *unc* genes (*unc*oordinated locomotion) result in gross defects in egg laying. The defects in locomotion of the *egl* mutants are of two general classes. Some (such as *egl-22* or *egl-30*) resemble mutants defective in muscle structure, which move slowly or are paralyzed (BRENNER 1974; EPSTEIN, WATERSTON and BRENNER 1974). The egg-laying defects of these mutants may reflect lesions in the sex muscles, possibly resulting from defects in gene products that function in both the body wall muscles and the sex muscles. Other *egl* mutants (such as *egl-3, egl-5, egl-21, egl-31, egl-33* and *egl-38*) do not resemble muscle mutants, e.g., the *egl-31* mutant is backward uncoordinated, the *egl-33* mutant is a kinker and the *egl-5* mutant is a coiler. These types of abnormalities in locomotion are likely to result from nervous system defects: (1) lesions in the ventral nervous system cause abnormalities in backward locomotion (SULSTON and HORVITZ 1981); (2) levamisole-resistant mutants (such as *unc-29* or *lev-1*), which appear to have defects in cholinergic neurotransmission (LEWIS *et al.* 1980b), are kinkers; and (3) studies of genetic mosaics have indicated that the coiler phenotype of the *unc-3* mutant probably results from a lesion in the nervous system (R. HERMAN, personal communication). The egg-laying defects of some of these *egl* mutants may result from lesions in the nervous system, and for at least one mutant, *egl-5* (which is defective in the

HSNs), it is clear that this is the case. The pleiotropic effects of such mutations could reflect defects in a component (*e.g.*, a specific neuron) common to the egg-laying and locomotory systems or they could reflect abnormalities in multiple components of the nervous system, *i.e.*, in neurons that function specifically in egg laying as well as in neurons that function specifically in locomotion. *egl-5* mutants, for example, presumably are defective in multiple types of neurons, since the egg-laying defect results from a loss of HSN function, which is known not to affect locomotion (*e.g.*, *egl-1* animals, which lack HSNs, exhibit normal locomotion). Alternatively, the egg-laying defects of these mutants need not be related to the nervous system. For example, *egl-38* animals are defective in the morphology of the vulva, and *egl-31* animals are defective in development of the sex muscles.

Some of the egg-laying-defective mutants have been examined for pleiotropies with respect to male morphology or behavior. The males of 11 of the 14 *egl* mutants examined appear phenotypically wild type (as seen with the dissecting microscope) and are capable of mating. However, *egl-5* and *egl-27* males have gross morphological abnormalities of the tail and do not mate, and *egl-2* males exhibit morphological abnormalities of the tail and mate with a low efficiency. It is of interest to determine whether mutations that perturb the development or functioning of neural components of the egg-laying system in hermaphrodites affect the nervous system in males. Three mutants [*egl-1*, *egl-5* and *her-1(n695)*] are egg-laying defective because the cell bodies of the HSNs are absent, and, by pharmacological criteria, a fourth mutant, *egl-10(n692)*, also appears to be defective in the HSNs. Males of these mutant strains were tested for mating ability. In contrast to *egl-5* males, *egl-1(n487)*, *egl-10(n692)* and *her-1(n695)* X0 males appear phenotypically wild type and are capable of mating. *egl-1* and *her-1(n695)* X0 males appear to mate with the efficiency of wild-type males, but *egl-10(n692)* males mate with slightly reduced efficiency.

The egg-laying-defective mutant with the most striking morphological pleiotropies carries a novel, dominant allele of *her-1*, *n695*. The *her-1(n695)* mutation causes the variable transformation of XX animals into abnormal males. In contrast, the previously identified recessive alleles of *her-1* transform X0 animals into phenotypic hermaphrodites (HODGKIN 1980). Like wild-type males, *her-1(n695)* XX animals lack the cell bodies of the HSN neurons. In wild-type males, the cell homologous to the HSNs undergo programmed cell death (SULSTON *et al.* 1983). Consistent with the observation that some *her-1(n695)* XX animals exhibit an obvious sexually transformed phenotype (*i.e.*, have a male body shape and size and a male-like tail), the lack of HSN cell bodies in *her-1(n695)* XX animals seems likely to result from expression of the normally male-specific program of HSN cell death. In support of this hypothesis, we have observed that mutations in the gene *ced-3* suppress the HSN defect (*ced-3* mutations result in the survival of cells that normally undergo programmed cell death; HORVITZ, ELLIS and STERNBERG 1982): in a *ced-3; her-1(n695)* double mutant, the HSN cell bodies are restored.

Mutations in the gene *tra-2* also affect sex determination (HODGKIN and BRENNER 1977). XX homozygotes for most *tra-2* alleles (*e.g.*, *n196* and *e1425*) are

phenotypically male. We have observed that heterozygotes for such alleles as well as homozygotes for a weaker allele, *e1875* (J. HODGKIN, personal communication), are hermaphrodites that exhibit a low penetrance egg-laying defect. Since these egg-laying-defective *tra-2* or *tra-2/+* hermaphrodites respond better to serotonin than to imipramine, they appear to have lost HSN function (C. TRENT, unpublished results). Furthermore, some *tra-2(n196)/+* heterozygotes lack HSN cell bodies. In addition, both the HSN and egg-laying defects of *tra-2(n196)/+* heterozygotes are suppressed in a double mutant with *ced-3*. The phenotypes of *her-1(n695)* and *tra-2(e1875)* animals and *tra-2/+* heterozygotes suggest that HSN development is a particularly sensitive indicator of sexual transformation: many of the XX animals of these genotypes appear to be hermaphrodites that express the normally male-specific program of HSN cell death.

Since we were primarily interested in isolating and characterizing mutations affecting the neural circuitry responsible for egg laying, we have focused some attention on mutants that, by pharmacological criteria, are likely to have defects in the HSNs. We have only begun to examine how genes control the development and functioning of the HSNs, but already it appears that some such genes may function only in HSN development (e.g., *egl-1*), whereas others clearly control many other developmental events involving both neural and non-neural tissues (e.g., *her-1, tra-2*). As in *her-1(n695)* and *tra-2* hermaphrodites, the lack of HSN cell bodies in *egl-1* hermaphrodites probably results from the expression of the normally male-specific program of HSN cell death: in *ced-3; egl-1* double mutant animals, both the ability to lay eggs normally and the presence of the HSN cell bodies are restored. *egl-1* hermaphrodites have no other obvious abnormalities. Thus, in contrast to the *her-1* and *tra-2* genes, which have central roles in sex determination (HODGKIN and BRENNER 1977; HODGKIN 1980), the *egl-1* gene may affect the expression of only one of the developmental programs involved in the differentiation of sexual phenotype and may define one target of the general sex-determining genes. Other genes involved in the development or functioning of the HSNs may also have roles in the development or functioning of other neurons. For example, *unc-86* mutations affect the development of multiple types of neurons including the HSNs, the microtubule cells and a number of dopaminergic neurons (SULSTON and HORVITZ 1981; CHALFIE, HORVITZ and SULSTON 1981); as discussed earlier, *egl-5* mutations probably affect the development or functioning of neurons involved in locomotion as well as the HSNs. Continued screening for HSN-defective mutants, based on a pharmacological assay of HSN function, may identify additional genes involved in the development or functioning of the HSNs. Such mutants should include animals that are weakly transformed in their sexual phenotype. Further analysis of HSN-defective mutants could provide insight into how genes specify and control the development of both a specific class of neuron and a specific synapse.

In addition to the mutants discussed, other egg-laying-defective mutants are likely to have lesions in the neural circuitry controlling egg laying. For example, two mutants [*daf-1(n690), daf-7(n696)*] that were isolated as egg-laying defective form dauer larvae constitutively, and many of the mutants isolated by others as

constitutive for dauer larva formation (RIDDLE, SWANSON and ALBERT 1981) are also defective in egg laying; the dauer-constitutive phenotype of these mutants appears to result from abnormalities in head neurons involved in the processing of environmental signals that modulate dauer larva formation (RIDDLE, SWANSON and ALBERT 1981; ALBERT, BROWN and RIDDLE 1981). Since the presence of bacteria in the environment normally inhibits dauer larva formation (CASSADA and RUSSELL 1975) and stimulates egg laying (TRENT 1982), these mutants may be abnormal in neurons that modulate both egg laying and dauer formation in response to the presence of a food supply. No head neurons provide either direct or indirect synaptic inputs to the vulval and uterine muscles of the egg-laying system (J. WHITE and R. HORVITZ, unpublished observations), which suggests that these dauer-constitutive egg-laying-defective mutants may be abnormal in neurons involved in a humoral regulation of egg laying. The further analysis of these mutants (as well as of *egl-4* and *egl-32* mutants, which, as discussed in RESULTS, are likely to have defects similar to those of the dauer-constitutive mutants), may identify the neurons involved as well as help reveal how these neurons function to control both egg laying and dauer larva formation.

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Corresponding editor: R. K. HERMAN