Mutations Affecting the Chemosensory Neurons of *Caenorhabditis elegans*

Todd A. Starich,* Robert K. Herman,* Claire K. Kari,* Wen-Hui Yeh,† Wendy S. Schackwitz,‡ Marcia W. Schuyler,* Joan Collet,* James H. Thomas‡ and Donald L. Riddle†

**Department of Genetics and Cell Biology, University of Minnesota, St. Paul, Minnesota 55108, †Division of Biological Sciences, University of Missouri, Columbia, Missouri 65211 and ‡Department of Genetics, University of Washington, Seattle, Washington 98195*

Manuscript received August 11, 1994
Accepted for publication September 27, 1994

ABSTRACT

We have identified and characterized 95 mutations that reduce or abolish dye filling of amphid and phasmid neurons and that have little effect on viability, fertility or movement. Twenty-seven mutations occurred spontaneously in strains with a high frequency of transposon insertion. Sixty-eight were isolated after treatment with EMS. All of the mutations result in defects in one or more chemosensory responses, such as chemotaxis to ammonium chloride or formation of dauer larvae under conditions of starvation and overcrowding. Seventy-five of the mutations are alleles of 12 previously defined genes, mutations which were previously shown to lead to defects in amphid ultrastructure. We have assigned 20 mutations to 13 new genes, called *dyf-1* through *dyf-13*. We expect that the genes represented by dye-filling defective mutants are important for the differentiation of amphid and phasmid chemosensilla.

THE nematode *Caenorhabditis elegans* is proficient at several behaviors that depend on chemosensory function (for reviews, see CHALFIE and WHITE 1988; BARGMANN 1993). For example, *C. elegans* worms are attracted to many water-soluble compounds, such as various salts, amino acids and cyclic nucleotides (WARD 1973; DUSENBERY 1974; BARGMANN and HORVITZ 1991a). Other compounds, repellents, are actively avoided (DUSENBERY 1975; BARGMANN *et al.* 1990). High concentrations (high osmolarity) of water-soluble compounds, which may be attractants at lower concentration, are also avoided by wild-type animals (CULOTTI and RUSSELL 1978). More recently it was shown that certain volatile organic molecules are attractants (BARGMANN *et al.* 1993).

Another chemosensory function is important for *C. elegans* larval development. In the presence of plentiful food and at low population density, newly hatched animals progress quickly to adulthood through four larval stages, L1–L4. Limited food and high population density, however, favor the formation of dauer larvae, which are alternative to the L3 and are specialized for dispersal and long-term survival (for reviews, see RIDDLE 1988; THOMAS 1993). Under favorable growth conditions, dauer larvae resume development, progressing to the fourth larval stage and then adulthood. The decision to form a dauer larva is influenced by temperature, food supply and a constitutively secreted, dauer-inducing pheromone that apparently serves as a measure of population density (GOLDEN and RIDDLE 1984a).

Corresponding author: Robert K. Herman, Department of Genetics and Cell Biology, University of Minnesota, 250 BioScience Center, 1445 Gortner Ave., St. Paul, MN 55108.
E-mail: bob-h molbio.cbs.umn.edu

The ultrastructure of the *C. elegans* nervous system has been reconstructed in its entirety from serial section electron micrographs (WARD *et al.* 1975; WARE *et al.* 1975; ALBERTSON and THOMSON 1976; WHITE *et al.* 1976, 1986; HALL and RUSSELL 1991). Of the 302 neurons in the nervous system, 60 have dendritic endings that are ciliated and are therefore likely to be sensory (WHITE *et al.* 1986). All of these sensory cilia are nonmotile. Most of the ciliated neurons are components of small sense organs or sensilla. Each sensillum consists of one or more ciliated nerve endings and two or three nonneuronal support cells, a sheath cell and one or two socket cells. The support cells provide a cylindrical channel that surrounds the dendritic endings of the neurons of the sensillum. Among the 60 ciliated neurons, 26 have endings located within channels that are open to the external environment; it has therefore been suggested that these neurons may be chemosensory and that the other ciliated neurons may be mechanosensory. The mechanosensory function of an additional set of neurons, the nonciliated touch cells, has been established by CHALFIE and SULSTON (1981). The 26 exposed neurons belong to three classes of sensilla. Eight pairs are members of the bilateral amphids in the head, two pairs belong to the bilateral phasmids in the tail and one is found in each of the six inner labial sensilla in the head.

The functions of many of the putative sensory neurons have been tested by analyzing the behavioral consequences of killing them with a laser microbeam. One pair of amphid channel neurons contributes in a major way and another four pairs contribute redundantly to the ability to chemotax to water-soluble attractants (BARGMANN and HORVITZ 1991a). Two additional pairs of amphid channel neurons have been implicated by laser killing studies in the avoid-

ance of repellents, including high osmolarity and garlic (BARGMANN *et al.* 1990). Chemotaxis to various volatile odorants appears not to require exposed neurons; instead, two pairs of nonchannel amphid neurons have been implicated (BARGMANN *et al.* 1993). Finally, four pairs of amphid channel neurons appear to be important in regulating dauer larva formation. When these neurons are killed in L1 larvae, the animals subsequently become dauer larvae regardless of environmental conditions (BARGMANN and HORVITZ 1991b). Recovery from the dauer stage requires the function of one pair of these amphid channel neurons. In sum, chemosensory functions have been identified for 10 pairs of amphid neurons, including all eight amphid channel neurons. No functions have as yet been identified for the other 10 exposed ciliated neurons: 6 in the inner labial sensilla and two pairs in the phasmids.

With the long-term aim of elucidating the genetic specification of nervous system development, structure and function, many *C. elegans* mutants defective in chemotaxis (LEWIS and HODGKIN 1977), avoidance of high osmolarity (CULOTTI and RUSSELL 1978) or dauer larva formation (ALBERT *et al.* 1981) have been identified. PERKINS *et al.* (1986) showed that many of these mutants are also abnormal in the filling of certain amphid and phasmid channel neurons with the fluorescent dye 5-fluorescein isothiocyanate (FITC). When living wild-type animals are exposed to a solution of FITC, six of the eight pairs of amphid channel neurons and both pairs of phasmid neurons fill with dye, thereby making the neuron processes and cell bodies readily visualizable by fluorescence microscopy (HEDGECOCK *et al.* 1985). PERKINS *et al.* (1986) showed that 28 mutations in 16 genes reduce or abolish dye filling of amphid and phasmid neurons and simultaneously lead to defects in chemotaxis, avoidance of high osmolarity or dauer formation.

Mutations that reduce dye filling of amphid and phasmid neurons, a phenotype we refer to as Dyf, appear to be indicative of defects in the chemosensory sensilla themselves, rather than functions that act downstream of chemosensory transduction. Mutations in each of the 16 genes that affect dye filling have been shown to cause defects in amphid ultrastructure (LEWIS and HODGKIN 1977; ALBERT *et al.* 1981; PERKINS *et al.* 1986). The structures of wild-type amphids and phasmids are shown diagrammatically in Figure 1. Each amphid consists of 12 ciliated neurons plus a sheath cell and a socket cell (WARD *et al.* 1975; WARE *et al.* 1975). The dendrites of all 12 neurons run together anteriorly as a lateral bundle. The socket cell is connected to the hypodermis by belt junctions and is lined with cuticle that is continuous with the external cuticle. Just posterior and attached to the socket is the sheath. The four nonchannel neurons leave the channel proximally and invaginate the sheath. Each of the 12 neurons is bipolar, with its axon extending into the nerve ring, the major neural integrative center (WHITE *et al.* 1986). The phasmid neurons are also bipolar, and their axonal processes extend anteriorly into the preanal ganglion (WHITE *et al.* 1986; HALL and RUSSELL 1991). Each phasmid has two ciliated neurons that run posteriorly into a channel formed by a sheath cell and either one (at hatching) or two (postembryonic) socket cells (SULSTON *et al.* 1980; HALL and RUSSELL 1991). Mutations in 11 genes that confer a Dyf phenotype are known to affect the structure of the sensory cilia of all amphid neurons (LEWIS and HODGKIN 1977; ALBERT *et al.* 1981; PERKINS *et al.* 1986). In most cases, all of the neuronal cilia seem to be affected. Mutations in three other genes that yield Dyf phenotypes (*che-12*, *che-14*, and *daf-6*) affect the structure of the socket and sheath cells (ALBERT *et al.* 1981; PERKINS *et al.* 1986). Mutations in the final two genes (*mec-1* and *mec-8*) appear to affect the bundling together of the anteriorly directed amphid neuronal processes (LEWIS and HODGKIN 1977; CHALFIE and SULSTON 1981; PERKINS *et al.* 1986).

In the work reported here, we have identified and genetically characterized 95 new mutations that confer a Dyf phenotype. Many of the mutants were initially recovered on the basis of an abnormality in chemosensory behavior, although others were identified solely on the basis of the Dyf phenotype. In general, the mutants displayed no other phenotypic abnormalities. Mobility, response to light touch, viability and fertility were essentially unaffected. Many of the mutations are new alleles of 12 already known genes, but other mutations define 13 new genes, which we have named *dyf-1* through *dyf-13*. Mutations in each of these genes lead to defects in chemosensory behavior. We predict that the *dyf* genes provide essential differentiation functions specific to sensilla.

MATERIALS AND METHODS

Growth media and culture and mating techniques were as described by SULSTON and HODGKIN (1988). Nematode strains were grown and mated at 20°.

Genes, alleles, rearrangements and strains: Mutations affecting dye filling that were identified and characterized in this work are listed in Table 1. Other genes and alleles used are listed below. The relative map positions of many of these markers are given in Figure 2; more detailed descriptions of the marker loci are given by HODGKIN *et al.* (1988).

LG (linkage group) I: *unc-74(x19)*, *dpy-5(e61)*, *bli-4(e947)*, *dpy-14(e188)*, *unc-13(e51, e1091)* (the *e51* allele was used unless otherwise noted), *caf-2(hf5)*, *daf-8(e1393)*, *unc-29(e193)*, *srf-2(yj262)*, *dpy-24(s71)*, *lin-11(n566)*, *unc-75(e950)*.

LG II: *unc-85(e1414)*, *dpy-10(e128)*, *unc-104(e1265)*, *rol-6(e187)*, *unc-4(e120)*, *unc-52(e444)*.

LG III: *unc-32(e189)*, *vab-7(e1562)*, *dpy-18(e364)*, *unc-25(e156)*, *bli-5(e518)*, *unc-64(e246)*.

LG IV: *dpy-9(e12)*, *unc-33(e204)*, *unc-17(e245)*, *caf-1(hf3)*, *dpy-13(e184)*, *unc-5(e53)*, *him-8(e1489)*, *unc-22(e66)*.

LG V: *unc-60(e667)*, *dpy-11(e224)*, *unc-23(e204)*, *unc-42(e270)*, *daf-11(m87)*, *sma-1(e30)*, *vab-8(e1017)*, *him-5(e1490)*, *unc-76(e911)*, *srf-4(ct109)*, *unc-51(e369)*, *rol-9(sc148)*.

LG X: *unc-1(e538)*, *daf-3(e1376)*, *dpy-3(e182)*, *unc-2(e55)*, *unc-20(e112)*, *lon-2(e678)*, *dpy-8(e130)*, *unc-6(e78)*, *dpy-7(e88)*, *dpy-6(e14)*, *unc-27(e155)*, *daf-12(m20)*, *vab-3(e648)*,

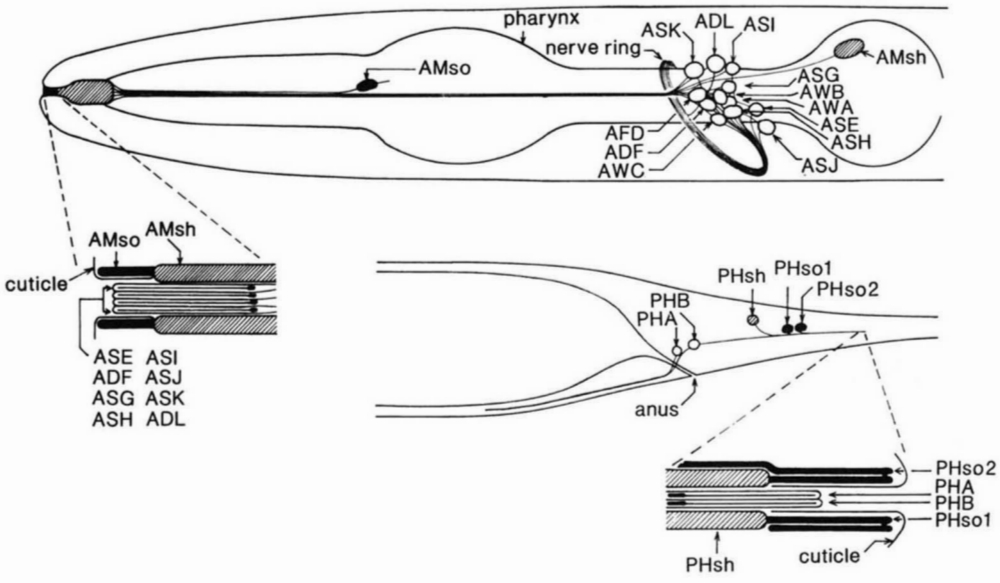

FIGURE 1.—Diagrams showing an amphid (above) and a phasmid (below), based on WARD *et al.* (1975), WARE *et al.* (1975), SULSTON *et al.* (1980, 1983), WHITE *et al.* (1986), PERKINS *et al.* (1986) and HALL and RUSSELL (1991). Anterior is to the left, and dorsal is up. AMsh, amphid sheath; AMso; amphid socket, PHsh, phasmid sheath; PHso, phasmid socket. Neurons that fill with FITC are ASK, ADL, ASI, ADF, ASH, ASJ, PHA and PHB (HEDGECOCK *et al.* 1985).

egl-15(n484), *lin-14(n179*ts*)*, *unc-9(e101)*, *srf-5(ct115)*, *unc-3(e151)*.

The deficiencies and duplications used are all listed in Table 4. The translocation-balancers *szT1(I;X)* and *eT1(III;V)*, the duplication-balancer *mnDp1(X;V)* and the chromosome balancer *mnC1(II)* were also used (HODGKIN *et al.* 1988).

PERKINS *et al.* (1986) identified mutations in 16 genes that reduced or abolished FITC filling of sensory neurons without strongly affecting animal movement. The reference alleles of these 16 genes that we used for complementation tests with our dye filling mutants were the following:

LG I: *che-14(e1960)*, *che-3(e1124)*, *mec-8(e398)*, *che-13(e1805)*.

LG II: *che-10(e1809)*, *daf-19(m86)*.

LG IV: *osm-3(p802)*, *daf-10(e1387)*.

LG V: *mec-1(e1066)*, *che-12(e1812)*, *osm-6(p811)*, *che-11(e1810)*.

LG X: *che-2(e1033)*, *osm-5(p813)*, *daf-6(e1377)*, *osm-1(p808)*.

Genetic nomenclature is described by HORVITZ *et al.* (1979). Unless otherwise noted, all strains are descended from the wild-type strain N2 (BRENNER 1974). Exceptions are dye-filling mutants derived from RW7097 (a derivative of RW7096, MORI *et al.* 1988) or from TR679 (COLLINS *et al.* 1987).

Assays for dye filling: Staining of living animals with FITC was carried out on plates by the method of HEDGECOCK *et al.* (1985). An alternative procedure made use of the lipophilic carbocyanine dye DiO (3,3′-dioctadecyloxacarbocyanine perchlorate; Molecular Probes). For staining with DiO, we followed the procedure described by HERMAN and HEDGECOCK (1990).

Identification of Dyf mutants: Mutants were identified in several different screens, as shown in Table 1, which gives the origin of each mutation that confers a dye-filling defect. Spontaneous mutants were recovered from the mutator strains RW7097 and TR679. In other screens, the mutagen EMS was used, according to the procedure described by SULSTON and HODGKIN (1988).

In some screens, mutants defective in the formation of dauer larvae upon starvation were first identified. In one version of this screen, N2 adults were treated with EMS, F_2 progeny were put singly onto standard growth plates, their progeny were allowed to exhaust the food supply and the plates were later inspected for the absence of dauer larvae (ALBERT *et al.* 1981). In a variant of this screen, the F_2 progeny of EMS-treated animals were placed individually into microtiter wells (Costar 96-well dishes) containing 50 μl of a 1% (w/w) suspension of *Escherichia coli* strain OP50 in S medium (GOLDEN and RIDDLE 1985). After 7–10 days of incubation at 25°, the starved populations in the wells were screened visually for the absence of dauer larvae. Candidate mutant hermaphrodites were crossed with *dpy-13(e184*sd*)/+* males, and both Dpy and non-Dpy segregants from semi-Dpy hermaphrodite cross progeny were placed in liquid culture medium containing exogenous partially purified pheromone (GOLDEN and RIDDLE 1984b). After 3–4 days, both Dpy and non-Dpy segregants that grew in pheromone were placed singly on plates to retest the dauer-defective phenotype. Approximately half of the mutants identified as defective in dauer formation upon starvation proved to be defective in dye filling. Three mutations (*e1383*, *e1384* and *m220*) were recovered from the screen involving starvation on plates, and 18 mutations (*m123*, *m126*, *m127*, *m128*, *m163*, *m175*, *m176*, *m177*, *m185*, *m186*, *m194*, *m195*, *m197*, *m198*, *m201*, *m202*, *m205* and *m226*) were derived from the procedure involving starvation in liquid.

We also selected for mutants able to grow in concentrations of pheromone (plus food) that promote dauer formation in wild-type animals. The pheromone solution was prepared as described by GOLDEN and RIDDLE (1984b) and stored as a 100× stock solution. L1 larvae hatched from alkaline hypochlorite-treated eggs (SULSTON and HODGKIN 1988) were suspended in 0.25% *E. coli* strain c1666 (from R. CURTISS III) and 2.5× pheromone (2.5 times the concentration of biological activity present in depleted liquid culture media) and incubated with shaking for 3 days at 25°. Adults that grew in pheromone were plated singly at 25°, and the resulting populations

TABLE 1

Origins and gene assignments of mutations that confer defects in dye filling

Gene	Previously identified mutations[a]	RW7097 Daf	TR679 Osm	EMS Daf	EMS Osm	EMS Dyf	EMS suppressor of *daf-11*	Total no. of alleles
che-2 X	*e1033*		*mn330*	*m127*		*mn395* *mn402*	*sa133*	6
che-3 I[b]	*e1124* *e1253* *e1379* *p801*	*m443* *m444* *m508* *m531* *m532* *m535* *m536*	*mn333* *mn334*	*m123* *m167* *m171* *m194* *m195* *m198* *m202* *m220*	*n1512*	*mn388* *mn394*	*sa129*	25
che-10 II	*e1809*	*m525*			*n1514* *n1519*	*mn403*		5
che-11 V	*e1810* *e1815*			*m162*		*mn387* *mn393* *mn404*		6
che-12 V	*e1812*					*mn389* *mn399*		3
che-13 I	*e1805*	*m523*			*n1520*			3
daf-6 X	*e1377*	*m506*		*m176* *m186* *m197* *m205*	*n1543*			7
daf-10 IV	*e1387* *m79* *p821*	*m534*	*mn321*	*e1384* *m126* *m163* *m226*		*mn401* *mn405*		11
osm-1 X	*a83* *e1803* *p808* *p816*	*m530* *m538*		*m128*		*mn390*	*sa118* *sa120* *sa123* *sa132*	12
osm-3 IV[c]	*e1806* *e1811* *p802*	*mn357*[d]			*n1540* *n1545*	*mn391*	*sa125* *sa131*	9
osm-5 X	*p813*			*m184*		*mn397* *mn425*	*sa126* *sa130*	6
osm-6 V	*p811*	*m511* *m533*		*m201*			*sa119*	5
dyf-1 I			*mn335*					1
dyf-2 III		*m543*		*m160*				2
dyf-3 IV			*mn331*	*m185*			*sa122*	3
dyf-4 V			*mn332*	*m158* *m177*				3
dyf-5 I						*mn400*		1
dyf-6 X			*mn346*	*m175*				2
dyf-7 X		*m537*						1
dyf-8 X		*m539*						1
dyf-9 V					*n1513*		*sa121*	2
dyf-10 I				*e1383*				1
dyf-11 X						*mn392*		1
dyf-12 X							*sa127*	1
dyf-13 II						*mn396*		1
Totals	23	19	8	27	8	19	14	118

[a] See PERKINS *et al.* (1986).
[b] We have shown that *caf-2(hf5) I* is Dyf and an allele of *che-3*. HARTMAN (1987) identified three alleles of *caf-2*.
[c] We have shown that *caf-1 (hf3) IV* is Dyf and an allele of *osm-3*. HARTMAN (1987) identified eight alleles of *caf-1*.
[d] Selected as Osm.

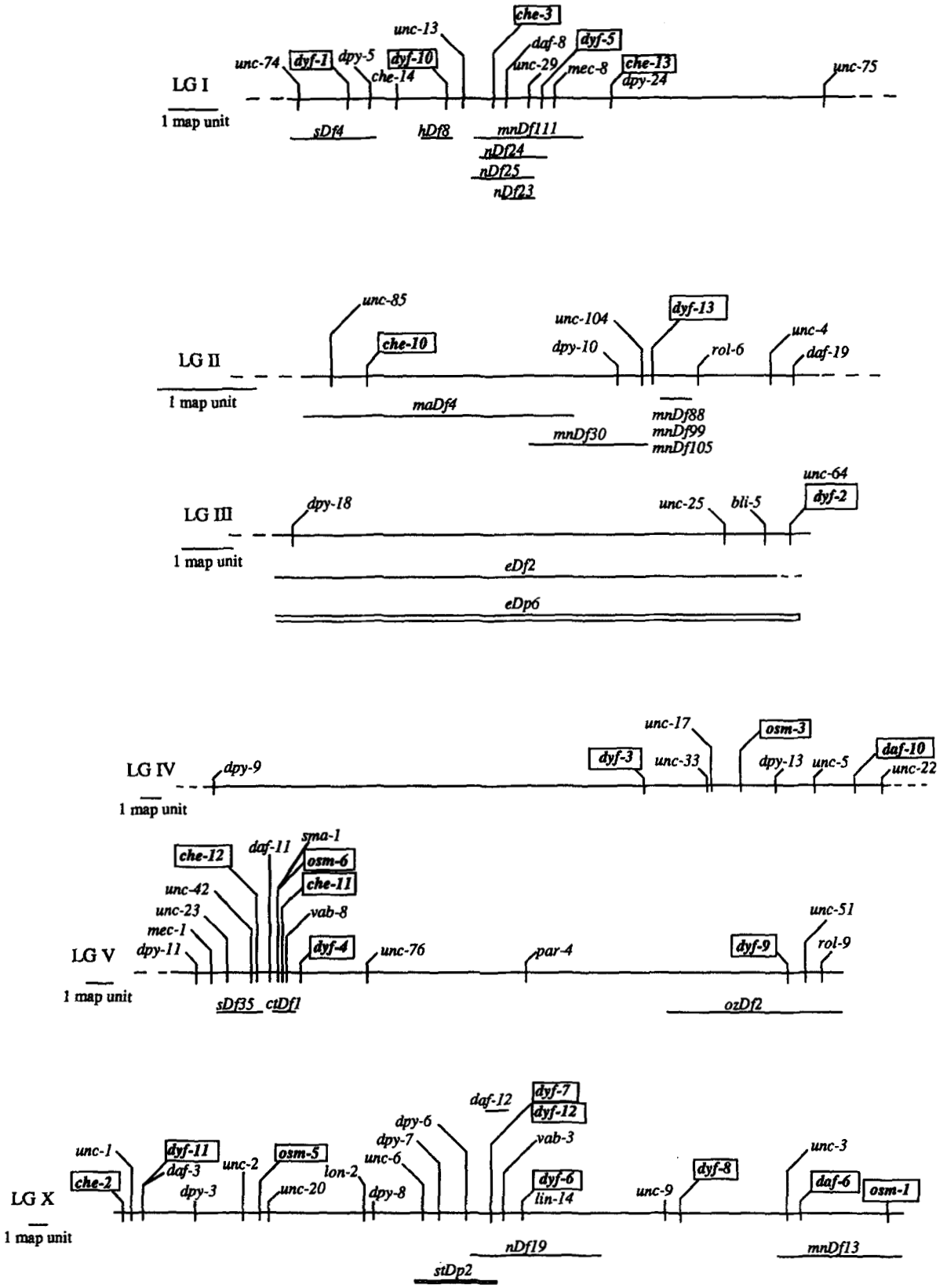

FIGURE 2.—Genetic linkage maps showing positions of the 25 genes (in bold letters and boxed) for which mutant alleles conferring a Dyf phenotype were identified in this work. Also shown are loci used in mapping, deficiencies and duplications. Note that the map scales, which are given on the left of each map, are different for different linkage groups.

were allowed to starve for observation of the dauer-defective phenotype. Mutant hermaphrodites were crossed to *dpy-13/+* males, and both Dpy and non-Dpy dauer-defective segregants were isolated as described above. This scheme yielded the following six EMS-induced mutations: *m158*, *m160*, *m162*, *m167*, *m171* and *m184*. Twenty-two dauer-defective mutants that arose spontaneously in RW7097 were also identified by this scheme, and 18 proved to be Dyf.

In other screens, the technique described by CULOTTI and RUSSELL (1978) was used to enrich for animals defective in the ability to avoid high osmotic pressure: animals that crossed an osmotic barrier made from 4 M fructose or 4 M NaCl were tested for dye filling. In another screen, *daf-11(m87)* animals were treated with EMS and grown at 15°. Mutation in *daf-11* leads to constitutive formation of dauer larvae at 25° (RIDDLE *et al.* 1981). The F_2 progeny of mutagenized animals were raised at 25° to identify animals in which the dauer-constitutive phenotype conferred by *daf-11(m87)* was suppressed (VOWELS and THOMAS 1992). Mutants defective in dye filling were then identified as a subclass (comprising 28%) of the suppressed mutants.

Finally, 19 EMS-induced mutants were identified in a direct screen for dye-filling defects, without prior enrichment for animals with a behavioral defect. The 19 mutants were found among 15,500 F_2 animals that were screened; 16 other potential mutants were sterile. The true incidence of mutants is likely to be significantly higher than we have measured because, lacking attraction to food, the chemosensory mutants tend to crawl off the agar surface and up the sides of the plastic growth plates, where they desiccate and die.

In all of the screens, care was taken to ensure independence of all mutations. For EMS-induced mutations, we saved only one descendant of each mutagenized parent. Independence of the spontaneous mutations was more difficult to ensure. Single animals from the mutator stocks were picked to initiate different lines; only one mutant per line was saved. This procedure ensured that rare mutants found in different lines were of independent origin.

Outcrossed lines were saved in the course of the genetic mapping of the mutations. The 20 mutations that were assigned to new loci were all crossed to N2 or its genetically marked derivatives at least twice before the behavioral and dye-filling assays reported in the RESULTS were performed. For each of the three mutations in this group that were identified as suppressors of *daf-11(m87)*, a double mutant involving the dye-filling-defective mutation and *unc-42*, which maps very near *daf-11*, was constructed; the dye-filling-defective mutation was then recovered from the double mutant after crossing to N2. This procedure ensured that the Dyf strain no longer carried *daf-11(m87)*.

Mapping to linkage groups and complementation testing between dyf mutations: Each mutation was first assigned to a linkage group, by standard methods (BRENNER 1974; SULSTON and HODGKIN 1988). Mapping was complicated somewhat by the already-noted tendency of *dyf* mutants to crawl off their growth plates. For example, allowance was made for the fact that significantly less than one quarter of the Dpy progeny of a *dpy/+; dyf/+* double heterozygote were generally found to be Dyf, even when the two loci were unlinked. After a *dyf* mutation was assigned to a linkage group, it was complementation tested against a representative allele of every gene on the same linkage group known to affect dye filling. For autosomal genes, this usually involved crossing a heterozygous male with a homozygous mutant hermaphrodite and checking male cross progeny for dye filling. In several cases, homozygous mutant males were used to ensure that all cross progeny were doubly heterozygous; the homozygous mutant males were self-progeny of *dyf* mutants that were also either *him-5 V* or *him-8 IV*. The *dyf* hermaphrodite parent in these tests sometimes carried an unlinked recessive marker mutation, in which case hermaphrodite cross progeny were also scored for dye filling. For all *X*-linked loci, mutant hermaphrodites, usually carrying an autosomal marker in addition to the *dyf* mutation, were mated to mutant males; hermaphrodite cross-progeny were then scored for dye filling.

Three-factor and two-factor genetic mapping: After the mutations were assigned to linkage groups and genes, all of the new *dyf* genes and many of the previously identified genes were mapped more precisely by standard three-factor (Table 2) and two-factor (Table 3) crosses. The final step in mapping was to conduct *dyf*-deficiency and *dyf*-duplication complementation tests.

Complementation tests between *dyf* mutations and deficiencies: The results of complementation tests between *dyf* mutations and deficiencies are given in Table 4. To prove that a mutation and a deficiency complemented, we demonstrated that the *dyf/Df* double heterozygote was in fact viable and non-Dyf (rather than simply relying on the absence of Dyf progeny in crosses between *dyf*- and *Df*-bearing parents). What follows are descriptions of how many of the complementation tests against deficiencies were performed. Not described are straightforward tests in which heterozygous *dyf* males (and not N2 males) crossed to heterozygous *Df* hermaphrodites yielded many healthy Dyf male progeny.

Males of genotype *unc-74 dyf-1/++* were crossed with *sDf4/bli-4 dpy-14* hermaphrodites. Unc-74 cross progeny were found to be Dyf. The animals were slow growing and about half as numerous as the Unc-74 progeny found in control crosses of *unc-74/+* males with the *sDf4/bli-4 dpy-14* hermaphrodites. They were fertile, however, and gave all Dyf self progeny. Analogous results, in all respects, were found in crosses between *dyf-1 dpy-5/++* males and *sDf4/bli-4 dpy-14* hermaphrodites. The *dyf-5*, *che-3(e1124)* and *che-13(e1805)* mutations were each tested with four deficiencies: *nDf23*, *nDf24*, *nDf25* and *mnDf111*, by the following general procedure. First, *dyf unc-75/++* males (where *dyf* stands for *che-3*, *che-13* or *dyf-5*) were mated with *Df/unc-13(e1091) lin-11* hermaphrodites, with two exceptions: *mnDf111/dpy-5 unc-13* hermaphrodites were used in matings with *che-3 unc-75/++* and *che-13 unc-75/++* males. The dye-filling phenotypes of many cross progeny from efficient matings were then checked. If all animals were non-Dyf, then individual wild-type hermaphrodites were put on separate plates to find *dyf unc-75/Df* animals, identified from the phenotypes of their self-progeny. Many non-Unc animals were picked from the broods of *dyf-5 unc-75/Df* hermaphrodites to show that the recessive lethal *Df* was present, and it was confirmed that the non-Unc animals were non-Dyf. Complementation tests between *dyf-13* and *mnDf30*, *mnDf88*, *mnDf99*, and *mnDf105* were all conducted as follows: *dyf-13 unc-4/+ +* males were crossed to *mnC1 dpy-10 unc-52/Df unc-4* hermaphrodites. Unc-4 hermaphrodite progeny were identified and shown to be non-Dyf.

Males of genotype *unc-25 dyf-2(m543)/+ +* were mated with dumpy and severely uncoordinated *eDf2; eDp6* hermaphrodites. Unc-25 hermaphrodite cross-progeny, genotype *unc-25 dyf-2/eDf2*, were non-Dyf and segregated Dyf self-progeny. Identical results were found using *unc-25 dyf-2(m160)*. These results were confirmed by a second procedure. Male progeny from a cross between N2 males and *eDf2; eDp6* hermaphrodites were mated with *unc-25 dyf-2(m160) III; dpy-11 V* hermaphrodites; many Unc-25 non-Dpy progeny (*unc-25 dyf-2/eDf2; dpy-11/+*) were scored, and all were found to be non-Dyf.

TABLE 2

Three-factor map data

Genotype of heterozygote	Recombinant phenotype	No.	Genotype of recombinant chromosome	Fraction of distance from left to right marker (or nearer marker)
Linkage group I				
dyf-1 + +/+ dpy-5 unc-13	Dpy non-Unc	7/7	*+ dpy-5 +*	
	Unc non-Dpy	6/6	*dyf-1 + unc-13*	Nearer to *dpy-5*
+ dyf-1 +/unc-74 + dpy-5	Dpy non-Unc	9/15	*+ + dpy-5*	
		6/15	*+ dyf-1 dpy-5*	
	Unc non-Dpy	9/13	*unc-74 dyf-1 +*	
		4/13	*unc-74 + +*	18/28
+dyf-10 +/dpy-5 + unc-75	Unc non-Dpy	4/4	*+ dyf-10 unc-75*	Nearer to *dpy-5*
+dyf-10 +/dpy-5 + unc-13	Dpy non-Unc	15/15	*dpy-5 dyf-10 +*	
	Unc non-Dpy	12/14	*+ + unc-13*	
		2/14	*+dyf-10 unc-13*	27/29
+ + dyf-5/dpy-5 unc-13 +	Dpy non-Unc	13/13	*dpy-5 + dyf-5*	
	Unc non-Dpy	8/8	*+ unc-13 +*	Nearer to *unc-13*
+ dyf-5 +/dpy-5 + unc-75	Dpy non-Unc	3/5	*dpy-5 + +*	
		2/5	*dpy-5 dyf-5 +*	
	Unc non-Dpy	7/10	*+ dyf-5 unc-75*	
		3/10	*++ unc-75*	5/15
+ dyf-5 +/unc-29 + dpy-24	Dpy non-Unc	13/15	*+ dyf-5 dpy-24*	
		2/15	*+ + dpy-24*	
	Unc non-Dpy	6/10	*unc-29 + +*	
		4/10	*unc-29 dyf-5 +*	6/25
+ + che-3(mn333)/dpy-5 unc-13 +	Dpy non-Unc	4/4	*dpy-5 + che-3*	
	Unc non-Dpy	6/6	*+ unc-13 +*	Nearer to *unc-13*
+ che-3(mn333) +/dpy-5 + unc-75	Unc non-Dpy	3/3	*+ che-3 unc-75*	
+ che-3(mn334) +/dpy-5 + unc-75	Unc-non-Dpy	4/6	*+ che-3 unc-75*	
		2/6	*+ + unc-75*	
	Dpy non-Unc	4/5	*dpy-5 + +*	
		1/5	*dpy-5 che-3 +*	3/14
+ che-3(mn333) +/unc-13 + daf-8	Daf non-Unc	1/2	*+ che-3 daf-8*	
		1/2	*+ + daf-8*	About 1/2
+ che-13(e1805) +/dpy-5 + unc-75	Dpy non-Unc	14/21	*dpy-5 che-13 +*	
		7/21	*dpy-5 +*	
	Unc non-Dpy	12/23	*+che-13 unc-75*	
		11/23	*+ + unc-75*	25/44
Linkage group II				
+ che-10(e1809)+/unc-85 + dpy-10	Dpy non-Unc	11/13	*+ che-10 dpy-10*	
		2/13	*+ + dpy-10*	
	Unc non-Dpy	10/11	*unc-85 + +*	
		1/11	*unc-85 che-10 +*	3/24
dyf-13 + +/+ rol-6 unc-4	Rol non-Unc	8/8	*+ rol-6 +*	
	Unc non-Rol	5/5	*dyf-13 + unc-4*	Nearer to *rol-6*
+ + dyf-13/unc-85 dpy-10 +	Dpy non-Unc	9/9	*+ dpy-10 +*	
	Unc non-DPy	7/7	*unc-85 + dyf-13*	Nearer to *dpy-10*
+ dyf-13 +/dpy-10 + unc-4	Dpy non-Unc	15/16	*dpy-10 + +*	
		1/16	*dpy-10 dyf-13 +*	
	Unc non-Dpy	18/23	*+ dyf-13 unc-4*	
		5/23	*+ + unc-4*	
dpy-10 dyf-13 unc-4/+ + +	Dpy non-Unc	13/14	*dpy-10 dyf-13 +*	
		1/14	*dpy-10 + +*	
	Unc non-Dpy	13/13	*+ + unc-4*	7/66
dpy-10 dyf-13 +/+ + unc-4	Dpy non-Dyf	7/7	*dpy-10 + unc-4*	Right of *dpy-10*
Linkage group III				
+ + dyf-2(m160)/dpy-18 unc-25 +	Unc non-Dpy	7/7	*+ unc-25 +*	
+ + dyf-2(m543)/dpy-18 unc-25 +	Unc non-Dpy	6/6	*+ unc-25 +*	
	Dpy non-Unc	9/9	*dpy-18 + dyf-2*	Nearer to *unc-25*
+ + dyf-2(m160)/unc-25 bli-5 +	Bli non-Unc	5/5	*+ bli-5 +*	Nearer to *bli-5*

TABLE 2

Continued

Genotype of heterozygote	Recombinant phenotype	No.	Genotype of recombinant chromosome	Fraction of distance from left to right marker (or nearer marker)
unc-25 + dyf-2(m543)/+ bli-5 +	Unc non-Dyf	7/9	*unc-25 bli-5* +	
		2/9	*unc-25* + +	
unc-25 + dyf-2(m160)/ + bli-5 +	Unc non-Dyf	1/3	*unc-25 bli-5* +	
		2/3	*unc-25* + +	8/12
Linkage group IV				
dyf-3(mn331) + +/+ *dpy-13 unc-22*	Dpy non-Unc	10/10	*dpy-13* + +	
	Unc non-Dpy	9/10	+ *dyf-3 unc-22*	
		1/10	+ + *unc-22*	Nearer to *dpy-13*
dyf-3(mn331) + +/+ *unc-33 dpy-13*	Dpy non-Unc	13/13	*dyf-3* + *dpy-13*	Nearer to *unc-33*
+ *dyf-3(m185)* +/*dpy-9* + *unc-5*	Unc non-Dpy	7/10	+ + *unc-5*	
		3/10	+ *dyf-3 unc-5*	
	Dpy non-Unc	8/10	*dpy-9 dyf-3* +	
		2/10	*dpy-9* + +	
+ *dyf-3(mn331)* +/*dpy-9* + *unc-5*	Unc non-Dpy	6/8	+ + *unc-5*	
		2/8	+ *dyf-3 unc-5*	
	Dpy non-Unc	5/6	*dpy-9 dyf-3* +	
		1/6	*dpy-9* + +	
+ *dyf-3(sa122)* +/*dpy-9* + *unc-5*	Unc non-Dpy	4/5	+ + *unc-5*	
		1/5	+ *dyf-3 unc-5*	
	Dpy non-Unc	2/4	*dpy-9 dyf-3* +	
		2/4	*dpy-9* + +	32/43
+ *dyf-3(m185)* +/*dpy-9* + *unc-17*	Dpy non-Unc	14/15	*dpy-9 dyf-3* +	
		1/15	*dpy-9* + +	
	Unc non-Dpy	13/16	+ + *unc-17*	
		3/16	+ *dyf-3 unc-17*	27/31
Linkage group V				
che-12(e1812) + +/+ *sma-1 unc-76*	Unc non-Sma	10/10	*che-12* + *unc-76*	Nearer to *sma-1*
+ *che-12(e1812)* +/*unc-42* + *sma-1*	Unc non-Sma	9/9	*unc-42* + +	Nearer to *unc-42*
+ *che-12(e1812)* +/*unc-42* + *vab-8*	Unc non-Vab	3/3	*unc-42* + +	
	Vab non-Unc	3/4	+ *che-12 vab-8*	
		1/4	+ + *vab-8*	1/7
+ + *osm-6(p811)/dpy-11 unc-42* +	Dpy non-Unc	5/5	*dpy-11* + *osm-6*	
	Unc non-Dpy	5/5	+ *unc-42* +	Nearer to *unc-42*
osm-6(p811) + +/+ *sma-1 unc-76*	Unc non-Sma	6/6	*osm-6* + *unc-76*	Nearer to *sma-1*
+ *osm-6(p811)* +/*unc-42* + *sma-1*	Unc non-Sma	27/27	*unc-42 osm-6* +	
	Sma non-Unc	20/20	+ + *sma-1*	Nearer to *sma-1*
+ *osm-6(p811)* +/*unc-42* + *vab-8*	Unc non-Vab	4/5	*unc-42* + *osm-6*	
		1/5	*unc-42* + +	4/5
osm-6(p811) + +/+ *sma-1 vab-8*	Sma non-Vab	1/1	+ *sma-1* +	
	Vab non-Sma	3/3	*osm-6* + *vab-8*	Nearer to *sma-1*
+ + *che-11(e1810)/dpy-11 unc-42* +	Dpy non-Unc	5/5	*dpy-11* + *che-11*	
	Unc non-Dpy	4/4	+ *unc-42* +	Nearer to *unc-42*
+ *che-11(e1810)* +/*unc-42* + *vab-8*	Unc non-Vab	5/5	*unc-42 che-11* +	Nearer to *vab-8*
+ + *che-11(e1810)/unc-42 sma-1* +	Unc non-Sma	5/5	*unc-42* + *che-11*	Nearer to *sma-1*
+ *che-11(e1810)* +/*sma-1* + *unc-76*	Unc non-Sma	5/7	+ *che-11 unc-76*	
		2/7	+ + *unc-76*	2/7
+ *che-11(e1810)* +/*sma-1* + *vab-8*	Vab non-Sma	4/4	+ *che-11 vab-8*	
	Sma non-Vab	3/3	*sma-1 che-11* +	
+ *che-11 (mn387)* +/*sma-1* + *vab-8*	Vab non-Sma	2/4	+ *che-11 vab-8*	
		2/4	+ + *vab-8*	
	Sma non-Vab	3/5	*sma-1 che-11* +	
		2/5	*sma-1* + +	8/16
+ + *dyf-4(mn332)/dpy-11 unc-23* +	Dpy non-Unc	4/4	*dpy-11* + *dyf-4*	
	Unc non-Dpy	2/2	+ *unc-23* +	Nearer to *unc-23*
+ + *dyf-4(mn332)/unc-42 sma-1* +	Unc non-Sma	5/5	*unc-42* + *dyf-4*	Nearer to *sma-1*
+ *dyf-4(mn332)* +/*sma-1* + *unc-76*	Unc non-Sma	5/5	+ *dyf-4 unc-76*	Nearer to *sma-1*
+ + *dyf-4(mn332)/unc-42 vab-8* +	Unc non-Vab	5/5	*unc-42* + *dyf-4*	Nearer to *vab-8*

TABLE 2

Continued

Genotype of heterozygote	Recombinant phenotype	No.	Genotype of recombinant chromosome	Fraction of distance from left to right marker (or nearer marker)
+ + *dyf-4(mn332)/sma-1 vab-8* +	Sma non-Vab	7/7	*sma-1* + *dyf-4*	
	Vab non-Sma	3/3	+ + *vab-8*	Nearer to *vab-8*
+ *dyf-9(n1513)*+*/dpy-11* + *unc-51*	Dpy non-Unc	23/24	*dpy-11 dyf-9* +	
		1/24	*dpy-11* + +	
	Unc non-Dpy	4/4	+ + *unc-51*	27/28
dyf-9(sa121) + +/+ *unc-51 rol-9*	Rol non-Unc	1/1	*dyf-9* + *rol-9*	Probably nearer to *unc-51*
Linkage group X				
dyf-11 + +/+ *dpy-6 unc-9*	Unc non-Dpy	15/15	*dyf-11* + *unc-9*	
	Dpy non-Unc	9/10	+ *dpy-6* +	
		1/10	*dyf-11 dpy-6* +	Nearer to *dpy-6*
dyf-11 ++/+ *unc-20 dpy-8*	Dpy non-Unc	8/8	*dyf-11* + *dpy-8*	
	Unc non-Dpy	5/4	+ *unc-20* +	Nearer to *unc-20*
dyf-11 + +/+*dpy-3 unc-2*	Dpy non-Unc	6/6	+ *dpy-3* +	
	Unc non-Dpy	5/5	*dyf-11* + *unc-2*	Nearer to *dpy-3*
+ *dyf-11* +/*unc-1* + *dpy-3*	Dpy non-Unc	3/4	+ *dyf-11 dpy-3*	
		1/4	+ + *dpy-3*	
	Unc non-Dpy	5/7	*unc-1* + +	
		2/7	*unc-1 dyf-11* +	3/11
+ + *dyf-12/unc-6 dpy-6* +	Dpy non-Unc	13/13	+ *dpy-6* +	
	Unc non-Dpy	12/12	*unc-6* + *dyf-12*	Nearer to *dpy-6*
+ *dyf-12* +/*dpy-6* + *unc-9*	Unc non-Dpy	10/14	+ *dyf-12 unc-9*	
		4/14	+ + *unc-9*	
	Dpy non-Unc	15/21	*dpy-6* + +	
		6/21	*dpy-6 dyf-12* +	10/35
+ *lin-14 unc-9/dyf-12* + +	Lin non-Unc	6/6	+ *lin-14* +	Nearer to *lin-14*
dpy-6 + *lin-14*/+ *dyf-12* +	Lin non-Dpy	9/12	+ + *lin-14*	
		3/12	+ *dyf-12 lin-14*	9/12
+ + *dyf-7/unc-6 dpy-6* +	Unc non-Dpy	15/15	*unc-6* + *dyf-7*	Nearer to *dpy-6*
+ *dyf-7* +/*dpy-6* + *unc-9*	Unc non-Dpy	7/8	+ *dyf-7 unc-9*	
		1/8	+ + *unc-9*	
	Dpy non-Unc	3/6	*dpy-6 dyf-7* +	
		3/6	*dpy-6* + +	4/14
+ + *dyf-6(m175)/unc-6 dpy-6* +	Unc non-Dpy	16/16	*unc-6* + *dyf-6*	
	Dpy non-Unc	19/19	+ *dpy-6* +	Nearer to *dpy-6*
+ *dyf-6(mn346)* +/*dpy-6* + *unc-9*	Unc non-Dpy	10/10	+ *dyf-6 unc-9*	
	Dpy non-Unc	6/9	*dpy-6* + +	
		3/9	*dpy-6 dyf-6* +	3/19
+ + *dyf-8/dpy-6 unc-9* +	Unc non-Dpy	12/12	+ *unc-9* +	Nearer to *unc-9*
+ *dyf-8* +/*unc-9* + *unc-3*	Unc-9 non-Unc-3	5/6	*unc-9* + +	
		1/6	*unc-9 dyf-8* +	1/6

All male progeny of matings between *osm-6(p811)*/+ males and *dpy-18/eT1(III); unc-60 dpy-11 sDf35/eT1(V)* hermaphrodites were non-Dyf. Therefore *dpy-18*/+; *unc-60 dpy-11 sDf35/osm-6* hermaphrodite progeny from the same cross were identified on the basis of the phenotypes of their self-progeny and shown to be non-Dyf. The identity of *dpyf-4/ctDf1* non-Dyf hermaphrodites was also confirmed by progeny testing, making use of the homozygous lethality of *ctDf1*.

Mutations in three *X*-linked *dyf* genes were complementation tested with *nDf19* as follows: *dyf/0* males were mated with *szT1*[*lon-2*]*/nDf19* hermaphrodites. The latter animals have a high incidence of Lon (but not non-Lon) male self progeny; the presence of non-Lon cross-progeny males therefore indicated a successful mating. Young hermaphrodite progeny were picked from plates on which mating had occurred. Animals of genotype *dyf/nDf19* were then identified by the absence of males and the segregation of homozygous *dyf* animals among their self-progeny. Finally, *mnDf13* and *daf-6(m186)* were complementation tested by the following procedure: *mnDp1(X;V)*/+: *mnDf13*/0 males were crossed to *unc-4 II; daf-6 X* hermaphrodites, and several non-Unc hermaphrodite progeny were found to be Dyf.

Testing for suppression of *dyf* mutations by chromosomal duplications: The duplication *eDp6* was tested for its ability to complement (*i.e.*, suppress) *dyf-2(m160)* by the following procedure: *unc-25 dyf-2*/+ + males were mated with *eDf2 III; eDp6(III; f)* hermaphrodites, which are dumpy and severely uncoordinated. Non-Unc hermaphrodite progeny were picked to identify *unc-25 dyf-2/eDf2; eDp6* animals, from which an *unc-25 dyf-2; eDp6* strain was founded. Animals of

TABLE 3

Two-factor map data

Genotype of heterozygous parent	Selected phenotype	No.	Recombinant chromosome	No.	Map units	95% confidence[a]
dpy-10 dyf-13/+ + II[b]	Dpy[c]	1043	*dpy-10 +*	7	0.3	0.1–0.7
unc-25 dyf-2(m543)/+ + III[d]	Unc[c]	513	*unc-25 +*	11	1.1	0.5–2.0
unc-25 dyf-2(m160)/+ + III[d]	Unc[c]	716	*unc-25 +*	7	0.5	0.2–1.1
unc-64 +/+ dyf-2(m160) III	Unc[e]	104	*unc-64 dyf-2*	0	0	0–1.8
+ unc-51/dyf-9(sa121) + V[f]	Unc[e]	121	*dyf-9 unc-51*	0	0	0.0–1.6
dpy-6 dyf-12/+ + X	Dpy[c]	442	*dpy-6 +*	25	2.8	1.9–4.1

[a] 95% confidence limits (MAINLAND *et al.* 1956).
[b] The + + chromosome also carried *unc-4.*
[c] Selected animals were picked from among the self-progeny of the heterozygous parent and scored for dye filling.
[d] The + + chromosome also carried *bli-5.*
[e] Selected animals were picked from among the self-progeny of the heterozygous parent and scored for their ability to segregate any Dyf self-progeny.
[f] The + *unc-51* chromosome also carried *rol-9.*

this genotype segregated only Unc Dyf and non-Unc non-Dyf self-progeny.

Finally, *stDp2(X;II)* was tested against three *dyf* loci. The male progeny of the cross between *stDp2/+; dpy-7/0* males and *dpy-6 dyf-12* hermaphrodites were Dpy Dyf and non-Dpy non-Dyf. Many of the non-Dpy male progeny of the cross between *stDp2/+; unc-6 dpy-6/0* males and *dpy-11 V; dyf-7(m537) X* hermaphrodites were non-Dyf; and all non-Unc male progeny of the cross between *stDp2/+; dpy-7/0* males and *unc-6 dyf-6(m175)* hermaphrodites were Dyf.

Chemotaxis assay: A tracking assay that makes use of a radial gradient of attractant, as described by WARD (1973), was used, with some modification. The buffer described by WARD was used in 4 ml of agarose per 85-mm diameter petri plate. After the agarose cooled, 25 μl (or 5 μl in some instances) of 0.5 M NH_4Cl, the attractant, was applied to the center of the plate; 12 hr later, an additional 25 μl (or 5 μl when 5 μl had been used initially) of attractant was added to the center of the plate. Three hours later, three worms were placed on each assay plate. The worms that were assayed were young adult, well-fed hermaphrodites that had been growing on standard growth plates. The animals were transferred from these plates to plates without a bacterial lawn 10 min before the assay to reduce the concentration of bacteria inadvertantly transferred to the assay plates. The three worms were placed at equal intervals on a circumference of the assay plate 35 mm from the center. After 15 min, the tracks of the worms on the assay plate, which were readily visible, were marked on the back of the plate. A track that led to within 9 mm of the center of the plate was scored as successful. The experi-

TABLE 4

Deficiency-duplication mapping

	Duplication or deficiency	
Mutation	Fails to complement	Complements
dyf-1 I	*sDf4*	
dyf-10 I	*hDf8*	
dyf-5 I	*nDf24, mnDf111*	*nDf23, nDf25*
che-3(e1124) I	*nDf24, nDf25, mnDf111*	*mDf23*
che-13(e1805) I		*nDf23, nDf24, nDf25, mnDf111*
che-10(mn403) II	*maDf4*	
dyf-13 II		*mnDf30, mnDf88, mnDf99, mnDf105*
dyf-2(m160) III		*eDf2, eDp6*
dyf-2(m543) III		*eDf2*
che-12(mn389) V	*sDf35*	
osm-6(p811) V	*ctDf1*	*sDf35*
che-11(e1810) V	*ctDf1*	
dyf-4(mn332) V		*ctDf1*
dyf-9(sa121) V	*ozDf2*	
dyf-12 X		*nDf19, stDp2*
dyf-7 X	*nDf19*	*stDp2*
dyf-6(m175) X	*nDf19, stDp2*	
dyf-6(mn346) X	*nDf19*	
daf-6(m186) X	*mnDf13*	

ments were performed in a 20° constant temperature room. On each day that chemotaxis assays were run, both N2 and at least one previously studied chemotaxis-defective mutant were run as controls (see RESULTS).

Other behavioral assays: Response to light touch was tested by the method of CHALFIE and SULSTON (1981) using the light, transverse touch of an eyebrow hair.

A qualitative assessment of male mating ability was made, using a slightly modified version of the method described by HODGKIN (1983): 7–10 males were placed on a 35-mm diameter plate with four *unc-17* hermaphrodites. For *X*-linked *dyf* loci, the tested males were the progeny of crosses between N2 males and *dyf* hermaphrodites. For autosomal *dyf* loci, double mutants were constructed, using either *him-5 V* or *him-8 IV*, which then segregated homozygous mutant male self-progeny for mating tests. Numbers of cross-progeny were counted for each cross and compared to appropriate control crosses involving N2, *him-5* or *him-8* males. At least two separate crosses were performed for each mutant tested.

Ability to form dauer larvae was assayed by collecting worm cultures grown to starvation on 85-mm diameter growth plates (sometimes three 50-mm diameter plates were used) in distilled water. The worms from each plate (or set of three plates) were washed, centrifuged and resuspended in 1 ml of 1% SDS for 1 hr, which kills nondauer animals (CASSADA and RUSSELL 1975). Survivors were washed by centrifugation and resuspension in distilled water three times and then placed on a fresh plate containing a lawn of *E. coli*. The number of surviving dauer larvae was estimated, and when dauer larvae were found, five were inspected by Nomarski microscopy to confirm the presence of the dauer-specific lateral alae (CASSADA and RUSSELL 1975). Each strain tested for dauer-forming ability was assayed at least twice. This assay is not quantitative; mutants with reduced but partial response to dauer pheromone may produce many dauer larvae on starved plates owing to high concentration of pheromone (RIDDLE 1988).

RESULTS

Identification of mutants: Mutants defective in dye filling, or Dyf, have been collected in a variety of screens, as shown in Table 1. Among a total of 95 mutations that we have characterized, 27 were spontaneous. Nineteen of these were obtained from the mutator strain RW7097, which has been shown to be active in the transposition of Tc1 (MORI *et al.* 1988). The other eight spontaneous mutants were recovered from the mutator strain TR679, which has been shown to exhibit active transposition of Tc1 as well as other families of transposons (COLLINS *et al.* 1987, 1989; YUAN *et al.* 1991; LI and SHAW 1993). The remaining 68 mutants were identified following treatment with EMS.

Many of the Dyf mutants were first identified on the basis of a defect in a chemosensory response. Forty-five were found among mutants selected for being defective in the formation of dauer larvae. Within this group, 21 EMS-induced mutants were originally recovered from starved broods, either on plates or in wells of microtiter dishes, which were devoid of dauer larvae. Six EMS-induced mutants derived from N2 and 18 spontaneous mutants from RW7097 were recovered among a set of mutants selected for their ability to grow in liquid culture in the presence of high levels of dauer-inducing pheromone (see MATERIALS AND METHODS). Eight additional EMS-induced Dyf mutants were found among a set of mutants originally identified as Osm, or defective in the ability to avoid high osmolarity (CULOTTI and RUSSELL 1978). An additional nine mutants, all spontaneous—one from RW7097 and eight from TR679, were collected in a screen that enriched for Osm animals, although no attempt was made to confirm the Osm phenotype before scoring the dye-filling phenotype for these animals.

Fourteen additional mutations were found among a collection of EMS-induced mutations recovered on the basis of suppressing *daf-11(m87)* (VOWELS and THOMAS 1992), which by itself leads to the formation of dauer larvae at 25° even when food is plentiful and the animals are not crowded (RIDDLE *et al.* 1981). Finally, 19 mutants were identified solely on the basis of their Dyf phenotype; for these mutants, no prior enrichment or selection was imposed.

Genetic mapping of mutations and gene assignments: All 95 mutations were shown to be recessive to wild type. Each mutant was crossed with N2 males and tested for possible *X*-linkage by scoring the dye-filling phenotype of male progeny. Each autosomal mutation was assigned to a linkage group by following the segregation of the *dyf* mutation from at least one marker mutation on each autosomal linkage group. Once assigned to a linkage group, each mutation was complementation tested against alleles of other *dyf* genes mapping to the same linkage group. Most mutations were complementation tested against representative mutations of several loci; all gene assignments based on complementation tests were unambiguous. Previous work (PERKINS *et al.* 1986) identified 16 genes at most that fit our definition of *dyf* genes, *viz.*, genes for which mutant alleles confer a defect in dye filling without strongly affecting overall animal morphology or ability to move. A reference allele of each of these 16 genes was used in the complementation tests involving our set of 95 mutations (see MATERIALS AND METHODS).

Seventy-five of our *dyf* mutations were assigned to one of 12 previously defined loci (Table 1). Twenty-one are alleles of *che-3 I*. This locus, previously represented by four alleles, is obviously a large target. We identified eight new alleles each of *daf-10* and *osm-1*; six new alleles each of *daf-6* and *osm-3*; five new alleles each of *che-2* and *osm-5*; four new alleles each of *che-10, che-11* and *osm-6*; and two new alleles each of *che-12* and *che-13*. These 12 genes are listed in Table 1 together with their previously identified alleles (23 altogether) and their new alleles. Only one of the 75 mutations assigned to the 12 previously identified *dyf* genes exhibited any novel properties. The exceptional mutation, *daf-10(mn321)*, was homozygous sterile and was therefore maintained as a heterozygote; this mutation has been

shown by S. STONE and J. SHAW (personal communication) to be a deficiency. It deletes the *daf-10* locus and fails to complement the closely linked gene *fem-3*.

Twenty mutations did not fall into previously identified *dyf* loci. These were assigned to 13 genes, called *dyf-1* through *dyf-13* (see Table 1). Two of these loci, *dyf-3* and *dyf-4*, are represented by three alleles each. Three others, *dyf-2*, *dyf-6* and *dyf-9*, have two alleles each. The remaining eight loci are each represented by a single mutant allele.

The total of 95 *dyf* mutations thus fall into 25 genes, 12 previously identified and 13 new. We have mapped the 13 new loci genetically by three-factor and two-factor crosses, and we have refined the map positions of 6 of the previously identified loci. Our map data are given in Tables 2 and 3, and the derived map positions, also based in part on deficiency mapping described in the next section, are presented in Figure 1. The distinct map positions of *dyf-1* through *dyf-13* lead us to conclude that they are indeed different from the previously identified genes, *i.e.*, no potential allelism has been missed because of intragenic complementation.

Representative alleles of a few additional genes were chosen, on the basis of mutant phenotype and map position, for dye filling assays and complementation tests. In this way we found that *caf-1* and *caf-2* mutants, which were originally identified because they are resistant to caffeine (HARTMAN 1987), are Dyf. Furthermore we found that *caf-1(hf3) IV*, which was originally mapped near *osm-3 IV*, failed to complement *osm-3* for dye filling, and that *caf-2(hf5) I*, which was originally mapped near *che-3 I*, failed to complement *che-3* for dye filling. (As expected, *caf-1* and *dyf-3 IV* complemented, as did *caf-3* and *dyf-5 I*.) We thus conclude that *caf-1* and *caf-2* mutations are *osm-3* and *che-3* alleles, respectively. Mutation in *daf-12 X*, which maps near *dyf-6*, *dyf-7*, and *dyf-12*, leads to an inability to form dauer larvae (RIDDLE *et al.* 1981). The *daf-12* mutant is non-Dyf (PERKINS *et al.* 1986), but we nonetheless tested *dyf-6(m175)*, *dyf-7*, and *dyf-12* for complementation, using *dyf/0* males and *unc-27 daf-12 egl-15* hermaphrodites; in each case all cross progeny hermaphrodites were non-Dyf. We also found that *dyf-6* (both alleles), *dyf-7* and *dyf-12* complement *vab-3*, which maps in the same region; mutations in *vab-3* lead to disorganization in anterior sensory anatomy (LEWIS and HODGKIN 1977) and variable reduction in dye filling (data not shown). A mutation in *unc-104*, a gene that encodes a kinesin-related protein (OTSUKA *et al.* 1991), confers a dye-filling defect and maps near *dyf-13* (HALL and HEDGECOCK 1991). We found that *unc-104/dyf-13* animals are non-Dyf. Finally, we found that the *daf-3/dyf-11 trans*-heterozygote is non-Dyf. Mutation in *daf-3*, which maps near *dyf-11 X*, leads to an inability to form dauer larvae (RIDDLE *et al.* 1981), but *daf-3* animals are non-Dyf (PERKINS *et al.* 1986). The genes *srf-2 I* (POLITZ *et al.* 1990), *srf-4 V* and *srf-5 X* (LINK *et al.* 1992) affect the surface cuticle and map near *dyf* genes. We tested a mutant allele of each for dye filling; all were non-Dyf.

Putting *dyf* mutations opposite chromosomal deficiencies: We have placed many *dyf* mutations opposite deficiencies, both to try to confirm map positions and to gain information about the nature of *dyf* mutations. Altogether, 14 of the 25 *dyf* loci have been uncovered by deficiencies. We have uncovered 12 genes: *dyf-1 I*, *dyf-10 I*, *dyf-5 I*, *che-3 I*, *che-10 II*, *che-12 V*, *osm-6 V*, *che-11 V*, *dyf-9 V*, *dyf-7 X*, *dyf-6 X*, and *daf-6 X* (Table 4 and Figure 1). In all of these cases, *dyf/Df* animals exhibited the Dyf phenotype. S. STONE and J. SHAW (personal communication) have shown that both *daf-10/Df* and *osm-1/Df* animals are Dyf. With the exception of *dyf-1*, all of the *dyf/Df* animals appear to have a phenotype indistinguishable from that of the *dyf* homozygotes, at least approximately, as judged by examination in the dissecting microscope. In contrast, *dyf-1/sDf4* animals were slow growing and sickly in addition to being Dyf, suggesting that *dyf-1(+)* has an essential developmental role and that the *dyf-1(mn335)* allele leads to a partial loss of function.

For 11 of the 25 *dyf* genes, no *dyf/Df* animals exhibiting a Dyf phenotype have been found. For most of these genes no appropriate deficiencies are available, but for three loci complications must be noted. Our mapping of *dyf-12 X* indicates that it should be deleted by *nDf19*, and yet *dyf-12/nDf19* animals are clearly non-Dyf (MATERIALS AND METHODS and Table 4). It is possible that *nDf19* is complex and does not delete *dyf-12*. Alternatively, two mutant copies of *dyf-12* may be required in the hermaphrodite to produce the fully mutant Dyf phenotype. (In the male, which presumably dosage compensates *dyf-12*, one mutant copy would confer the Dyf phenotype.) Another surprising result involves *dyf-2*, which maps near the right end of linkage group III (Figure 1). For both *dyf-2* alleles, *dyf-2/eDf2* animals were clearly non-Dyf (Table 4); on the other hand, *eDp6(III;f)*, which was apparently generated in the same event that produced *eDf 2* and which is thought to be complementary to *eDf 2* in structure (HODGKIN 1980), carries *dyf-2(+)*, since *dyf-2/dyf-2; eDp6* animals are non-Dyf (MATERIALS AND METHODS and Table 4). We conclude that either *eDf 6* and *eDp2* are not complementary, each carrying *dyf-2(+)*, or two mutant copies of *dyf-2* (either allele) are required to produce the fully mutant Dyf phenotype. Finally, we note that *dyf-13* maps near a region rich in deficiencies, none of which fails to complement the *dyf-13* mutation. In this case, however, *dyf-13* maps close to or in a small segment that has not been deleted by any known deficiencies (Figure 2), so we have tentatively placed it in that segment. We cannot be sure that *dyf-13/Df* animals would have a Dyf phenotype, however.

Dye-filling phenotypes of mutants representing new

TABLE 5

Dye filling of amphid and phasmid neurons

Genotype	FITC		DiO		Other traits
	Amphid	Phasmid	Amphid	Phasmid	
N2	6	2	6	2	
dyf-1	<1, faint	0	0	0	
dyf-2(m160)	<1, faint	0	0	0	
dyf-2(m543)	~1	0	0	0	
dyf-3(m185)	0	0	0	0	
dyf-3(mn331)	0	0	0	0	
dyf-3(sa122)	0	0	0	0	
dyf-4(m158)	0	0	0	0	
dyf-4(m177)	0	0	0	0	
dyf-4(mn332)	0	0	0	0	
dyf-5	~1, faint	0–2	0		Slightly short
dyf-6(m175)	0	0	0	0	
dyf-6(mn346)	0	0	0	0	
dyf-7	0	0	0	0	
dyf-8	0	0	0	0	Slightly short
dyf-9(n1513)	~1, faint	0	0	0	
dyf-9(sa121)	~1	0	0	0	
dyf-10	~1, faint	0	0	0	
dfy-11	0	0	0	0	Slightly short
dyf-12	<1	0	0	0	
dyf-13	1–2	0	1–2	0	Slow growth, small broods

At least 10 animals were scored for each dye for each genotype. Values are average numbers of dye-filled neurons per amphid or phasmid. Two amphid neurons. (ASI especially) stain weakly with DiO in N2 animals (see Figure 3).

genes: We have carefully characterized the defects in dye-filling conferred by each of the 20 mutations that have been assigned to the 13 new *dyf* genes. Table 5 gives the results for both FITC and DiO. Figure 3 illustrates the DiO filling of an N2 animal. HEDGECOCK *et al.* (1985) and HERMAN (1987) have published micrographs illustrating FITC staining. All of the mutants are severely defective in dye filling (Table 5) and very easy to score. Some exhibit occasional staining, usually faint and usually a single amphid neuron. Both *dyf-2* alleles and both *dyf-9* alleles are examples. The only mutant that exhibits consistent filling of one or two amphid neurons by both FITC and DiO is *dyf-13(mn396)*.

Four of the 20 mutations may cause abnormalities not directly related to the deficiency in dye filling (Table 5): *dyf-5*, *dyf-8* and *dyf-11* animals are slightly shorter than N2; the shortness might be difficult to score reliably for individual animals, but it is clear when scoring a brood. *dyf-13* animals are distinctly slower to mature than N2 and have brood sizes that are variable and smaller than those of N2 or any of the other 20 mutants. We cannot be sure that these abnormalities are not caused by secondary mutations, despite repeated outcrossing.

Behavioral phenotypes of mutants representing new genes: Hermaphrodites carrying mutations in new *dyf* genes were assayed for chemotaxis in radial gradients of NH_4Cl and all were found to be chemotaxis-defective (Table 6). More than 70% of N2 animals gave a positive response in our assays. Mutations in *che-2*, *osm-1* and *osm-6* were previously shown to cause extreme chemotaxis defects (LEWIS and HODGKIN 1977; CULOTTI and RUSSELL 1978; PERKINS *et al.* 1986), and we found that only 0–2% of animals carrying mutations in these genes gave a chemotactic response in our assay (Table 6). This level of tracking may represent complete absence of chemotaxis. All animals tested were capable of normal mobility when prodded. Previously studied *mec-8* mutants, which show reduced FITC and DiO filling (PERKINS *et al.* 1986; LUNDQUIST and HERMAN 1994), were reported to be capable of some chemotaxis (PERKINS *et al.* 1986). We found that *mec-8(u74)* animals are chemotaxis defective, but not as severely as *che-2*, *osm-1*, or *osm-6* animals (Table 6). Although all 20 of the mutants representing the 13 new *dyf* genes were defective in chemotaxis compared with wild type, only a few seemed to be as defective as *che-2*, *osm-1* and *osm-6*. These included two of the *dyf-3* alleles, as well as *dyf-7* and *dyf-8*. The mutants that seemed to be best at chemotaxis were *dyf-13* and the two *dyf-9* mutants. Although 53% of the *dyf-13* animals gave a positive response in our tracking assay, the difference between this response and that of N2 was highly significant ($P < 0.001$). We note that the *dyf-13* mutant also gave

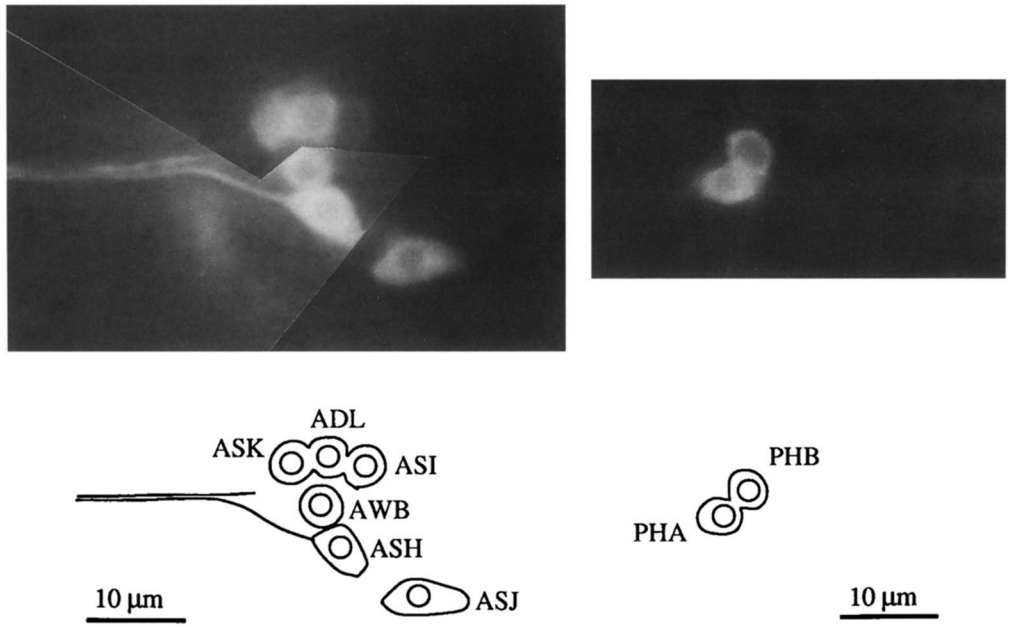

FIGURE 3.—Micrographs of an amphid and a phasmid of N2 (wild type) stained with DiO. Anterior is to the left, and dorsal is up. The amphid was photographed at two planes of focus because AWB and ASH are more laterally situated than the other stained cell bodies, and the picture is a montage of two photographs. The neurons that fill with DiO are the same as those that fill with FITC except that AWB fills with DiO and ADF does not (C. BARGMANN, personal communication); ASI fills very weakly and AWB fills somewhat weakly.

the greatest residual dye filling among the 20 mutants (Table 5). Three of the 20 mutants in this group, *dyf-5*, *dyf-11* and *dyf-13*, were recovered strictly on the basis of a defect in dye filling; *dyf-5* and *dyf-13* were among the better trackers.

We have also scored the 20 *dyf* mutants for ability to form dauer larvae. The majority of the mutants were clearly defective, but most showed some ability to form dauers. As noted in the MATERIALS AND METHODS, our assay may not be sensitive to partial defects in dauer formation. There is a reasonable correlation between ability to chemotax and ability to form dauer larvae. For example, *dyf-9(sa121)*, *dyf-12(sa127)* and *dyf-13(mn396)* were all fairly good trackers and quite capable of forming dauers. The ability of mutant males to mate was also assayed. All males were capable of some mating, although generally less well than wild type. PERKINS *et al.* (1986) reported, from similar tests of male mating, that several of the previously identified dye-filling defective mutants were male sterile. We have not tested any of our new alleles of the previously identified genes for their effects on male mating. Finally, all of the 20 *dyf-1*–*dyf-13* mutants were unaffected in their response to light touch. The control in this case was *mec-8(u74)* animals, which are nonresponsive to light touch (CHALFIE and SULSTON 1981).

DISCUSSION

As pointed out by PERKINS *et al.* (1986), a virtue of the Dyf phenotype is that it can be scored in single animals very reliably, whereas defects in chemosensory behaviors must generally be scored using populations. Although some of the *dyf* mutants in this work showed relatively weak chemotaxis defects, the mapping and complementation testing with respect to the Dyf phenotype was in every case straightforward. The 25 loci we have studied are therefore useful as general genetic markers. Because all previously identified Dyf mutants, representing 16 genes, have turned out to exhibit ultrastructural defects in amphid sensilla (LEWIS and HODGKIN 1977; ALBERT *et al.* 1981; PERKINS *et al.* 1986), we expect that mutations in the 13 new *dyf* genes defined in this work also cause abnormalities in amphid structure, whether in the ciliated endings of the neurons, in the bundling of amphid processes or in the structure or connections of socket and sheath cells. Therefore it would be valuable to investigate the ultrastructure of the sensilla of the new *dyf* mutants.

Among the 95 new mutations, 75 turned out to be alleles of 12 genes that were already defined by mutations conferring a Dyf phenotype. Seven of these genes were previously represented by a single allele; to each of these genes we have added at least two, and as many as six, new alleles. To the other five genes, which were already represented by two to four alleles each, we have added 6–21 new alleles. The new alleles, many of which are expected to carry transposon insertions, should be useful in the future analysis of these genes. Indeed, some have already proved useful in the cloning and molecular analysis of *osm-1* and *daf-10* (S. STONE and J.

TABLE 6

***dyf* mutant behaviors**

Genotype	Chemotaxis			Dauer formation[d]	Male mating[e]
	n[a]	Frequency[b]	95% confidence[c]		
N2	391	0.71	0.66–0.76	++	4
dyf-1	87	0.18	0.11–0.28	+	2
dyf-2(m160)	67	0.24	0.14–0.36	+	4
dyf-2(m543)	90	0.07	0.02–0.14	0	4
dyf-3(m185)	108	0.12	0.06–0.19	+	4
dyf-3(mn331)	120	0.03	0.01–0.07	+	2
dfy-3(sa122)	84	0.02	0.00–0.08	+	1
dfy-4(m158)	87	0.16	0.09–0.25	+	4
dyf-4(m177)	60	0.30	0.19–0.43	+	4
dyf-4(mn332)	90	0.16	0.09–0.25	0	3
dyf-5	87	0.28	0.19–0.39	+	2
dyf-6(m175)	54	0.22	0.12–0.35	0	3
dyf-6(mn346)	81	0.09	0.04–0.17	0	4
dyf-7	108	0.00	0.00–0.03	0	1
dyf-8	105	0.02	0.00–0.07	0	1
dyf-9(n1513)	54	0.31	0.12–0.45	+	1
dyf-9(sa121)	72	0.39	0.28–0.51	++	3
dyf-10	105	0.14	0.09–0.23	++	4
dyf-11	90	0.07	0.02–0.14	+	3
dyf-12	60	0.28	0.17–0.41	++	3
dyf-13	105	0.53	0.43–0.63	++	2
che-2(e1033)	128	0.02	0.01–0.07		
mec-8(u74)	54	0.26	0.15–0.39		
osm-1(p808)	42	0.00	0.00–0.08		
osm-6(p811)	120	0.02	0.00–0.06		

[a] n is the no. of animals assayed.

[b] The fraction of animals that tracked to the attractant (see MATERIALS AND METHODS).

[c] 95% confidence limits, based on binomial distributions (MAINLAND *et al.* 1956).

[d] ++, ≥20% as many dauers formed as for N2; +, ~1–20% as many dauers formed as for N2; 0, <1% as many dauers formed as for N2.

[e] The scale is very similar to that defined by HODGKIN (1983): 4, 30–100% as efficient mating as N2; 3, 10–30%; 2, 1–10%; 1, <1%.

SHAW, personal communication), *osm-3* (M. SHAKIR and S. SIDDIQUI, personal communication) and *osm-6* (J. COLLET, R. HERMAN and J. SHAW, unpublished experiments). We also found that *caf-1* and *caf-2* mutations, which confer resistance to caffeine (HARTMAN 1987), should be added to the list of *osm-3* and *che-3* alleles, respectively. We have not tested any of our mutants for caffeine resistance, but the obvious suggestion is that amphid and phasmid defects may hinder caffeine uptake. C. JOHNSON and W. GRANT (personal communication) have found that many mutations conferring dye-filling defects, particularly in *che-3* and *osm-3*, also confer resistance to the anthelmintic drug ivermectin.

We recovered no new alleles of four genes shown by PERKINS *et al.* (1986) to affect dye filling. For two of these genes, *mec-1* and *che-14*, the mutant Dyf phenotypes seen by PERKINS *et al.* (1986) were very weak, with the neurons frequently staining normally. Such a weak phenotype would probably have been overlooked in our screens. More than 60 alleles of *mec-1* have in any case been identified on the basis of their effect on response to light touch (CHALFIE and AU 1989). Mutations in a third gene, *mec-8*, do not completely abolish amphid neuron staining; typically, two amphid neurons fill with dye (PERKINS *et al.* 1986; LUNDQUIST and HERMAN 1994), and this may have reduced our chances of recovering new alleles. Mutations in *mec-8* also result in an inability to respond to light touch and have been recovered on the basis of this phenotype (CHALFIE and SULSTON 1981; CHALFIE and AU 1989; LUNDQUIST and HERMAN 1994).

The fourth gene for which no new alleles were recovered is *daf-19*. Mutations in this gene lead to both a Dyf phenotype and, particularly at high temperature, constitutive dauer formation (D. RIDDLE, unpublished experiments; MALONE and THOMAS 1994). It is not surprising that we did not recover *daf-19* mutants in our experiments if such mutants usually form dauer larvae constitutively. The ultrastructural abnormality in the *daf-19(m86)* mutant was unique: all cilia were absent

(PERKINS *et al.* 1986). All of the other cilia-defective mutants showed residual, albeit abnormal, cilia. Because the laser-killing of a set of four pairs of amphid channel neurons leads to constitutive dauer formation, BARGMANN and HORVITZ (1991b) have suggested that the responsible neurons must generate a signal to prevent dauer formation under favorable growth conditions. It is thus possible that any mutation that destroys the function of the amphid neurons would lead to constitutive dauer formation and therefore not be recovered in a straight screen for Dyf mutants. To combat this problem one could initiate a Dyf screen using a mutant that is defective for dauer formation at a later step in the dauer pathway; at least some non-Dyf dauer-defective mutations suppress the induction of dauer larvae by neuron-killing (BARGMANN and HORVITZ 1991b). It seems unlikely that a large class of constitutive dauer-forming Dyf mutants remains to be discovered, however, because among the known constitutive dauer-formers, only *daf-19* mutants are Dyf.

Mutations in several other genes not represented in our collection are known to affect dye-filling, but these mutations are also associated with other, more striking phenes, such as uncoordinated movement, which we consciously avoided. HEDGECOCK *et al.* (1985), *e.g.*, noted that mutations in *unc-33* or *unc-44*, genes that affect the outgrowth of many axons, result in weak dye filling. Similarly, mutations in *unc-104*, which encodes a kinesin-like protein (OTSUKA *et al.* 1991; HALL and HEDGECOCK 1991), or *unc-101*, which encodes a clathrin-associated protein (LEE *et al.* 1994), result in both severe uncoordination and abolishment of dye filling. Mutants such as these are more readily identified on the basis of their uncoordinated phenotype. On the other hand, it might be profitable in future screens for Dyf mutants to look for mutants that are defective in dye filling of phasmid neurons only, a class we did not seek. Mutations in two genes, *lin-17* and *lin-44*, are known to cause this phenotype, in each case because the fates of the postembryonically derived phasmid socket cells are altered (STERNBERG and HORVITZ 1988; HERMAN and HORVITZ 1994). Such mutants are unlikely to show known chemosensory defects, since phasmids appear to be less important than amphids for chemosensation.

Among the 25 genes for which we found mutations, 14 are now represented by three or more alleles, 3 have two alleles each and 8 are defined by single mutant alleles. The spectra of genes identified in our different screens were all similar. The large proportion of single-mutant genes strongly suggests that additional genes in the Dyf class remain to be discovered. It seems plausible that at least some of the mutations in the allele-sparse set of genes are special alleles rather than simple gene knockouts, in order to account for the low incidence of multiple alleles. The one *dyf-13* mutation may be responsible for slow growth and reduced fertility, which suggests that *dyf-13(+)* plays a role in developmental processes beyond those involved in chemosensation. We also suggest from the sickly phenotype of *dyf-1/Df* animals that *dyf-1(+)* may have an essential developmental role. The *dyf-12* mutation also did not behave as a simple null: *dyf-12/Df* appears to be non-Dyf, as if two mutant copies of *dyf-12* are required to produce the Dyf phenotype. This is similar to the behavior of a set of recessive suppressors of *glp-1* (in the genes *sog-1*, *sog-4* and *sog-6*), which do not suppress when placed opposite deficiencies (MAINE and KIMBLE 1993). The obvious suggestion is that the *sog* and *dyf-12* mutations are weak gain-of-function alleles, required in two doses to confer a mutant phenotype. Both alleles of *dyf-2* may be acting similarly, although in this case we cannot be sure that *dyf-2* is deleted by *eDf2*. It would not be surprising if the null phenotype of some *dyf* genes, possibly including *dyf-12* and *dyf-2*, is wild type, given the high gene estimates from the *C. elegans* genome sequencing project compared with estimates of genes giving recessive visible and lethal phenotypes (WATERSTON *et al.* 1992). If this idea were correct for *dyf-2* and *dyf-12*, *e.g.*, it should be possible to obtain phenotypic revertants of the *dyf-2* and *dyf-12* mutations by intragenic suppression. For at least six of the *dyf* genes, it should be possible to screen directly for new alleles that fail to complement existing mutations, and such screens should yield null alleles because for each of these genes—*dyf-1*, *dyf-5*, *dyf-6*, *dyf-7*, *dyf-9* and *dyf-10*—we have shown that *dyf/Df* is viable and Dyf.

Only two of the genes studied here have been subjected to mosaic analysis. With respect to the dye-filling phenotype, it was concluded that the focus of *osm-1* action is within the dye-filling neurons themselves and that the focus of *daf-6* action is within the sheath cell (HERMAN 1987). These conclusions are consistent with the electron microscopic studies of these genes (ALBERT *et al.* 1981; PERKINS *et al.* 1986). Mosaic analysis of other *dyf* genes would be useful. Furthermore, once an anatomical focus for a mutant has been established, the analyzed gene might prove useful as a cell-specific marker for the mosaic analysis of other, closely linked genes.

We thank E. LUNDQUIST for help with some experiments. This work was supported by National Institutes of Health (NIH) research grants GM-22387 (R.K.H.), HD-11239 (D.L.R.) and GM-48700 (J.H.T.). J.C. was a recipient of a fellowship from the Catalonian Comissionat per a Universitats i Recerca. Some nematode strains were supplied by the Caenorhabditis Genetics Center, which is supported by a contract between the NIH National Center for Research Resources and the University of Minnesota.

LITERATURE CITED

ALBERT, P. S., S. J. BROWN and D. L. RIDDLE, 1981 Sensory control of dauer larva formation in *Caenorhabditis elegans*. J. Comp. Neurol. **198:** 435–451.

ALBERTSON, D. G., and J. N. THOMSON, 1976 The pharynx of *Caenorhabditis elegans*. Philos. Trans. R. Soc. Lond. B Biol. Sci. **275:** 299–325.

BARGMANN, C. I., 1993 Genetic and cellular analysis of behavior in *C. elegans*. Annu. Rev. Neurosci. **16:** 47–71.

BARGMANN, C. I., and H. R. HORVITZ, 1991a Chemosensory neurons with overlapping functions direct chemotaxis to multiple chemicals in *C. elegans*. Neuron **7:** 729–742.

BARGMANN, C. I., and H. R. HORVITZ, 1991b Control of larval development by chemosensory neurons in *Caenorhabditis elegans*. Science **251:** 1243–1246.

BARGMANN, C. I., J. H. THOMAS and H. R. HORVITZ, 1990 Chemosensory cell function in the behavior and development of *Caenorhabditis elegans*. Cold Spring Harb. Symp. Quant. Biol. **55:** 529–538.

BARGMANN, C. I., E. HARTWIEG and H. R. HORVITZ, 1993 Odorant-selective genes and neurons mediate olfaction in *C. elegans*. Cell **74:** 515–527.

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

CASSADA, R. C., and R. L. RUSSELL, 1975 The dauerlarva, a post-embryonic developmental variant of the nematode *Caenorhabditis elegans*. Dev. Biol. **46:** 326–342.

CHALFIE, M., and M. AU, 1989 Genetic control of differentiation of the *Caenorhabditis elegans* touch receptor neurons. Science **243:** 1027–1033.

CHALFIE, M., and J. E. SULSTON, 1981 Developmental genetics of the mechanosensory neurons of *Caenorhabditis elegans*. Dev. Biol. **82:** 258–270.

CHALFIE, M., and J. WHITE, 1988 The nervous system, pp. 337–391 in *The Nematode Caenorhabditis elegans*, edited by W. B. WOOD. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

COLLINS, J., B. SAARI and P. ANDERSON, 1987 Activation of a transposable element in the germ line but not the soma of *Caenorhabditis elegans*. Nature **328:** 726–728.

COLLINS, J., E. FORBES and P. ANDERSON, 1989 The Tc3 family of transposable genetic elements in *Caenorhabditis elegans*. Genetics **121:** 47–55.

CULOTTI, J. G., and R. L. RUSSELL, 1978 Osmotic avoidance defective mutants of the nematode *Caenorhabditis elegans*. Genetics **90:** 243–256.

DUSENBERY, D. B., 1974 Analysis of chemotaxis in the nematode *Caenorhabditis elegans* by countercurrent separation. J. Exp. Zool. **188:** 41–47.

DUSENBERY, D. B., 1975 The avoidance of D-tryptophan by the nematode *Caenorhabditis elegans*. J. Exp. Zool. **193:** 413–418.

GOLDEN, J. W., and D. L. RIDDLE, 1984a The *Caenorhabditis elegans* dauer larva: developmental effects of pheromone, food, and temperature. Dev. Biol. **102:** 368–378.

GOLDEN, J. W., and D. L. RIDDLE, 1984b A pheromone-induced developmental switch in *Caenorhabditis elegans*: temperature-sensitive mutants reveal a wild-type temperature-dependent process. Proc. Natl. Acad. Sci. USA **81:** 819–823.

GOLDEN, J. W., and D. L. RIDDLE, 1985 A gene affecting production of the *Caenorhabditis elegans* dauer-inducing pheromone. Mol. Gen. Genet. **198:** 534–536.

HALL, D. H., and E. M. HEDGECOCK, 1991 Kinesin-related gene *unc-104* is required for axonal transport of synaptic vesicles in *C. elegans*. Cell **65:** 837–847.

HALL, D. H., and R. L. RUSSELL, 1991 The posterior nervous system of the nematode *Caenorhabditis elegans*: serial reconstruction of identified neurons and complete pattern of synaptic interactions. J. Neurosci. **11:** 1–22.

HARTMAN, P. S., 1987 Caffeine-resistant mutants of *Caenorhabditis elegans*. Genet. Res. **49:** 105–110.

HEDGECOCK, E. M., J. G. CULOTTI, J. N. THOMSON and L. A. PERKINS, 1985 Axonal guidance mutants of *Caenorhabditis elegans* identified by filling sensory neurons with fluorescein dyes. Dev. Biol. **111:** 158–170.

HERMAN, R. K., 1987 Mosaic analysis of two genes that affect nervous system structure in *Caenorhabditis elegans*. Genetics **116:** 377–388.

HERMAN, M. A., and H. R. HORVITZ, 1994 The *Caenorhabditis elegans* gene *lin-44* controls the polarity of asymmetric cell divisions. Development **120:** 1035–1047.

HERMAN, R. K., and E. M. HEDGECOCK, 1990 Limitation of the size of the vulval primordium of *Caenorhabditis elegans* by *lin-15* expression in surrounding hypodermis. Nature **348:** 169–171.

HODGKIN, J., 1980 More sex-determination mutants of *Caenorhabditis elegans*. Genetics **96:** 649–664.

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

HODGKIN, J., M. EDGLEY, D. L. RIDDLE and D. G. ALBERTSON, 1988 Genetics, pp. 491–584 in *The Nematode Caenorhabditis elegans*, edited by W. B. WOOD. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

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

LEE, J., G. D. JONGEWARD and P. W. STERNBERG, 1994 *unc-101*, a gene required for many aspects of *Caenorhabditis elegans* development and behavior, encodes a clathrin-associated protein. Genes Dev. **8:** 60–73.

LEWIS, J. A., and J. A HODGKIN, 1977 Specific neuroanatomical changes in chemosensory mutants of the nematode *Caenorhabditis elegans*. J. Comp. Neurol. **172:** 489–510.

LI, W., and J. E. SHAW, 1993 A variant Tc4 transposable element in the nematode *C. elegans* could encode a novel protein. Nucleic Acids Res. **21:** 59–67.

LINK, C. D., M. A. SILVERMAN, M. BREEN, K. E. WATT and S. A. DAMES, 1992 Characterization of *Caenorhabditis elegans* lectin-binding mutants. Genetics **131:** 867–881.

LUNDQUIST, E. A., and R. K. HERMAN, 1994 The *mec-8* gene of *C. elegans* affects muscle and sensory neuron function and interacts with three other genes: *unc-52, smu-1* and *smu-2*. Genetics **138:** 83–101.

MAINE, E. M., and J. KIMBLE, 1993 Suppressors of *glp-1*, a gene required for cell communication during development in *Caenorhabditis elegans*, define a set of interacting genes. Genetics **135:** 1011–1022.

MAINLAND, D. L., L. HERRERA and M. I. SUTCLIFFE, 1956 *Statistical Tables for use with Binomial Samples, Contingency Tests, Confidence Limits and Sample Size Estimates*. Department of Medical Statistics, New York University School of Medicine, New York.

MALONE, E. A., and J. H. THOMAS, 1994 A screen for nonconditional dauer-constitutive mutations in *Caenorhabditis elegans*. Genetics **136:** 879–886.

MORI, I., D. G. MOERMAN and R. H. WATERSTON, 1988 Analysis of a mutator activity necessary for germline transposition and excision of Tc1 transposable elements in *Caenorhabditis elegans*. Genetics **120:** 397–407.

OTSUKA, A. J., A. JEYAPRAKASH, J. GARCIA-ANOVEROS, L. Z. TANG, G. FISK *et al.*, 1991 The C. elegans *unc-104* gene encodes a putative kinesin heavy chain-like protein. Neuron **6:** 113–122.

PERKINS, L. A., E. M. HEDGECOCK, J. N. THOMSON and J. G. CULOTTI, 1986 Mutant sensory cilia in the nematode *Caenorhabditis elegans*. Dev. Biol. **117:** 456–487.

POLITZ, S. M., M. PHILIPP, M. ESTEVEZ, P. J. O'BRIEN and K. J. CHIN, 1990 Genes that can be mutated to unmask hidden antigenic determinants in the cuticle of the nematode *Caenorhabditis elegans*. Proc. Natl. Acad. Sci. USA **87:** 2901–2905.

RIDDLE, D. L., 1988 The dauer larva, pp. 393–412 in *The Nematode Caenorhabditis elegans*, edited by W. B. WOOD. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

RIDDLE, D. L., M. M. SWANSON and P. S. ALBERT, 1981 Interacting genes in nematode dauer larva formation. Nature **290:** 668–671.

STERNBERG, P. W., and H. R. HORVITZ, 1988 *lin-17* mutations of *Caenorhabditis elegans* disrupt certain asymmetric cell divisions. Dev. Biol. **130:** 67–73.

SULSTON, J., and J. HODGKIN, 1988 Methods, pp. 587–606 in *The Nematode Caenorhabditis elegans*, edited by W. B. WOOD. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

SULSTON, J. E., D. G. ALBERTSON and J. N. THOMSON, 1980 The *Caenorhabditis elegans* male: postembryonic development of nongonadal structures. Dev. Biol. **78:** 542–576.

SULSTON, J. E, E. SCHIERENBERG, J. G. WHITE and J. N. THOMSON, 1983 The embryonic cell lineage of the nematode *Caenorhabditis elegans*. Dev. Biol. **100:** 64–119.

THOMAS, J. H., 1993 Chemosensory regulation of development in *C. elegans*. BioEssays **15:** 791–797.

VOWELS, J. J., and J. H. THOMAS, 1992 Genetic analysis of chemosensory control of dauer formation in *Caenorhabditis elegans*. Genetics **130:** 105–123.

WARD, S., 1973 Chemotaxis by the nematode *Caenorhabditis elegans*: Identification of attractants and analysis of the response by use of mutants. Proc. Natl. Acad. Sci. USA **70:** 817–821.

WARD, S., J. N. THOMSON, J. G. WHITE, S. BRENNER, 1975 Electron microscopical reconstruction of the anterior sensory anatomy of the nematode *Caenorhabditis elegans*. J. Comp. Neurol. **160:** 313–338.

WARE, R. W., D. CLARK, K. CROSSLAND and R. L. RUSSELL, 1975 The nerve ring of the nematode *Caenorhabditis elegans*: sensory input and motor output. J. Comp. Neurol. **162:** 71–110.

WATERSTON, R., C. MARTIN, M. CRAXTON, C. HUYNH, A. COULSON *et al.*, 1992 A survey of expressed genes in *Caenorhabditis elegans*. Nature Genet. **1:** 114–123.

WHITE, J. G., E. SOUTHGATE, J. N. THOMSON and S. BRENNER, 1976 The structure of the ventral nerve cord of *Caenorhabditis elegans*. Philos. Trans. R. Soc. Lond. B Biol. Sci. **275:** 327–348.

WHITE, J. G., E. SOUTHGATE, J. N. THOMSON and S. BRENNER, 1986 The structure of the nervous system of *Caenorhabditis elegans*. Philos. Trans. R. Soc. Lond. B Biol. Sci. **314:** 1–340.

YUAN, J., M. FINNEY, N. TSUNG and H. R. HORVITZ, 1991 Tc4, a *Caenorhabditis elegans* transposable element with an unusual fold-back structure. Proc. Natl. Acad. Sci. USA **88:** 3334–3338.

Communicating editor: I. GREENWALD