The Genetics of Feeding in *Caenorhabditis elegans*

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ABSTRACT

The pharynx of *Caenorhabditis elegans* is a nearly self-contained neuromuscular organ responsible for feeding. To identify genes involved in the development or function of the excitable cells of the pharynx, I screened for worms with visible defects in pharyngeal feeding behavior. Fifty-two mutations identified 35 genes, at least 22 previously unknown. The genes broke down into three broad classes: 2 *pha* genes, mutations in which caused defects in the shape of the pharynx, 7 *phm* genes, mutations in which caused defects in the contractile structures of the pharyngeal muscle, and 26 *eat* genes, mutants in which had abnormal pharyngeal muscle motions, but had normally shaped and normally birefringent pharynxes capable of vigorous contraction. Although the Eat phenotypes were diverse, most resembled those caused by defects in the pharyngeal nervous system. For some of the *eat* genes there is direct evidence from previous genetic mosaic and pharmacological studies that they do in fact affect nervous system. In *eat-5* mutants the motions of the different parts of the pharynx were poorly synchronized. *eat-6* and *eat-12* mutants failed to relax their pharyngeal muscles properly. These pharyngeal motion defects are most easily explained as resulting from abnormal electrical excitability of the pharyngeal muscle membrane.

WITH the important exception of recent work on ion channel function in Drosophila [reviewed by PAPAZIAN *et al.* (1988)], classical genetic analysis has played little role in the understanding of excitable cell function. Yet genetics has much to offer neurobiologists. Genetic analysis allows identification of functionally important molecules independent of their abundance, straightforward isolation of the genes that encode those molecules, and determination of the consequences of gene dysfunction in the whole organism. These strengths would be useful for identifying ion channels and the molecules that modulate them, and for determining how these molecules together produce distinctive excitability properties that lead to normal behavior.

The genetic studies of Drosophila ion channels prove the truth of these assertions. For instance, the *Shaker* potassium channel, identified by the hyperexcitability of mutant flies, has become the best understood of all voltage-gated ion channels (HILLE 1992). Indeed, genetic investigations of electrical excitability in Drosophila have dominated the discovery of new potassium channels [see, *e.g.*, WARMKE, DRYSDALE and GANETZKY (1991)]. The availability of molecular probes has allowed the isolation of potassium channel genes from other species [reviewed by KACZMAREK (1991)]. The value of the Drosophila system suggests that other model systems with complementary strengths might help in understanding excitability. In this paper I report a genetic investigation, the results of which suggest that the pharynx of *Caenorhabditis elegans* has potential to become such a system.

The pharynx, which is responsible for getting food into the worm, is a simple, self-contained neuromuscular pump (ALBERTSON and THOMSON 1976; AVERY and HORVITZ 1989). It contains 80 nuclei: 5 belonging to gland cells, 18 to miscellaneous structural cells, 37 to muscle cells, and 20 to pharyngeal neurons (ALBERTSON and THOMSON 1976; SULSTON *et al.* 1983). The entire structure is surrounded by a basement membrane, which isolates it from the rest of the worm (ALBERTSON and THOMSON 1976). The 20 neurons constitute the pharyngeal nervous system, which regulates feeding (AVERY and HORVITZ 1987, 1989). The pharyngeal nervous system is self-contained. There is one bilaterally symmetric pair of connections between the pharyngeal nervous system and the extrapharyngeal nervous system (ALBERTSON and THOMSON 1976), but this connection is dispensable for normal feeding: the only known consequence of its elimination is that feeding fails to be regulated by certain stimuli sensed by the extrapharyngeal nervous system (J. E. SULSTON, M. C. CHALFIE, J. H. THOMAS and G. GARRIGA, personal communication; L. AVERY and D. RAIZEN, unpublished). The simplicity of the pharynx and its functional independence make it possible to study how the organ as a whole produces and controls behavior.

Feeding is of course essential for life. Of the 20 pharyngeal neurons, however, only M4 is essential for life (AVERY and HORVITZ 1987). A worm lacking all 19 remaining neurons is viable and fertile (AVERY and

HORVITZ 1989). Although they are not essential for life, these neurons are important for normal feeding, and for normal growth rates and fertility levels. In at least three cases (I5, MC, and M3), killing just one type of pharyngeal neuron causes a change in feeding behavior together with a decrease in growth rate (AVERY and HORVITZ 1989; AVERY 1993). The effects are more striking when multiple neuron types are killed (AVERY and HORVITZ 1989). Therefore I expected to be able to isolate mutations in genes necessary for pharyngeal nervous system function by screening for viable mutants with abnormal feeding behavior.

In fact, most of the 35 genes identified in this screen had phenotypes similar to those seen when pharyngeal neurons are killed with a laser microbeam. However, mutations in a small class of three genes, *eat-5*, *eat-6* and *eat-12*, produced changes in pharyngeal muscle motions different from those expected to result from nervous system dysfunction. A mutation in another gene, *eat-11*, causes hypersensitivity to an acetylcholine agonist. Their mutant phenotypes suggest these four genes may be involved in controlling the excitability of pharyngeal muscles.

MATERIALS AND METHODS

General methods: General methods for handling and observing worms were as described by SULSTON and HODGKIN (1988), except that worms were routinely grown on NGMSR agar, instead of NGM. NGMSR differs from NGM in containing 200 μg/ml streptomycin sulfate, 10 μg/ml nystatin, and 2% agar instead of 1.7%. (Streptomycin and nystatin reduce bacterial and fungal contamination. The higher agar concentration delays the burrowing of the worms into the agar.) The plates were seeded with a spontaneous streptomycin-resistant mutant of *Escherichia coli* OP50 (BRENNER 1974). Worms were routinely kept at 20°

Mutations used:

Chromosome I: bli-4(e937), dpy-5(e61), dpy-14(e188), eat-5(ad464), eat-9(e2337), eat-11(ad541), eat-15(ad602), eat-16(ad702), eDf3, eDf4, eDf5, eDf6, eDf9, eDf12, eDf15, eDf16, hDp62, let-75(s101), let-201(e1716), let-202(e1720), let-203(e1717), lin-10(e1439), lin-11(n566), nDf23, nDf24, nDf25, phm(ad699), phm-2(ad538, ad597), qDf3, sDp2, unc-11(ad571, e47), unc-13(e51, e450), unc-29(e403, e1072), unc-54(e190, e1092), unc-57(ad516, ad592, e402), unc-59(e261), unc-75(e950), unc-89(ad539, e1460, e2338), unc-101(m1).

Chromosome II: dpy-10(e128), eat-2(ad453, ad465, ad570, ad692), eat-3(ad426), exp-1(sa6), jDf1, jDf4, lin-7(e1413), mnDf16, mnDf30, mnDf31, mnDf39, mnDf67, mnDf68, mnDf69, mnDf88, mnDf89, mnDf96, ric-2(ad596), rol-6(e187), unc-4(e120), unc-52(e444), unc-104(e1265, rh43, rh142).

Chromosome III: daf-2(e1368), daf-7(e1372), dpy-1(e1), dpy-17(e164), dpy-18(e364), dpy-19(e1259), eat-4(ad537, ad572), eat-8(ad599, ad697), eDf2, eDp6, emb-9(hc70), fem-2(e2105), glp-1(e2144, q339), lin-12(n137 n720), lon-1(e185), mec-12(e1605), nDf20, phm-3(ad493), qDf2, qDp3(III; f), sma-2(e502), unc-16(e109), unc-32(e189), unc-36(ad698, e251, e2341), unc-45(r450), unc-93(e1500), vab-7(e1562).

Chromosome IV: bli-6(sc16), ced-2(e1752), cha-1(p1152), deb-1(st555), dpy-9(e12), dpy-13(e184), dpy-20(e1282, e2017), eat-1(ad427, e2343), eat-7(ad450), eat-10(ad606), eat-12(ad695), eDf18, eDf19, let-59(s49), lin-1(e1777), lin-45(sy96), pha-3(ad607), sDf2, sDf8, sDf9, sDf10, sDf60, sDf62, sDf63, sDf64, sDf67, sem-3(n1655, n1905, s1734), unc-8(n491), unc-17(e113, e245), unc-24(e138), unc-26(ad473, ad701, ad706, e205, e2340), unc-30(e191), unc-31(e169, e928), unc-44(e362).

Chromosome V: act(ad468 ad767, st15), ali-1(e1934), ctDf1, dpy-11(e224), eat-6(ad467), egl-10(n692), him-5(e1490), phm(n2251), rol-3(e754), sDf20, sDf26, sDf27, sDf28, sDf35, sqt-3(sc63), unc-46(e177), unc-76(e911).

Chromosome X: aex-3(ad418, ad696, sa5), che-2(e1033), dpy-3(e27), dpy-6(e14), eat-13(ad522), eat-14(ad573), eat-17(ad707), egl-15(n484), egl-17(e1313), let-2(g37), lin-15(n309, n765), lon-2(e678), maDf1, mec-4(e1611), mnDf4, mnDf41, nDf19, pha-2(ad472), phm(ad598), sma-5(n678), sup-10(n183), unc-1(e538, e1598), unc-2(ad463, e55, e2342), unc-3(e151), unc-6(e78), unc-10(ad591, e102), unc-18(ad486, e81), unc-20(e112), unc-58(e665), unc-78(e1217), unc-110(e1913).

Balancers: eT1(III; V), dpy-10(e128) unc-52(e444) mnC1 II, nT1(IV; V), dpy-19(e1259ts,mat) glp-1(q339) qC1 III, lon-2(e678) szT1(X; I).

Parental strains for mutant screens: All but three of the new mutations described here were isolated in an *unc-31(e928)* mutant background. Three *unc-31* parent strains were used: DA456, DA509 and DA640. DA456 was constructed by R. HOSKINS (personal communication) from CB928, the original *unc-31(e928)* isolate (BRENNER 1974), by crossing once with N2, the Bristol strain used as the standard wild type in *C. elegans* genetics, and then reisolating an *unc-31* homozygote. DA509 was constructed from DA456 by repeating the N2 backcross 10 times. DA640 is isogenic with a different wild strain, RC301, which has many restriction fragment length differences from N2 (HODGKIN and BARNES 1991; T. M. BARNES, personal communication). DA640 was constructed by outcrossing a *dpy-20(e1282ts) unc-31(e928) unc-30(e191)* triple mutant against RC301 10 times and separating the *unc-31* mutation from *dpy-20* and *unc-30* by recombination.

Isolation of mutations: The F_1 or F_2 self-progeny of *unc-31* hermaphrodites mutagenized with ethane methylsulfonate (EMS) or x-rays were screened for feeding-defective mutants. Mutants were recognized on dissecting microscope examination by their visibly abnormal feeding motions or starved appearance. Mutants with a starved appearance were examined by Nomarski microscopy, and only those with abnormal feeding motions were kept. Constipated, egg-laying-defective, or flaccid paralyzed (*i.e.*, probable body wall muscle-defective) mutants whose pumping was slow were not kept, since these defects can cause slow pumping (my unpublished observations).

Worms to be screened were gotten by one or more of five methods: F1V (F_1 visible), F2V (F_2 visible), F2SG (F_2 slow growth), F1BA (F_1 brood arrest), and F1BV (F_1 brood visible). In the F1V screen, designed to enrich for dominant mutations, mixed viable F_1 self-progeny of mutagenized hermaphrodites were individually examined. In the F2V screen, mixed viable F_2 progeny were individually examined. These methods were biased against strong feeding-defective mutants, which grew slowly and were unable to reach a size that allowed convenient recognition before their healthy nieces and cousins ate all the food on the dish. The F2SG, F1BV, and F1BA screens were designed to circumvent this problem. In the F2SG screens, slow growing F_2s were enriched, either by $1 \times g$ velocity sedimentation or hand-picking, allowed to grow, and examined individually. [For velocity sedimentation, worms anesthetized in about 300 μl 10 mM sodium azide in M9 buffer (SULSTON and HODGKIN, 1988) were layered onto 3 ml 10% Ficoll, 500 μg/ml kanamycin. After about 10 min adults had settled to

the bottom. About 0.5 ml liquid containing small worms was removed from the top with a Pasteur pipet.] In the F1BA and F1BV screens single gravid adult F_1 progeny of mutagenized *unc-31* hermaphrodites were placed on individual plates, allowed to lay eggs for a day, then removed. In the F1BV screen, the plates were screened for ones that bore about 1/4 viable feeding-defective mutant progeny. In the F1BA screen, the plates were screened for ones that bore about 1/4 progeny that appeared to be arrested as starved larvae, and these starved larvae were transferred to a new plate where they did not have to compete with their sisters and nieces for food. The large majority of these apparently arrested larvae were truly arrested and were eventually discarded. Some however were merely slow-growing and gave rise to feeding-defective mutant strains. The F1BV and F1BA screens were the most successful and were the source of most of the mutations (see Table 1). Those F_1s screened by both the F1BA and F1BV screens are the most useful for looking at the statistical properties of feeding-defective screens, since together the two screens should detect viable feeding-defective mutations with near 100% efficiency.

The M4I (M4-independent) screen selected for mutants able to survive without the essential pharyngeal neuron M4 (AVERY and HORVITZ 1987). M4 was laser-killed in 679 newly hatched F_2 self-progeny of mutagenized *unc-29(e1072amber)* hermaphrodites, and worms that grew up were retested for ability to survive without M4. [The *unc-29* mutation was used to reduce problems with worms crawling off the plate (AVERY and HORVITZ 1987).] Because of larval lethal mutations, the effective size of the screen was about 400 F_2s. No high-penetrance M4-independent mutants were isolated, but among the worms that survived was one homozygous for a new *eat-6* mutation, *ad792*. Preliminary experiments suggest that about 10% of *eat-6(ad467)* worms can survive laser killing of M4 (data not shown).

All mutations in the same gene were derived from independently mutagenized P_0s, with the possible exception of the two *eat-1* mutations *e2343* and *ad427*, which both arose from the same large mixed brood. These are treated as independent, since they have different properties. (*ad427* is stronger than *e2343*.)

Two mutations, *unc-57(ad516)* and *phm(n2251)* were gifts from J. H. THOMAS and E. JORGENSEN, respectively. Table 1 summarizes the sources of mutations.

Initial analysis of mutants: I began the analysis of each mutation isolated in the *unc-31* background in the same way. First, the new mutation was outcrossed by mating with *unc-31/+* males and re-isolating the mutation in the F_2 progeny. Usually it was possible to separate the mutation from *unc-31*. During this outcross *X*-linkage and linkage to the *unc-31IV* were detected. I discarded any mutations that did not act like single Mendelian loci, or whose phenotypes proved too difficult to score. The outcross was then repeated with N2 males, and a single F_2 feeding-defective mutant homozygote was used to create a strain. If the mutation did not produce a strong Unc phenotype, I usually constructed a *him-8* or *him-5* double mutant as a source of homozygous males (HODGKIN, HORVITZ and BRENNER 1979).

After I had become familiar with some feeding-defective mutants, I was often able to recognize that a mutation was likely to be a new allele of a previously known gene based on its phenotype. When this occurred a complementation test was done, and if the new mutation was indeed allelic, it was kept but not further analyzed.

New mutations that were not recognized as allelic with known mutations and that had not been localized to *IV* or *X* during backcrossing were mapped to a chromosome using the protocol described by TRENT, TSUNG and HORVITZ (1983), except that instead of using two strains MT464 and MT465 marked on three chromosomes each, I used the single strain DA438 *bli-4(e937)*, *I*; *rol-6(e187)* *II*; *daf-2(e1368ts)* *vab-7(e1562)* *III*; *unc-31(e928)* *IV*; *dpy-11(e224)* *V*; *lon-2(e678)* *X*, marked on all six chromosomes. In addition to simplifying mapping, DA438 allowed a more stringent test of genetic simplicity than MT464 and MT465, since pseudolinkage between any pair of chromosomes would have been detected.

Mapping and complementation tests: Genetic map data, summarized in Table 2, were obtained using standard techniques or variations on standard techniques (SULSTON and HODGKIN 1988).

ad596 was assigned to the gene *ric-2* on the basis of its genetic map location (see Table 2, Figure 2) and a complementation test done by J. RAND (personal communication). The Phm mutant strain DA468 was shown by L. A. SCHRIEFER and R. H. WATERSTON (personal communication) to carry mutations in the closely linked *act-2* and *act-3* genes (FILES, CARR, and HIRSH 1983; WATERSTON, HIRSH and LANE 1984), to which I assigned the allele names *ad468* and *ad767*, respectively. Since these two mutations are inseparable for practical purposes, I treat them as a single mutation *act(ad468 ad767)* from here on. It is not known whether the *act(ad468 ad767)* mutant phenotype is caused by *act-2(ad468)*, *act-3(ad767)*, or both.

All other gene assignments were based on genetic criteria: two mutations were considered to affect the same gene if they failed to complement and were closely linked. All complementation tests and deletion mapping were done using protocols that allowed positive identification of the desired heterozygote. In almost every case (the exceptions are noted in RESULTS AND DISCUSSION) these heterozygotes were fertile. To test for second-site noncomplementation, self-progeny broods of noncomplementing mutant heterozygotes were examined whenever possible. A double heterozygote *a/+*; *b/+* between two unlinked noncomplementing recessive mutations should produce at least 5/16 phenotypically wild-type progeny, while there should be no wild-type progeny if the two mutations are closely linked. All noncomplementing mutations were closely linked by this test.

Male mating was not carefully tested. If 10 homozygous mutant males produced abundant cross progeny in a mating with marked hermaphrodites, the mutant is scored as + for male mating in Table 3. Mating was usually tested as part of genetic mapping with the hermaphrodite partner strain DA438 (described above). Male mating "+" corresponds roughly to HODGKIN's ME2 or ME3, and "−" to ME0 or ME1 (HODGKIN 1983).

Estimation of gene numbers: Gene numbers were estimated by a maximum likelihood method. Likelihoods and confidence intervals were calculated using a distribution (derived below) for the number of distinct genes detected, with the total number of genes that could be detected and the number of mutations isolated as parameters. This method is slightly more rigorous than the usual Poisson method of estimating gene numbers, but the main purpose of deriving the distribution was to allow the calculation of confidence intervals.

Suppose there are t genes in which mutations are detected at equal frequencies. m independent mutations have been isolated, and those m mutations identify g distinct genes from among the t. Each gene can be thought of as a letter in an alphabet with t letters. A particular list of mutations in a particular order constitutes a word. Let c_{tgm} be the number of words m letters long that contain exactly g distinct

letters. Such a word is derived from a word $m-1$ letters long by the addition of a new letter. There are two ways this can be done. First, the old word could have been one of the $c_{t,g-1,m-1}$ words that contain $g-1$ distinct letters, and one of the $t-g+1$ letters not in the old word was added to it. Second, the old word could have been one of the $c_{t,g,m-1}$ that contain g distinct letters, and one of these g letters was added to it. This results in the following recursive formula for c_{tgm}:

$$c_{tgm} = (t-g+1)c_{t,g-1,m-1} + gc_{t,g,m-1}.$$

It is obvious that $c_{tgm} = 0$ whenever $g > m$ or $g > t$, and that $c_{t1m} = t$ for any t and m. These equations, together with the recursion, allow the computation of c_{tgm} for any t, g and m. Since the total number of words m letters long is simply t^m, the probability p_{tgm} of identifying exactly g distinct genes in a collection of m independent mutations isolated from a set of t genes detected with equal probability is:

$$p_{tgm} = \frac{c_{tgm}}{t^m}.$$

Starting with experimental values for g and m, I used this distribution to compute confidence intervals and maximum likelihood estimates for t. It might be argued that such estimates are pointless, based as they are on an implausible assumption of equal frequencies for gene detection. However, the estimates are useful as lower bounds. One always expects to detect more genes if mutation and detection frequencies are equal than if they are unequal. More precisely, the cumulative distribution function (CDF) of g, the probability of detecting g or fewer genes, is minimized when all t genes are detected at equal frequencies. Since confidence intervals are computed from the CDF, the lower bound of the confidence interval is conservative.

An independent estimate of the number of genes was derived from the recovery of 6/12 previously known *unc* genes that produce an Eat phenotype as described in RESULTS AND DISCUSSION. The confidence interval for that estimate was derived from the binomial distribution, which is correct under the assumption that each of the twelve genes had an equal probability of showing up in the feeding-defective screen, and that this probability is equal to that that any feeding-defective gene would have been identified. The first assumption is conservative: if there is in fact a range of probabilities, the distribution of the number of *unc*s recovered would become narrower, leading to a narrower confidence interval. Thus the only important assumption is that the genes that mutate to an Unc feeding-defective phenotype are representative of all genes that mutate to feeding-defective in their probability of recovery.

Photography: The pictures in Figures 1 and 4a were taken using a Bio-Rad MRC-600 confocal microscope with the transmitted light option. These images were then processed using the free software X windows image viewer XV (BRADLEY 1992), modified to allow reading of MRC-600 PIC files and some simple image processing. The images in Figure 1, a, c, e, and g, were all taken with identical microscope settings and were identically processed, so they can be directly compared. Figure 1, panels b, c, f, and h, were also obtained and processed identically to each other. The remaining pictures were photographed on Kodak Technical Pan film and developed with Diafine developer. Scale bars were measured from simultaneous pictures of a stage micrometer.

Arecoline sensitivity: Plates containing the acetylcholine agonist arecoline were made by placing 0.6 ml of a 20× concentrated stock solution on a 12 ml NGMSR plate and waiting until the liquid had soaked in. To test strains for sensitivity to arecoline, I placed five gravid adult hermaphrodites on a bacteria-seeded NGMSR plate containing 5 mM arecoline. The plate was examined and the age of the oldest F_1 and F_2 progeny noted daily either until all the bacteria had been eaten, or until at least two weeks had passed. If the plate starved (*i.e.*, the worms ate all the bacteria), the time to starvation was also noted. (Although it is also affected by the efficiency of reproduction, time to starvation is a useful crude measure of the rapidity with which worms consume food.) Wild-type worms and strains homozygous for the reference alleles of the following genes have been tested: *cha-1*, *eat-1*, *eat-2*, *eat-3*, *eat-4*, *eat-6*, *eat-7*, *eat-8*, *eat-9*, *eat-10*, *eat-11*, *eat-12*, *phm-3* and *unc-36*. *eat-3*, *eat-10* and *eat-11* growth was arrested by 5 mM arecoline. Most *eat-2* and *unc-36* worms arrested immediately after hatching as first-stage larvae, but a few were able to pass the L1 block and become fertile adults. Because the mutations slow growth in the absence of arecoline, and 5 mM arecoline slows wild-type growth, this experiment alone could not distinguish hypersensitivity from an additive effect of the drug and the mutation. *eat-3* and *eat-10* in particular have very strong effects on growth even in the absence of arecoline, and I felt that their sensitivity to arecoline could not be interpreted. To test whether *eat-2*, *eat-11* and *unc-36* were truly hypersensitive, I repeated the experiment, but varied the concentration of arecoline. Only *eat-11* was hypersensitive to lower concentrations than 5 mM. Details are described in RESULTS AND DISCUSSION.

Videotape analysis: L1 hermaphrodites were mounted for Nomarski microscopy (SULSTON and HORVITZ 1977) and videorecordings made with a Hitachi KP-160 black-and-white solid-state videocamera and a Panasonic AG-1960 Super-VHS videocassette recorder. After viewing the tapes and selecting a few seconds during which the worm pumped and there was little motion to blur the pharyngeal muscles, I played the tape back at 1/30 original speed into a RasterOps SPARC Card TV in a Sun SPARCStation 2. There are 30 frames/sec, each frame consisting of two interleaved images, the first comprising the odd lines, and the second the even lines. The AG-1960 at 1/30 speed plays these alternately, thus displaying a new image twice a second, showing the pharynx as it was at 1/60-sec intervals. I wrote programs to capture these images and store them as computer files, then later to automatically adjust contrast and brightness and translate into GIF format. Finally, they were examined sequentially using XV (BRADLEY 1992), and the motion of the corpus and terminal bulb in each image recorded as either relaxing, contracting, or stationary. In addition, the position was estimated at every change of motion (*e.g.*, from relaxing to stationary) as either fully relaxed, fully contracted, slightly relaxed, or slightly contracted.

Two points must be emphasized to avoid overinterpretation of the plots of Figure 3. First, although the abscissa, time, is quantitative, the ordinate, state of contraction, is partially schematic, since I recorded the extent of contraction only at changes of motion, and distinguished only four extents of contraction. Between changes the motion is arbitrarily shown as linear. Second, I have analyzed very few worms by this method. My purpose was to represent in static form the pharyngeal motions I had seen while watching behavior. I chose for analysis worms whose motions appeared typical. Therefore, although the plots are quantitatively accurate pictures of the timing of pharyngeal motions in one individual and qualitatively typical of the motions of worms of the indicated genotype, I do not know that they are quantitatively typical.

Antibody staining of embryos: Antibody staining methods were adapted from those described by PRIESS and THOMSON (1987) and SULSTON and HODGKIN (1988) as follows. Embryos were picked off a plate into 5 μl of the buffer described by SULSTON *et al.* (1983) for *Turbatrix aceti* embryos on a slide coated with poly-L-lysine, covered with a cover slip, frozen on an aluminum slab on dry ice, and the cover slip pried off with a razor blade. The slides were fixed with methanol and acetone at −20°, rehydrated through an ethanol series, washed three times with PBS + 0.5% Tween 20, incubated 1 hr in primary antibody, washed three times with PBS + 0.5% Tween 20, incubated 2 hr at room temperature and overnight at 4° in fluorescein-labeled secondary antibody, then mounted under a coverslip in a drop of DABCO (diazabicyclo[2.2.2]octane) in buffered glycerol and viewed with epifluorescence optics.

RESULTS AND DISCUSSION

Isolation of feeding-defective mutants: The screen for feeding-defective mutants was very simple in essence: *unc-31* hermaphrodites were mutagenized, and their F_2 self-progeny examined for defective feeding. (I also isolated a few F_1 feeding-defective worms, in the hope of obtaining dominant mutations.) The *unc-31* background, although not essential, made the screen easier since *unc-31* mutants usually lie still, and their pharynxes pump rapidly and continuously (L. AVERY and H. R. HORVITZ, unpublished data). Not surprisingly, strong feeding-defective mutants grow slowly, and most screens incorporated some trick to prevent their being overgrown by their wild-type nieces (described in MATERIALS AND METHODS).

Feeding-defective worms were recognized in two ways. First, the motions of the grinder, a cuticular specialization in the terminal bulb of the pharynx that breaks bacteria up (Figure 1b), can be easily seen using a dissecting microscope. I looked for worms in which its motion was visibly abnormal. Second, I looked for worms that had a starved appearance. Worms whose pharyngeal nervous systems have been partially or completely destroyed (AVERY and HORVITZ 1987; AVERY and HORVITZ 1989; AVERY 1993), are small and pale (*e.g.*, Figure 1, g and h). Of course, a small, pale mutant need not be starved, and a starved mutant need not have a feeding defect. (It might, for instance, have an intestinal defect that interferes with nutrient uptake.) I therefore used Nomarski differential interference contrast microscopy to examine the feeding motions of mutants isolated on the basis of starved appearance.

Starved appearance turned out to be the more useful of the two phenotypes. Every mutation isolated on the basis of abnormal grinder motion also caused a starved appearance. Furthermore, the majority of mutants with an obviously starved appearance had feeding defects. Figure 1 shows the appearance of wild type (Figure 1, a and b) and of weak (Figure 1, c and d), medium (Figure 1, e and f), and strong (Figure 1, g and h) feeding-defective mutant adult hermaphrodites. Feeding-defective mutants consistently had lighter intestinal color than wild type. The mutants also tended to be smaller and thinner than wild type, and adult hermaphrodites generally contained fewer eggs. Wild-type worms that are starved because of food deprivation or pharyngeal nervous system defects have a similar appearance (data not shown).

I isolated 49 mutations by screening for feeding-defective mutants. Two more, *ad592* and *n2251*, were gifts from J. H. THOMAS and E. JORGENSEN, respectively, and one, *ad792*, was isolated in a screen for mutants that could survive without the essential pharyngeal neuron M4 (AVERY and HORVITZ 1987). The screens are summarized in Table 1. By genetic mapping (Table 2) and complementation tests these 52 mutations were assigned to 35 genes (Table 3) distributed over all six chromosomes (Figure 2). At least 22 of the genes were previously unknown: *aex-3*, *eat-1* through *eat-17*, *pha-2*, *pha-3*, *phm-2* and *phm-3*. [*aex-3* was independently discovered by J. H. THOMAS (1990).] Table 4 shows how the mutations were distributed among genes. The distribution was not significantly different from a Poisson.

How many feeding genes are there? Feeding-defective mutants arose at high frequency. This is best seen by examining the results of the F1BA and F1BV screens, whose design ensured a nearly 100% efficiency of recovery. Using a standard EMS mutagenesis protocol, known to have an approximately 5×10^{-4} probability of inactivating an average gene (BRENNER 1974; GREENWALD and HORVITZ 1980), I isolated 10 mutations in a screen of 317 F_1 progeny of EMS-mutagenized hermaphrodites (Table 1), a frequency of 0.016 (0.008–0.028 with 95% confidence) per mutagenized haploid genome. The high frequency suggested that many genes (about 30) were capable of mutating to the feeding-defective phenotype.

The large number of genes was confirmed by mapping and complementation tests: the 52 feeding-defective mutations defined 35 genes: 26 *eat*s, 2 *pha*s and 7 *phm*s. I found only one allele of most of these genes, suggesting there are many more genes to be discovered (Table 4). By assuming visible mutations in all genes were recovered with equal probability (see MATERIALS AND METHODS), the number of feeding genes could be estimated at 60 (44–95 with 95% confidence), of which 25 are still unknown. Since in reality different genes probably give rise to visible mutations at differing frequencies, and some genes involved in feeding might not give rise to visible feeding-defective mutations at all (they might be redundant or essential, for instance), this number is a lower bound.

The number of genes that mutate to feeding-defective can be estimated by an independent method. Mutations in 12 *unc* genes (*unc-2*, *unc-10*, *unc-11*,

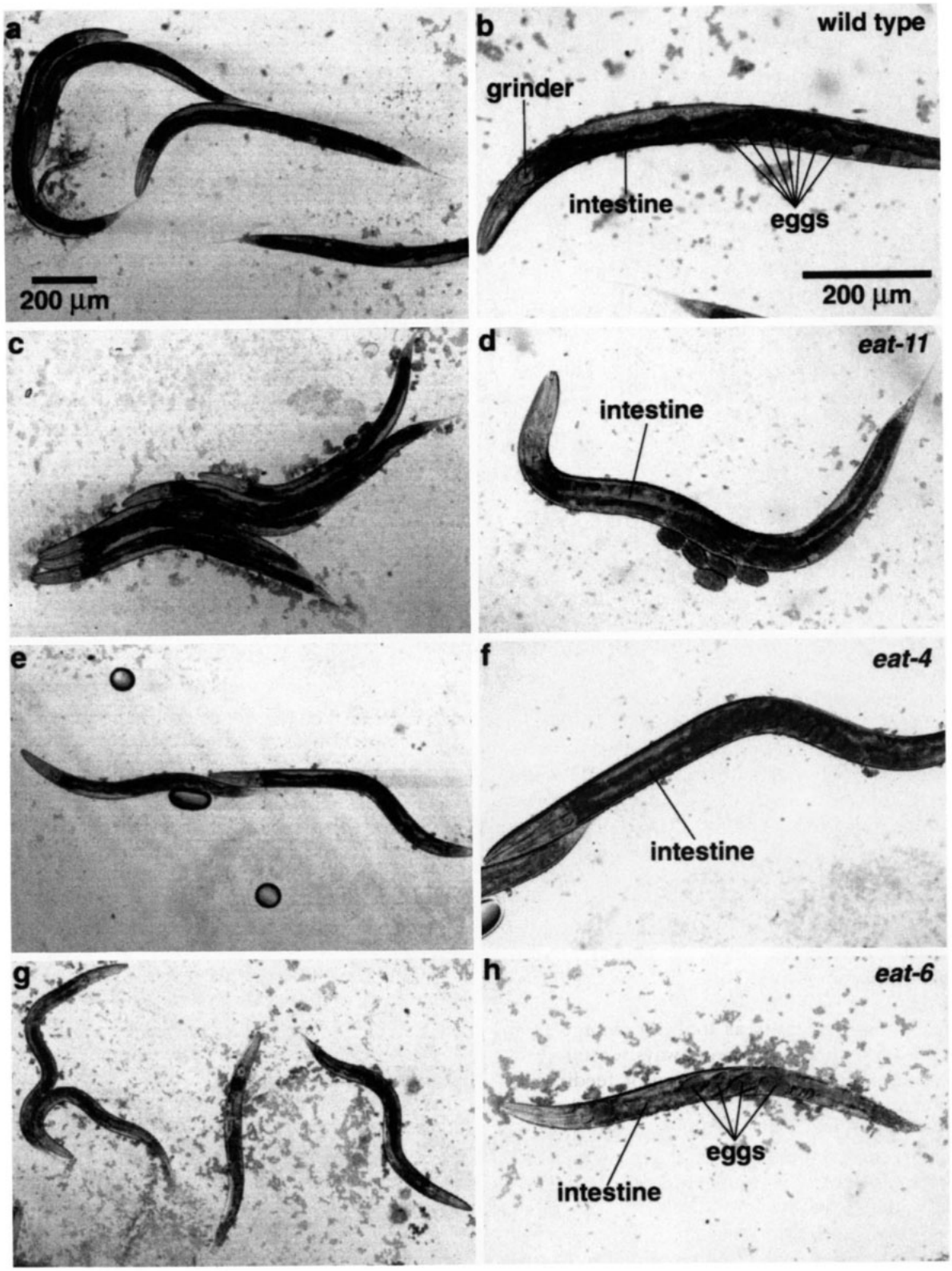

FIGURE 1.—Starved phenotypes of feeding-defective mutants. The appearance of wild-type (a, b), and representative Eat mutants: *eat-11*(*ad541*) *I*, a weak Eat mutant (c, d), *eat-4*(*ad572*) *III*, a weak to medium-strong Eat mutant, and *eat-6*(*ad467*) *V*, a medium-strong Eat mutant. Panels a, c, e and g show low magnification (scale bar in a) bright-field images of several worms, and panels b, d, f and h higher magnification (scale bar in b) Nomarski images of single worms, anterior to the left and ventral down. The Nomarski images are negatives for easier comparison with the bright-field images. Starved worms have fewer refractile granules in intestinal cells, making them appear pale. Strongly starved mutants are small and thin, and tend to retain fewer eggs than wild type. A strong starved phenotype such as that of *eat-6* homozygotes can be easily distinguished from wild type by an inexperienced observer. With practice the medium-strength starved phenotype of *eat-4* mutants can also be reliably scored. A weak phenotype such as that of *eat-11* mutants is difficult for even an experienced observer to score reliably, although a brood of *eat-11* worms can easily be distinguished from a brood of wild-type worms.

TABLE 1

Mutant screen summary

Mutagenesis	No. screened[a]	Screen(s)	Mutants isolated
EMS	2,350	F2V	8
EMS[b]	22,000	F2SG	6
		F2V	0
EMS[b]	2,558	F1V	4
6000 rad x-ray	4,785	F2V	1
7500 rad x-ray	4,000	F2V	0
EMS	317	F1BV	7
		F1BA	3
7500 rad x-ray	390	F1BV	1
9000 rad x-ray	1,138	F1BV	10
		F1BA	8
EMS	400	M4I	1
EMS	Unknown	Gifts	2

This table summarizes the sources of feeding-defective mutations. Each row represents a group of progeny of mutagenized worms screened by one or more methods. The screens, described in detail in MATERIALS AND METHODS, are F1V (F_1 visible), F2V (F_2 visible), F2SG (F_2 slow growth), F1BV (F_1 brood visible), F1BA (F_1 brood arrest), and M4I (M4-independent).

[a] For the F2SG screen, the "No. screened" is the number of F_2 worms from which slow growing worms were selected. For F2V, it is the number of F_2s directly examined for feeding defects. For F1V, it is the number of F_1s directly examined for feeding defects. For F1BV and F1BA, it is the number of F_1 broods examined for feeding-defective F_2 progeny.

[b] About ¼ of these 22,000 were the progeny of 1,450 worms screened by the F1V method.

unc-13, *cha-1/unc-17*, *unc-18*, *unc-26*, *unc-32*, *unc-37*, *unc-57*, *unc-75* and *unc-104*) were known to cause an Eat phenotype (HODGKIN *et al.* 1988; J. H. THOMAS, personal communication; L. AVERY, unpublished). I isolated new alleles of six, half of them. This suggests the 35 genes found were half the total that could be found, thus providing an estimate of 70 (44–165 with 95% confidence).

These two independent estimates suggest that there are at least 60 genes necessary for proper feeding behavior, half or more of them still genetically uncharacterized.

Use of feeding mutants as tools to change food intake: Behavior changes in many ways when worms are deprived of food. This collection of feeding-defective mutants allows one to distinguish whether the failure to ingest (and the ensuing starvation) or the failure of sensory neurons to detect food in the environment influences a particular behavior. For instance, the defecation cycle stops in the absence of bacteria (THOMAS 1990). THOMAS (1990) used *eat-2* and *unc-89* mutants to show that bacterial intake has only a small effect on the period of the defecation cycle.

Food deprivation biases worms towards entering the dauer state (GOLDEN and RIDDLE 1984). However, the collection of 52 mutants, ranging from ones that are barely detectably starved to others that are so defective in feeding they require extraordinary effort to maintain as homozygous strains, includes only two that caused a dauer-constitutive phenotype: the two *aex-3* mutants. All 52 formed dauers when deprived of food. Another effect of food deprivation is to prevent egg-laying, so that the eggs hatch inside and eat the mother, forming a bag of worms. None of the mutants formed bags of worms in the presence of food. I therefore propose that bacteria influence dauer formation and egg-laying via sensory neurons, not (or at least not solely) because of effects on the nutritional state of the worm.

Summary of the mutant phenotypes: Most of the 35 genes fit clearly into three broad categories: *pha*s, *phm*s and *eat*s. Mutations in *pha* genes caused defects in the shape of the pharynx (Figure 4). *phm* mutations caused defects in the contractile structures of the pharyngeal muscle (Figure 5); their defining characteristic was abnormal pharyngeal muscle birefringence. *eat* mutations caused defects in pharyngeal muscle motions, but mutants had normally shaped and normally birefringent pharynxes capable of vigorous contraction. The only genes whose classifications were uncertain were *phm-2*, whose mutant phenotype could be equally well described as a pharynx deformity or a muscle abnormality (Figure 6), and *eat-14*, mutation of which had an effect on pharyngeal muscle motions somewhat similar to that of *phm-2*, but caused no obvious deformity or birefringence abnormality.

Descriptions of feeding mutant phenotypes: Except where otherwise indicated, the phenotype described below is that of the reference allele (Table 3). For ease of description I have grouped together some genes that produce similar phenotypes. The grouping need not imply a fundamental similarity of mechanism. Where I am fairly certain that a mutation identifies a new gene, I have given it a full gene name beginning with *eat*, *pha* or *phm*, *e.g.*, *eat-11*, *pha-2* and *phm-3*. Three mutations without gene names, *phm(ad598)*, *phm(ad699)* and *phm(n2251)*, have been less characterized and may be alleles of known genes. However, with the possible exception of *phm(ad598)* (see below), these mutations identify new feeding-defective genes in the sense that no allelic mutations have been previously described to produce a viable, fertile, feeding-defective phenotype.

Individual descriptions of *eat* genes:

eat-10 I, *eat-13 X*, *eat-15 I*, *eat-16 I*, *eat-17 X*: Mutations in these genes caused a phenotype I call "slippery pharynx" (AVERY 1993). Bacteria in the corpus and isthmus were not efficiently moved posteriorly during pharyngeal pumping. In *eat-13*, *eat-15* and *eat-17* mutants all three parts of the pharynx were affected: corpus and isthmus were slippery, and the timing of terminal bulb contractions was also slightly abnormal. *eat-10* and *eat-16* mutants had strong slip-

TABLE 2

Map data

Gene	Data
act(ad468 ad767) V	<2 map unit from *act(st15)* *dpy-11* (9/18) *ad468 ad767* (4/18) *him-5* (5/18) *unc-76* *dpy-11* (6/22) *rol-3* (16/22) *ad468 ad767* *(ad468 ad767 sqt-3)* (11/11) *egl-10* *(ad468 ad767 sqt-3 egl-10)* (1/1) *unc-76* *sDf20*, *sDf35* and *ctDf1* complement *ad468 ad767*[a]
eat-1 IV[b]	0.43 (0.26–0.7) map unit from *dpy-20* 0.11 (0.04–0.26) map unit from *sem-3* *unc-24* (18/33) *eat-1* (15/33) *unc-31* *unc-24* (19/20) *eat-1* (1/20) *dpy-20* *eat-1* (4/11) *dpy-20* (7/11) *unc-31* *eat-1* (2/13) *let-59* (11/13) *dpy-20* *eat-1* (5/19) *sem-3* (14/19) *dpy-20* *eDf18*, *eDf19*, *sDf8*, *sDf10*, and *sDf60* do not uncover *eat-1* *sDf2*, *sDf62*, *sDf63*, and *sDf67* uncover *eat-1*
eat-2 II	0.34 (0.21–0.49) map unit from *unc-52* *(eat-2 lin-7)* (2/2) *unc-52* *eat-2* (2/2) *(lin-7 unc-52)* *eat-2* (16/25) *lin-7* (9/25) *unc-52* *jDf1* and *jDf4* do not uncover *eat-2*
eat-3 II	<5 map unit from *rol-6* *dpy-10* (13/17) *ad426* (4/17) *unc-4* *mnDf30*, *mnDf67*, *mnDf69*, and *mnDf88* do not uncover *eat-3* *mnDf16*, *mnDf68*, and *mnDf89* uncover *eat-3*
eat-4 III	0.18 (0.09–0.33) map unit from *unc-32* *dpy-17* (26/26) *unc-32 eat-4)* *(eat-4 unc-16)* (23/23) *dpy-18* *sma-2* (2/2) *(unc-32 eat-4)* *unc-32* (3/12) *eat-4* (9/12) *emb-9* *sma-2* (1/1) *(unc-32 eat-4 emb-9)* *unc-32* (10/10) *(eat-4 glp-1)* *unc-32* (9/9) *(lin-12 eat-4)* *nDf20* does not uncover *eat-4* *qDp3* covers *eat-4* *qDf2* uncovers *eat-4*[c]
eat-5 I	1.3 (0.5–2.7) map unit from *dpy-5* 0.9 (0.3–2.2) map unit from *unc-29* *dpy-5* (11/22) *eat-5* (11/22) *unc-29* *dpy-5* (11/11) *(unc-13 eat-5)* *dpy-5* (4/4) *(eat-5 dpy-14)* *(eat-5 dpy-14)* (18/18) *unc-29* *dpy-14* (51/69) *let-75* (2/69) *eat-5* (16/69) *unc-13*
eat-6 V	*dpy-11* (7/12) *ali-1* (5/12) *eat-6* *dpy-11* (31/37) *eat-6* (3/37) *him-5* (3/37) *unc-76* *sqt-3* (1/3) *eat-6* (2/3) *unc-76* *sqt-3* (10/24) *eat-6* (14/24) *egl-10* *sDf20*, *sDf35*, and ±*f11* do not uncover *eat-6*
eat-7 IV	*dpy-9* (7/15) *eat-7* (8/15) *lin-1* *eat-7* is right of *ced-2*
eat-8 III	<5 map unit from *dpy-1* *(eat-8 dpy-1)* (9/9) *lon-1* *unc-45* (1/12) *eat-8* (6/12) *dpy-1* (5/12) *phm-3* *unc-45* (1/13) *fem-2* (3/13) *daf-7* (9/13) *eat-8*
eat-9 I	7 (2–19) map unit from *unc-54* 9 (3–22) map unit from *dpy-5* *dpy-5* (5/9) *eat-9* (4/9) *unc-54* *unc-101* (5/5) *(unc-59 eat-9)* *(eat-9 let-201)* (6/6) *unc-54* *let-202* (3/12) *eat-9* (9/12) *unc-54* *(eat-9 let-203)* (9/9) *unc-54* *unc-101* (6/6) *(eat-9 unc-59)* *eDf3* uncovers *eat-9* *eDf4*, *eDf5*, *eDf6*, *eDf9*, *eDf12*, *eDf15*, and *eDf16* do not uncover *eat-9*
eat-10 IV	<0.6 map unit from *dpy-9* *(eat-10 dpy-9)* (8/8) *lin-1*
eat-11 I	2.0 (0.7–4.3) map unit from *unc-29* 1.3 (0.4–3.3) map unit from *dpy-5* *dpy-5* (9/9) *(unc-13 eat-11)* *dpy-5* (4/4) *(eat-5 eat-11)* *(eat-11 eat-5)* (9/9) *unc-29* *dpy-14* (6/6) *(eat-5 eat-11)* *(eat-11 lin-10)* (8/8) *unc-29* *nDf24* and *nDf25* uncover *eat-11* *nDf23* partially fails to complement *eat-11*[d]
eat-12 IV[e]	*(eat-12 unc-24)* (22/22) *(eat-1 unc-31)* *(eat-12 unc-24)* (10/10) *(pha-3 unc-31)* *dpy-13* (19/45) *eat-12* (26/45) *unc-31* *unc-17* (42/42) *(dpy-13 eat-12)* *bli-6* (14/20) *eat-12* (6/20) *unc-24* *lin-45* (1/2) *eat-12* (1/2) *unc-24* *unc-44* (5/5) *(deb-1 eat-12)* *unc-8* (5/7) *eat-12* (2/7) *unc-24*
eat-13 X	0.4 (0.15–0.7) map unit left of *unc-58* *lon-2* (9/9) *(unc-6 eat-13)* *(unc-10 unc-110)* (9/9) *eat-13*
eat-14	*lon-2* (26/26) *(unc-6 eat-14)* *lon-2* (2/3) *unc-6* (1/3) *eat-14* *egl-15* (3/4) *eat-14* (1/4) *sma-5* *nDf19* and *maDf1* uncover *eat-14*
eat-15 I	*dpy-5* (3/3) *(eat-5 eat-15)* *(eat-15 eat-5)* (4/4) *unc-29* *dpy-14* (5/7) *eat-15* (2/7) *eat-5* *hDp62* and *sDp2* do not cover *eat-15*
eat-16 I	Linked to *bli-4* *eat-5* (10/10) *(unc-29 eat-16)* *unc-29* (6/15) *eat-16* (9/15) *lin-11*
eat-17 X	*unc-3* (26/26) *(lin-15 eat-17)*[f] *lin-15* (22/26) *eat-17* (4/26) *sup-10*[f] *lin-15* (12/17) *(let-2 eat-17)* (5/17) *sup-10* *mnDf41* uncovers *eat-17* *mnDf4* does not uncover *eat-17*

TABLE 2

Continued

Gene	Data
pha-2 X	0.6 (0.2–1.3) map unit from *unc-1* 0.1 (0.05–0.2) map unit from *egl-17* *pha-2* (22/22) *(lon-2 dpy-6)* *pha-2* (16/31) *unc-20* (15/31) *lon-2* *pha-2* (6/6) *(unc-1 dpy-3)* *(pha-2 unc-1)* (2/2) *dpy-3* *egl-17* (3/10) *pha-2* (7/10) *unc-1* *(che-2 pha-2)* (17/17) *unc-1* *(che-2 egl-17)* (11/11) *pha-2*
pha-3 IV	*eat-1* (4/13) *pha-3* (9/13) *unc-31* *sDf8*, *sDf9*, and *sDf64* do not uncover *pha-3* *sDf10*, *sDf62*, and *sDf67* uncover *pha-3*
phm(ad598) X	*(ad598 lon-2)* (7/7) *unc-6*
phm(ad699) I	<5 map unit from *bli-4*
phm(n2251) V	4.0 (2.7–5.7) map unit left of *dpy-11* *n2251* (19/32) *unc-46* (13/32) *dpy-11* *sDf26*, *sDf27*, and *sDf28* uncover *n2251*
phm-2 I	2 (0.04–9) map unit from *bli-4* 0.18 (0.08–0.35) map unit left of *unc-75* *dpy-5* (8/12) *eat-5* (4/12) *(unc-29 phm-2)* *eat-5* (12/12) *(unc-29 phm-2)* *unc-29* (7/7) *(lin-11 phm-2)* *unc-29* (32/32) *(phm-2 unc-75)* *unc-29* (10/22) *lin-11* (12/22) *(unc-75 phm-2)*
phm-3 III	5 (1–16) map unit from *dpy-17* 4.2 (2.4–6.8) map unit from *dpy-1* *(phm-3 daf-2)* (10/10) *dpy-17* *phm-3* (9/22) *daf-2* (13/22) *dpy-17* *unc-45* (12/12) *(dpy-1 phm-3)* *unc-45* (13/22) *dpy-1* (9/22) *phm-3* *(unc-45 dpy-1)* (19/19) *(phm-3 mec-12)* *phm-3* (4/8) *mec-12* (4/8) *daf-2* *eDf2* does not uncover *phm-3*
ric-2 II[g]	0.07 (0.03–0.14) map unit right of *dpy-10* 0.05 (0.02–0.12) map unit left of *unc-104* *(ric-2 dpy-10)* (10/10) *unc-4* *exp-1* (7/7) *(dpy-10 ric-2)*[f] *dpy-10* (7/12) *ric-2* (5/12) *unc-104* *mnDf16*, *mnDf31*, *mnDf39*, *mnDf68*, and *mnDf88* do not uncover *ric-2* *mnDf30* and *mnDf96* uncover *ric-2*
unc-2 X[h]	*unc-2* (6/20) *lon-2* (14/20) *lin-15*
unc-36 III[i]	*dpy-17* (17/21) *unc-36* (4/21) *unc-32*
unc-57 I[j]	0.6 (0.2–1.5) map unit from *dpy-5* 1.1 (0.5–2.1) map unit from *unc-11* *unc-11* (9/16) *unc-57* (7/16) *dpy-5*
unc-89 I[k]	<3 map unit from *dpy-5* *(unc-89 dpy-5)* (14/14) *unc-13* *unc-11* (5/20) *unc-89* (15/20) *dpy-5* *qDf3* does not uncover *unc-89*

This table summarizes data regarding the location of mutations on the *C. elegans* genetic map. Except where otherwise noted, the reference allele of each gene was used. The data are of three types: two-point measurements of recombination distances, three- or more-point determinations of gene order and relative distances, and complementation tests with deficiencies and duplications. Two-point measurements are reported in map units, each representing a 1% probability of recombination, with 95% confidence intervals in parentheses. Three- and four-point measurements are given as "*gene1 (l/t) gene2 (r/t) gene3*" or "*gene1 (t/t) (gene2 gene3)*". The first format means that of *t* independent recombination events between *gene1* and *gene3*, *l* were between *gene1* and *gene2*, and *r* were between *gene2* and *gene3*. The second format means that all of *t* independent recombination events between *gene1* and *gene2* were between *gene1* and *gene3*. Although the data do not definitively order *gene2* and *gene3*, their order within the parentheses is significant.

[a] These data probably do not imply that *ad468 ad767* lies outside the deficiencies, since a deficiency acts like a wild-type allele in complementation tests with dominant actin mutations.

[b] *e2343* was used for all *eat-1* mapping.

[c] These data were obtained by R. LEE.

[d] The *eat-11/nDf23* heterozygote shows an Eat phenotype, but it is weaker than that of an *eat-11* homozygote.

[e] All *eat-12* mapping was done by R. LEE.

[f] These data were obtained by D. RAIZEN.

[g] *ric-2(ad596)* was used in these experiments.

[h] *unc-2(e2342)* was used in these experiments.

[i] *unc-36(e2341)* was used in this experiment.

[j] *unc-57(ad592)* was used in these experiments.

[k] *unc-89(e2338amber)* was used in these experiments.

pery isthmus and corpus phenotypes, but I did not notice any abnormality in terminal bulb action.

eat-1 IV, *eat-11 I*, *unc-36 III*: In addition to feeding, each of these genes affected locomotion and body shape. Mutations in all three caused worms to look slightly longer and thinner than normal. *eat-1* worms pumped very slowly and irregularly, and moved somewhat sluggishly. *unc-36* mutations caused slow, irregular pumping in addition to a slippery corpus and isthmus. Movement was very sluggish and loopy. Among these three genes, *eat-11* mutations produced the mildest phenotype. Mutants had a slippery corpus and slightly loopy movement, but the rate of pumping and the rate of movement were normal.

eat-11 mutants were hypersensitive to the cholinergic agonist arecoline, which increases pharyngeal muscle contraction (AVERY and HORVITZ 1990; L. AVERY, unpublished). Wild-type worms grew well on medium containing 5 mM arecoline: a plate inoculated with five adult hermaphrodites produced thousands

TABLE 3

Feeding-defective mutations

Gene	Mutations	Mutagen	Screen[a]	Feeding phenotype	Other phenotypes	Male mating
eat genes: 41 mutations, 26 genes						
aex-3 X	***ad418***	EMS	F2V	Slow, long-lasting pumps in *unc-31* background	Defecation defective, also dauer-constitutive in *unc-31* background	+
	ad696	EMS				
eat-1 IV	***ad427***[b]	EMS	F2V	Slow pumping	Long, thin appearance. Sluggish movement	ND
	e2343[b]	EMS	F2V			
eat-2 II	***ad465***	EMS	F2SG	Slow, regular pumping		
	ad453	EMS	F1V			
	ad570	X-ray	F1BV			
	ad692	EMS	F1BV			
eat-3 II	***ad426***	EMS	F2V	Slow, irregular pumping	Sluggish movement. Seldom recovers from dauer. Low fertility, some embryonic lethality	ND
eat-4 III	***ad572***	X-ray	F1BV	Weak relaxation-defective		+
	ad537	X-ray	F1BA			
eat-5 I	***ad464***	EMS	F2SG	Corpus and terminal bulb contractions unsynchronized		+
eat-6 V	***ad467***	EMS	F2SG	Strong relaxation-defective	Slightly Dpy	+
	ad601	X-ray	F1BV			
	ad792	EMS	M4I			
eat-7 IV	***ad450sd***	EMS	F1V	Falls asleep. Does not feed while asleep	While asleep, does not move or defecate	ND
eat-8 III	***ad599***	X-ray	F1BV	Brief, rare pumps	Slight coiler Unc	+
	ad697	EMS	F1BV			
eat-9 I	***e2337***	EMS	F2V	Irregular pumping		+
eat-10 IV	***ad606***	X-ray	F1BA	Slippery isthmus and corpus		+
eat-11 I	***ad541***	X-ray	F1BV	Slippery corpus	Long. Slight coiler Unc. Highly sensitive to arecoline	+
eat-12 IV	***ad695sd***	EMS	F1BV	Relaxation-defective	Slightly Dpy. Adult males often have blocked anus, or protruding spicules	−
eat-13 X	***ad522***	X-ray	F1BV	Slippery, slight relaxation defect		+
eat-14 X	***ad573***	X-ray	F1BV	Grinder fails to come to full relaxed position between pumps		+
eat-15 I	***ad602***	X-ray	F1BV	Slippery corpus, isthmus, and terminal bulb		+
eat-16 I	***ad702***	EMS	F1BA	Slippery isthmus and corpus	Partially suppresses *unc-31* Unc phenotype. Most die early in adulthood with transparent, shrunken appearance	ND
eat-17 X	***ad707***	EMS	F1BV	Slippery isthmus and corpus. Terminal bulb contractions mistimed		+
ric-2 II	*ad596*	X-ray	F1BA	Very slow, irregular pumping	Strong Unc: jerky backward motion	ND
unc-2 X	*e2342*	EMS	F2V	Slow, irregular pumping	Unc: kinker	+[c]
	ad463	EMS	F2SG			
unc-10 X	*ad591*	X-ray	F1BA	Slow, irregular pumping	Unc: strong coiler	+[c]
unc-11 I	*ad571*	X-ray	F1BA	Slow, irregular pumping	Unc: jerky backward motion	+[c]
unc-18 X	*ad486*	EMS	F1V	Very slow, irregular pumping	Unc: paralyzed, kinky	−[c]
unc-26 IV	*ad473*	EMS	F2SG	Slow, irregular pumping	Unc: paralyzed, kinky	−[c]
	ad701	EMS	F1BA			
	ad706	EMS	F1BV			
	e2340	EMS	F2V			
unc-36 III	*ad698*	EMS	F1BA	Slow, irregular pumping	Long, thin appearance. Unc: Sluggish, coiler	−[c]
	e2341	EMS	F2V			

TABLE 3

Continued

Gene	Mutations	Mutagen	Screen[a]	Feeding phenotype	Other phenotypes	Male mating
unc-57 I	*ad592*	X-ray	F1BV	Slow, irregular pumping	Unc: Strong kinker, active	+[c]
	ad516	EMS	JT			
pha genes: 2 mutations, 2 genes						
pha-2 X	***ad472***	EMS	F2SG	Deformed pharynx		+
pha-3 IV	***ad607***	X-ray	F1BA	Deformed pharynx		ND
phm genes: 9 mutations, 7 genes						
act V	*ad468 ad767*	EMS	F1V	Phm: patchy birefringence		ND
phm(ad598) X	***ad598***	X-ray	F1BA	Phm: disorganized terminal bulb birefringence	Unc: progressive flaccid paralysis	−
phm(ad699) I	***ad699***	EMS	F1BV	Phm: weak terminal bulb birefringence		+
phm(n2251) V	***n2251***	EMS	EJ	Phm: weak terminal bulb birefringence	Constipated: weak Exp contractions	ND
phm-2 I	***ad538***	X-ray	F1BV	Grinder cannot come to full forward position	Protruding male spicules	−
	ad597	X-ray	F1BV			
	ad597	X-ray	F1BV			
phm-3 III	***ad493***	X-ray	F2V	Phm: weak pharyngeal birefringence		+
unc-89 I	*e2338am*	EMS	F2V	Phm: generally weak birefringence, with bright peripheral arcs in terminal bulb		+
	ad539	X-ray	F1BV			
	ad539	X-ray	F1BV			

Mutations identified in the Eat screen are listed by gene. Genes are grouped into three broad categories: *pha*s, which had grossly deformed pharynxes, *phm*s, which had defective pharyngeal muscle, and *eat*s, which had abnormal pharyngeal motions, but no obvious defects in pharyngeal morphology or muscle contractile structures. Phenotypic descriptions are for the reference allele with the exception of *ric-2*, where the description is of *ad596*. Most of these genes were identified for the first time in this screen. When this is the case, the reference allele is shown in boldface. Others were new alleles of previously known genes. The reference alleles for these are *unc-2(e55)*, *unc-10(e102)*, *unc-11(e47)*, *unc-18(e81)*, *unc-26(e205)*, *unc-36(e251)*, *unc-57(e406)* and *unc-89(e1460)*. *ad468 ad767* is a special case, in that it is a mutation in the actin genes *act-2* and *act-3* (L. A. SCHRIEFER *and* R. H WATERSTON, personal communication), but has a phenotype different from all previously known mutations in the actin gene cluster.

[a] Screens were as follows: F2V: visible Eats among F_2 progeny of mutagenized POs. F2SG: slow-growing worms among F_2. F1V: visible Eats among F_1. F1BV: ¼ visible Eats in the broods of single F_1s. F1BA: ¼ larval arrest in the broods of single F_1s. M4I: M4 independent F_2. JT: gift from J. H. THOMAS. EJ: gift from E. JORGENSEN. See MATERIALS AND METHODS for details.

[b] *e2343* and *ad427*, although not definitely known to be independent, have different phenotypes.

[c] Male mating ability quoted from HODGKIN 1983.

of progeny and exhausted the food in 8 days, 3 days more than were required in the absence of arecoline. In contrast, 1 mM arecoline completely arrested growth of *eat-11* mutants in the L1 stage. In the absence of arecoline, *eat-11* worms grew to starvation in seven days. A minimum concentration of 1 mM was required to produce a visible effect on wild-type feeding. *eat-11* mutants showed a similar or stronger effect at 0.2 mM. This hypersensitivity was particularly striking because in the absence of arecoline the *eat-11* phenotype was quite mild, and indeed difficult to score.

eat-2 II, *eat-8 III*, *eat-9 I*: Mutations in these three genes caused slow pumping, but had little other effect. *eat-2* mutants had slow, strong, often regular pumping. In *eat-8* mutants pumps were brief and rare, but otherwise normal, except that they were sometimes so brief as to be only coordinated twitches. *eat-8* worms had slightly loopy movement. *eat-9* mutation caused slow, irregular pumping. There was no other obvious defect.

aex-3 X: *aex-3* was discovered independently by THOMAS (1990), who isolated *aex-3(sa5)* based on its defect in the aBoc (anterior body wall muscle contraction) and Exp (expulsion) steps of the defecation cycle. Like most of the *eat* mutations, *ad418* and *ad696* were first detected in an *unc-31* background. The *unc-31*; *aex-3* double mutant had three defects: very slow, irregular pumping (Eat), dauer-constitutivity (Daf-c), and failures of the aBoc and Exp steps of the defecation cycle [Aex; THOMAS (1990)]. The Aex phenotype was equally strong in *unc-31*; *aex-3* double mutants and *aex-3* single mutants. The *aex-3* single mutant, however, had nearly normal feeding (there was a

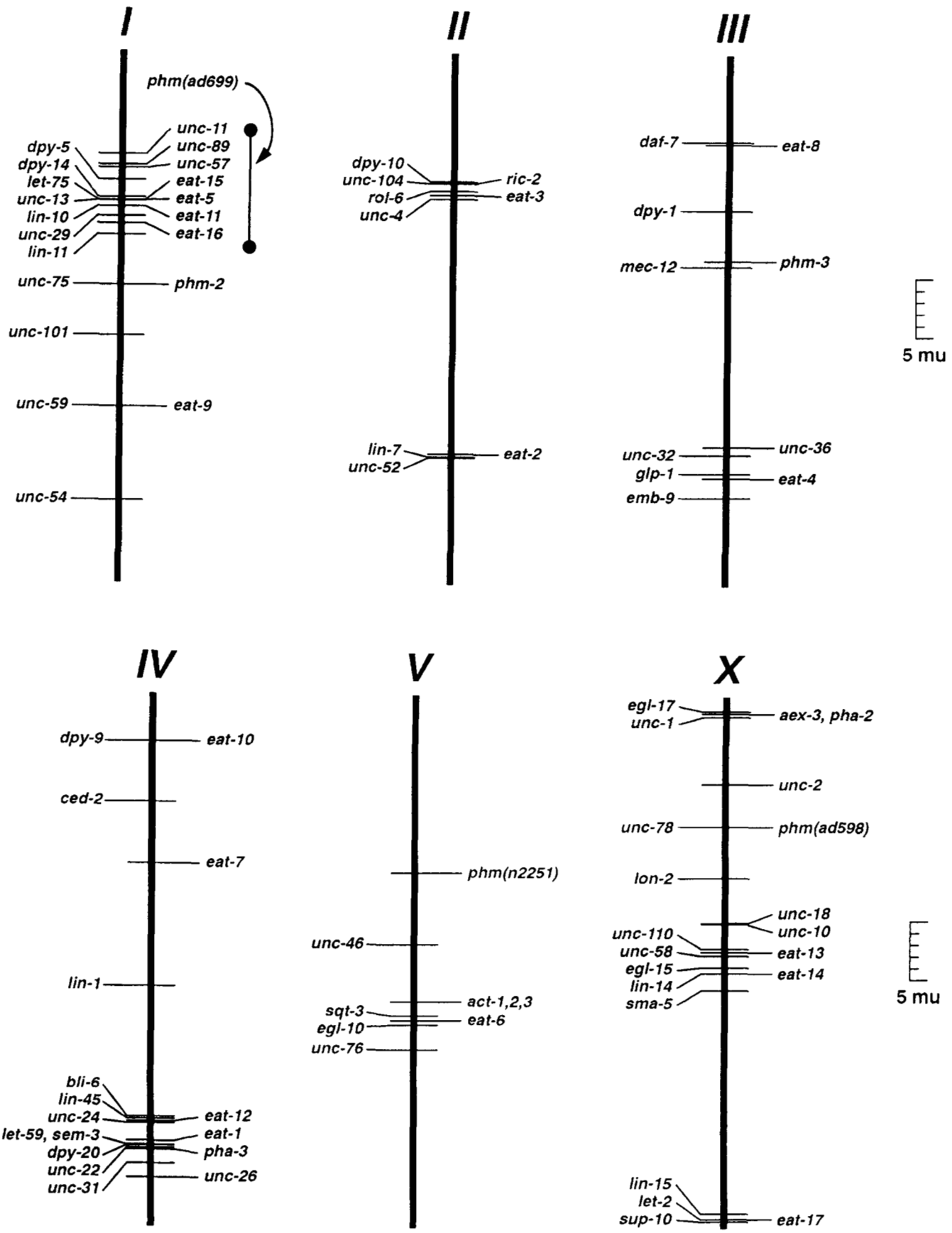

FIGURE 2.—Locations on the *C. elegans* genetic map of genes isolated in the Eat screen. Each chromosome is represented by a vertical line with the left end at the top. Genes identified by mutations found in this paper are shown to the right of the chromosome, and representative markers used for mapping to the left. The scales at right give distance in map units (mu), 1 mu corresponding to 1% recombination of 1 centimorgan. This figure is only intended as an overview of the locations of the genes, not as a comprehensive representation of all information about their positions. For complete information, see Table 2.

TABLE 4

Distribution of feeding-defective mutations by gene and phenotypic class

Mutations per gene	*eat*	*pha*	*phm*	All genes
1	16	2	5	23
2	7	0	2	9
3	1	0	0	1
4	2	0	0	2
Total genes	26	2	7	35
Total mutations	41	2	9	52

barely noticeable irregularity in pumping) and was not Daf-c.

The Eat and Daf-c phenotypes in the *unc-31* background were caused by the *aex-3* mutation, not a simultaneously induced mutation in a different gene. This was shown by two observations. First, each of the three double mutants *unc-31*; *ad418*, *unc-31*; *ad696*, and *unc-31*; *sa5* was Eat, Daf-c, and Aex. [*sa5* was originally isolated in an *unc-31*(+) background by its Aex phenotype (THOMAS 1990).] Second *unc-31*; *ad418/sa5* and *unc-31*; *ad418/ad696* heterozygotes were Eat Daf-c Aex.

eat-5 I: The *eat-5* mutation uncoupled the contractions of the corpus and terminal bulb (Figure 3b). In normal worms, all parts of the pharynx except the posterior isthmus contract nearly simultaneously (Figure 3a). A peristaltic wave of posterior isthmus contraction occurs after some but not all pumps (AVERY and HORVITZ 1987). The synchronization of the corpus and terminal bulb is very robust; it remains after the entire pharyngeal nervous system is killed (AVERY and HORVITZ 1989), in the presence of several drugs that affect feeding (data not shown), in the presence of relaxation-defective mutations (below) and even when the pharynx is dissected out of the worm (data not shown). In *eat-5* mutants the corpus and terminal bulb each contracted approximately normally, but they were no longer synchronized. The corpus usually pumped faster than the terminal bulb. Posterior isthmus peristalsis occurred after some but not all terminal bulb pumps. The unsynchronized corpus and terminal bulb contraction phenotype was fully penetrant in young larvae, and the *eat-5* mutation delayed growth during the larval stages. Pumping was synchronized in a few L4 worms, and about half of the *eat-5* adults pumped normally. *eat-5* adults whose pumping was unsynchronized were small and starved, while those with synchronized pumping were large and appeared well fed. These results suggested that either lack of synchronization or possibly the slow terminal bulb pumping decreased the intake of bacteria.

eat-4 III: In *eat-4*(*ad572*) mutants long-lasting, feeble pumps in which the lumen of the corpus failed to open to its full extent were interspersed with normal strong, brief pumps (Figure 3d). Long weak corpus contractions corresponded to long terminal bulb contractions of normal strength. The weaker allele *eat-4*(*ad537*) caused slightly long-lasting pumps, a mild slippery isthmus, and a temporary L1 arrest, but *ad537* adults were almost indistinguishable from wild type. *eat-4*(*ad572/ad537*) heterozygotes had a phenotype intermediate between those of the homozygotes. *eat-4*(*ad572*)/*qDf2* heterozygotes are similar in phenotype to *eat-4*(*ad572*) homozygotes (R. LEE, personal communication), so *ad572* may be a null mutation.

eat-12 IV: The semidominant mutation *eat-12*(*ad695sd*) delayed terminal bulb relaxation (Figure 3c). During the long terminal bulb contractions the adjacent muscles of the posterior isthmus and pharyngeointestinal valve also remained contracted, holding the empty lumen open. This suggests that the muscles were electrically excited rather than held in the contracted position by some mechanical impediment. *eat-12*/+ heterozygotes had a similar but weaker phenotype. The basis of *eat-12* semidominance is unknown, as there are no deficiencies known to uncover the region.

eat-6 V: *eat-6*(*ad467*) and *eat-6*(*ad792*) mutants were strongly defective in pharyngeal muscle relaxation (Figure 3f). *eat-6*(*ad601*) produced similar but weaker effects (Figure 3e). Although *ad467* homozygotes had a uniform, very starved appearance (Figure 1, g and h), the exact pumping defect varied from worm to worm. Figure 3f is typical. During the approximately five seconds recorded, the terminal bulb returned to its fully relaxed position only twice: once about three seconds into the trace, and once at the end. Figure 3f is also typical in that the corpus appears less strongly affected than the terminal bulb. However, corpus motions were abnormal: the relaxations were slow, giving the peaks a triangular rather than a sawtooth appearance (compare Figure 3a), and at one point the corpus failed to relax fully between pumps. In the most extreme cases pumps turned into yawns during which the entire pharynx: corpus, isthmus, and terminal bulb; remained contracted (data not shown). These yawns could last at least a minute, more than 300 times as long as normal.

Paradoxically, pharyngeal contractions in *eat-6* mutants were often feeble; Figure 3e contains some examples. A possible explanation is that the pharyngeal muscles wore themselves out. Long tetanic contractions induced in wild-type pharynxes by muscarinic agonists (AVERY and HORVITZ 1990) often end after a few minutes with a slow, gradual, unsynchronized relaxation, and the muscle cells often appear damaged (unpublished observations). Normally pharyngeal muscles would not stay contracted for more than

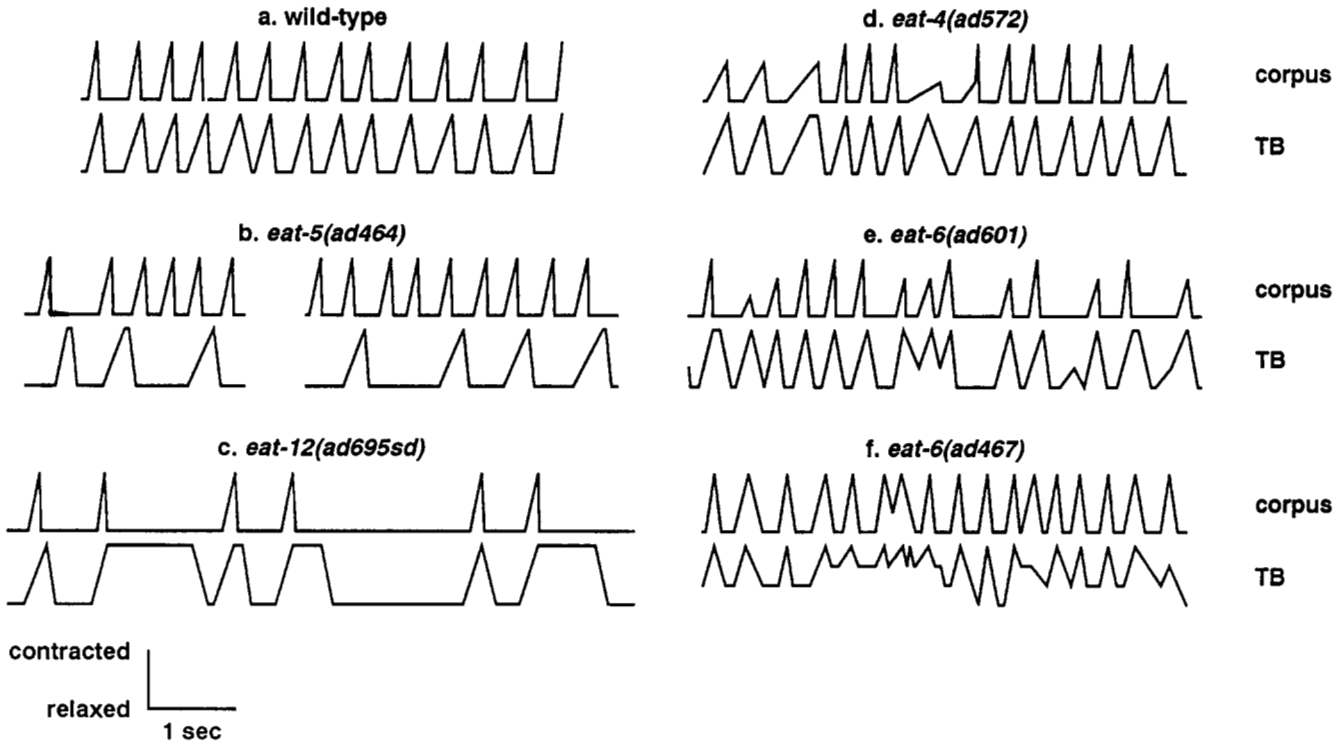

FIGURE 3.—Timing diagrams of wild-type and mutant pumping. Each of the six panels represents the pumping motions of a single worm as measured by analysis of videotapes. (The *eat-5* panel shows two bouts of pumping that were separated by a few seconds during which pharyngeal motions could not be seen clearly.) Times are accurate to about 17 msec (*i.e.*, less than the thickness of the line), but the *y*-axis is partially schematic (see MATERIALS AND METHODS). Only four positions were recognized: relaxed, slightly contracted, mostly contracted, and fully contracted. Continuous contraction or continuous relaxation is arbitrarily represented as linear (except at a few points where there was a striking change in speed). (a) In wild type the contractions of the corpus and terminal bulb begin at the same time, and usually end at roughly the same time, although the terminal bulb contraction seems to last slightly longer. The relaxation of the corpus is extremely abrupt, sometimes appearing to be complete within a single video field (17 msec), giving rise to the characteristic sawtooth form of the corpus trace. The terminal bulb relaxation is always much slower. There is a robust one-to-one correspondence between corpus and terminal bulb contractions. (b) In *eat-5* mutants this correspondence is broken. Corpus action seems normal, but the terminal bulb pumps at a lower rate. Although five of the terminal bulb contractions in the traces began in near synchrony with a corpus contraction, the first two did not. (c) In *eat-12* worms there is a normal correspondence between the beginning of corpus and terminal bulb contractions, but the terminal bulb may stay contracted for over a second. Corpus motions appear normal. (d) Characteristic of *eat-4* mutants are long lasting, feeble corpus contractions interspersed with normal pumps. The terminal bulb remains tightly synchronized with the corpus; the long feeble corpus contractions are coupled with long (but strong) terminal bulb contractions. (e, f) Two alleles of *eat-6* are shown, a weak one (*ad601*) (e) and a strong one (*ad467*) (f). Most characteristic of *eat-6* mutants is a failure of the muscle to relax fully. Although the terminal bulb and corpus are both affected, the effect is more obvious in the terminal bulb. The motions of the terminal bulb and corpus are nevertheless very precisely synchronized. (Some apparent exceptions in these plots are probably only due to the greater ease with which small motions of the terminal bulb can be seen.)

about 200 msec. Perhaps they are damaged by longer contractions.

The corpus and terminal bulb remained tightly synchronized in *eat-6* worms. This synchronization is only partially evident in the timing schematics of Figure 3, e and f. In the original videotapes most or all corpus and terminal bulb contractions, even those that began from a partially contracted position, could be seen to begin within 17 msec of each other as in wild-type. (The resolution of the measurements was 17 msec.) As in wild type, the beginnings of the relaxations were not so tightly synchronized, but still corresponded very well. Even during yawns erratic partial relaxations occurred; these partial relaxations were perfectly synchronized throughout the pharynx, each slight closing of the corpus lumen being accompanied by a slight forward movement of the grinder. This tight synchronization suggests these motions reflect fluctuations in membrane potential, since other processes are too slow to communicate through the length of the pharynx in so short a time. (For instance, a typical small molecule with a diffusion coefficient of 10^{-5} cm^2/sec would require about 10 sec to diffuse the 150 μm length of the pharynx.)

eat-7 IV: This is a mysterious and amusing mutation. *eat-7* homozygotes had a moderately starved appearance. *eat-7*/+ heterozygotes had a slightly weaker phenotype on average, but most were indistinguishable from *eat-7* homozygotes. Since there are no known *eat-7* deficiencies, the basis of semidominance is not known. Pumping was occasionally slow or irregular, but this abnormality seemed far too weak to account

for the degree of starvation. A partial solution to the mystery was suggested by the observation that all the worms on *eat-7* plates that had been undisturbed for a day lay still without moving, pumping or defecating. Within a minute of examination, however, they began to move, feed, and defecate normally. In fact, they became indistinguishable from wild-type worms except for their starved appearance. My hypothesis is that *eat-7* worms, when undisturbed, fell asleep and remained asleep for long periods. (I use the word "sleep" here only for the inactive state of *eat-7* mutants. Wild-type worms have no unconscious state, and there is no reason to think "sleep" in *eat-7* has any fundamental similarity to vertebrate sleep.) While asleep, *eat-7* worms did not move, defecate, or eat. Because they failed to eat for so much of their lives (wild-type worms feed continuously except during molting lethargus), they became starved. I have not had much luck pursuing this hypothesis further, because I have not been able to identify the stimulus that wakes them up, and it is very difficult to study a phenomenon that vanishes when looked at. They could be awakened immediately by light touch or by tapping the plate, but eventually woke up even if carefully handled. Furthermore, an *eat-7*; *mec-4* double mutant woke up as rapidly as the *eat-7* single mutant, despite a presumed lack of the sensory neurons that sense light touch, which degenerate in *mec-4* mutants (DRISCOLL and CHALFIE 1991). Tests for the ability of light to wake *eat-7* mutants were either inconclusive or negative.

ric-2 II, *unc-2 X*, *unc-10 X*, *unc-11 I*, *unc-18 X*, *unc-26 IV*, *unc-57 I*: Mutations in these seven genes caused slow, irregular pumping and a strong Unc phenotype. To them should be added at least six more genes: *unc-13*, *cha-1/unc-17*, *unc-32*, *unc-37*, *unc-75* and *unc-104*, which cause generally similar phenotypes (HODGKIN *et al.* 1988, J. H. THOMAS, personal communication, and my unpublished observations) and ought to turn up in the Eat screen but have not yet, presumably because it has not reached saturation. Of these genes, at least *cha-1/unc-17* (RAND 1989), *unc-32* (D. THIERRY-MIEG and A. SPENCE, personal communication), *unc-37* [also known as *let-76*; ROSE and BAILLIE (1980)], and *unc-104* (HALL and HEDGECOCK 1991) are essential. Mutations in *unc-13*, *cha-1/unc-17*, *unc-75* and *unc-104* cause slow, irregular pumping. In viable *cha-1/unc-17* and *unc-75* mutants the severity of the phenotype varies from time to time in the same animal, and pumps are generally brief. There is almost no pumping in the lethal mutant *cha-1(m324)* (AVERY and HORVITZ 1990). Some worms carrying a viable mutation in *unc-104* arrest with the anterior pharyngeal lumen stuffed full of bacteria; this defect is characteristic of worms that lack function of the motor neuron M4 (AVERY and HORVITZ 1987). The reference allele of *unc-32*, *e189*, causes slightly irregular pumping. Viable *unc-37* mutants are also reported to pump abnormally (J. H. THOMAS, personal communication). The Unc phenotypes vary considerably within this group in a way that defies simple classification (Table 3).

eat-3 II: *eat-3* showed every sign of being a housekeeping gene required for many different functions. In addition to very slow irregular pumping, the mutants had sluggish movement, low fertility, partial embryonic lethality, and very poor recovery from the dauer state. Other mutants are known that fail to recover from dauer, but a dauer larva mutant for any of these genes remains a living, apparently healthy dauer under recovery conditions (ALBERT, BROWN and RIDDLE 1981; ALBERT and RIDDLE 1988; C. I. BARGMANN and H. R. HORVITZ, personal communication). *eat-3* dauers, in contrast, died under recovery conditions, suggesting they received the signal to recover, but were unable to carry out the developmental program it triggered.

eat-3 had a strong maternal rescue effect. The only detectable phenotype of *eat-3* homozygous progeny of an *eat-3*/+ heterozygous mother was slightly slow, irregular pumping. Zygotic *eat-3*(+) was sufficient to produce normal worms, since *eat-3*/+ progeny of an *eat-3* homozygote were completely normal.

eat-14 X: In *eat-14* worms the grinder failed to return to its fully relaxed position between pumps. Unlike the relaxation-defective *eat-6* and *eat-12* mutants, *eat-14* had no effect on the timing of pharyngeal motions: the frequency and length of pumps were normal in mutants. The dissecting microscope phenotype was very similar to that of *phm-2* (below), but *eat-14* pharynxes did not have the birefringence abnormalities or terminal bulb lumen deformation of *phm-2*.

Many *eat* mutations are likely to affect nervous system function: The evidence is strongest for those that also produced an Unc phenotype, because previous characterization of these genes suggests their Unc phenotypes result from defects in the ventral cord motor neurons that control locomotion (WHITE *et al.* 1976; CHALFIE *et al.* 1985). The complex locus *cha-1/unc-17* encodes choline acetyltransferase, necessary for the synthesis of the neurotransmitter acetylcholine (RAND 1989). *unc-104* encodes a kinesin-like molecule necessary for the formation of neuronal processes (HALL and HEDGECOCK 1991). Genetic mosaic analysis by KENYON (1986) and by YUAN and HORVITZ (1990) showed that for normal movement *unc-26* and *unc-36* must function in ABp descendants, which include the ventral cord motor neurons that control locomotion. *ric-2*, *unc-10*, *unc-11*, *unc-13*, *cha-1/unc-17*, *unc-18*, *unc-32*, *unc-36*, *unc-75* and *unc-104* mutants are resistant to acetylcholinesterase

inhibitors (A. ALFONSO-PIZARRO and J. RAND, personal communication; R. HOSONO and T. SASSA, personal communication) but do not turn up in selections for mutants resistant to the acetylcholine agonist levamisole (LEWIS *et al.* 1980). Such a result can be explained if the ventral cord motorneurons in these mutants release less than normal amounts of acetylcholine.

Eat mutants that move normally may have nervous system defects that are confined to the pharyngeal nervous system. Most have defects in feeding behavior like those caused by killing subsets of the pharyngeal nervous system. For instance, slippery pharynx mutations can be phenocopied by killing the 12 GREEN pharyngeal neuron types (AVERY and HORVITZ 1989) or some subsets of them (AVERY 1993). I suspect the slippery pharynx phenotype is caused by incorrect coordination of pharyngeal muscle motions, especially relaxation, on time scales of tens of milliseconds (AVERY 1993). Slow or irregular pumping, another common Eat phenotype, can be phenocopied by killing the MC sensory neurons (AVERY and HORVITZ 1989).

While the general similarity between Eat phenotypes and the effects of pharyngeal nervous system defects allows an argument that many of the *eat* genes likely affect pharyngeal nervous system function, the aberrant behaviors were not sufficiently distinctive that specific candidate defective neurons could be identified on this basis alone. One possible exception is *eat-4*. *eat-4* mutants had long, feeble corpus contractions interspersed with normal brief strong ones (Figure 3d). A very similar effect is produced by killing the motor neuron M3 [AVERY (1993) and data not shown]. If the effect of *eat-4* mutations is to inactivate M3 neurons, inactivation must result from blocking the ability of M3 to function rather than to develop, since M3 neurons were present in *eat-4* mutants. It is, however, unlikely that the sole effect of *eat-4* inactivation is to knock out M3 function, because *eat-4(ad572)* worms were more severely feeding-defective than wild type worms in which M3 has been killed. A possible explanation is that *eat-4*(+) is necessary for the function of a group of neurons that includes M3, and all members of the group affect pharyngeal muscle in the same way. It is also possible that *eat-4*(+) is necessary in the muscle for a process that is stimulated by M3 but not entirely dependent on it.

Individual descriptions of *pha* genes:

pha-2 X: *pha-2* adults had obviously misshapen pharynxes in which the cells of the anterior terminal bulb and isthmus appeared to have mixed (Figure 4b). The terminal bulb was smaller than normal, and the grinder was in an anterior position, instead of in the center as usual. The isthmus was thicker than normal

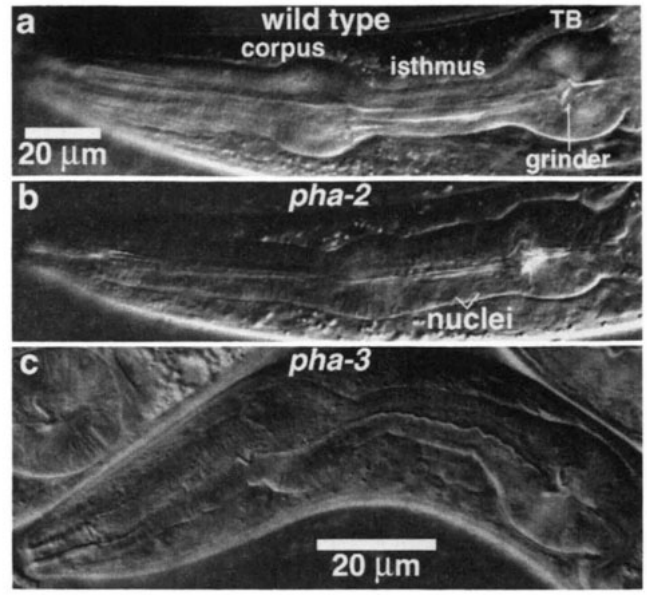

FIGURE 4.—Shapes of wild-type and Pha mutant pharynxes. Nomarski differential interference contrast pictures of wild-type (a), *pha-2* (b), and *pha-3* (c) pharynxes. Anterior is to the left. The scale bar in c applies to b as well. *pha-2* worms have nuclei in the isthmus, where they are never found in wild type. The smaller size of the *pha* mutant pharynxes is probably a consequence of starvation: these are among the very strongest feeding-defective mutants isolated. They grow extremely slowly, and never become as large even as the *eat-6* mutant worms in Figure 1, g and h. TB, terminal bulb.

and had nuclei in it. [In wild-type the isthmus has no nuclei except at its posterior end (ALBERTSON and THOMSON 1976).] In young *pha-2* larvae the gross shape of the pharynx was normal. However, the normal reproducible placement of pharyngeal nuclei was disrupted, so that nuclei could not be easily identified, and most *pha-2* larvae arrested in the L1 stage. Occasional larvae passed the L1 stage; these generally grew up to become extremely starved adults. *pha-2* was weakly cold-sensitive, in that more larvae passed the L1 block at 25° than at 15° or 20°. The frequency and timing of pumping were normal.

One possible explanation of the shape abnormalities would be that the pm6 muscles of the anterior terminal bulb were transformed into pm5 isthmus muscle. To test this, I stained *pha-2* embryos with the monoclonal antibody 3NB12, which stains pm5 and pm7 (posterior terminal bulb muscles) but not pm6 (PRIESS and THOMSON 1987). *pha-2* embryos were indistinguishable from wild-type by this method.

pha-3 IV: In *pha-3* mutants the isthmus, whose diameter normally is constant throughout its length (Figure 4a), instead tapered from the terminal bulb, becoming quite thin where it joined the corpus (Figure 4c). The metacorpus lumen was open when relaxed. The procorpus was sometimes straight and thin, rather like the normal isthmus. *pha-3* worms took in few bacteria and grew very slowly. However, unlike *pha-2* worms, most *pha-3* worms did grow. The *pha-3*

null phenotype is probably lethal or sterile. *pha-3/Df* worms, although viable, were sterile, unlike *pha-3* homozygotes. The deficiency heterozygotes probably grew more slowly than *pha-3* homozygotes, although it was difficult to get enough worms to be sure. The pharyngeal deformation was not obviously more severe in *pha-3/Df* than in *pha-3* homozygotes.

Individual descriptions of *phm* genes:

act(ad468 ad767sd) V: The *ad468 ad767* double mutation was isolated in an F_1 screen for dominant mutations, and it was in fact semi-dominant: many *ad468 ad767/+* heterozygotes were slightly starved. *ad468 ad767* homozygotes had a strong Phm phenotype. Pharyngeal contractions were so feeble as to be almost invisible in the dissecting scope, and *ad468 ad767* worms grew very slowly and produced only very small broods. The strength of the mutant phenotype depended solely on the number of copies of the mutation: *Df/+* heterozygotes are wild-type, and *ad468 ad767/Df* heterozygotes are weakly Phm, like *ad468 ad767/+*. *ad468 ad767* pharyngeal muscle was strongly birefringent, but the birefringence appeared patchy. This appearance is deceptive, because by changing the orientation of the polarization, most of the pharyngeal muscle could be shown to be birefringent (Figure 5, b and c). I explain this by supposing that the orientation of birefringent fibers varied from place to place within the muscle cell.

The birefringence phenotype was similar to that of semi-dominant actin mutations (WATERSTON, HIRSH and LANE 1984), and *ad468 ad767* mapped near the *act-1 act-2 act-3* cluster on chromosome V (Table 2). In fact, L. A. SCHRIEFER and R. H. WATERSTON (personal communication) showed by sequencing the actin genes that *ad468 ad767* carries point mutations in *act-2* and *act-3*. *ad468 ad767* is unique among actin mutations in one regard: homozygotes moved normally, suggesting that *ad468 ad767* specifically affected pharyngeal muscle. All previously known actin mutations were isolated on the basis of their effects on body muscle. Furthermore, *ad468* is the first known mutation in *act-2*. These results suggest *act-2* may function specifically in pharyngeal muscle, while *act-1* and *act-3* function in both pharyngeal and body wall muscle. However, it is not known whether the *act(ad468 ad767)* mutant phenotype is caused by *act-2(ad468)*, *act-3(ad767)*, or both.

phm(ad598) X, phm(ad699) I, phm(n2251) V, phm-3 III, unc-89 I: These mutations all caused weak or disorganized terminal bulb birefringence and feeble pharyngeal contractions. In wild-type worms the pm6 and pm7 muscles of the terminal bulb have radially oriented, strongly birefringent fibers (Figure 5a). In the mutants, this birefringence was weak, and there were often arcs of birefringence at the periphery of the terminal bulb instead (Figure 5a). These peripheral arcs were particularly strong in *unc-89* mutants, where WATERSTON, THOMSON and BRENNER (1980) have shown that they contain longitudinally rather than radially oriented myosin thick filaments. Most or all of these mutations probably affect corpus and isthmus as well as terminal bulb muscle, because contractions were feeble in these parts of the pharynx. (Indeed, Figure 5a also shows that corpus birefringence was weaker in *phm-3* worms than in wild type.) The more intricate structure of terminal bulb birefringence made abnormalities more obvious there. A possible exception is *phm(n2251)*, whose corpus contractions appeared vigorous, despite the fact that grinder motion was almost imperceptible.

All five genes appeared to affect pharyngeal muscle

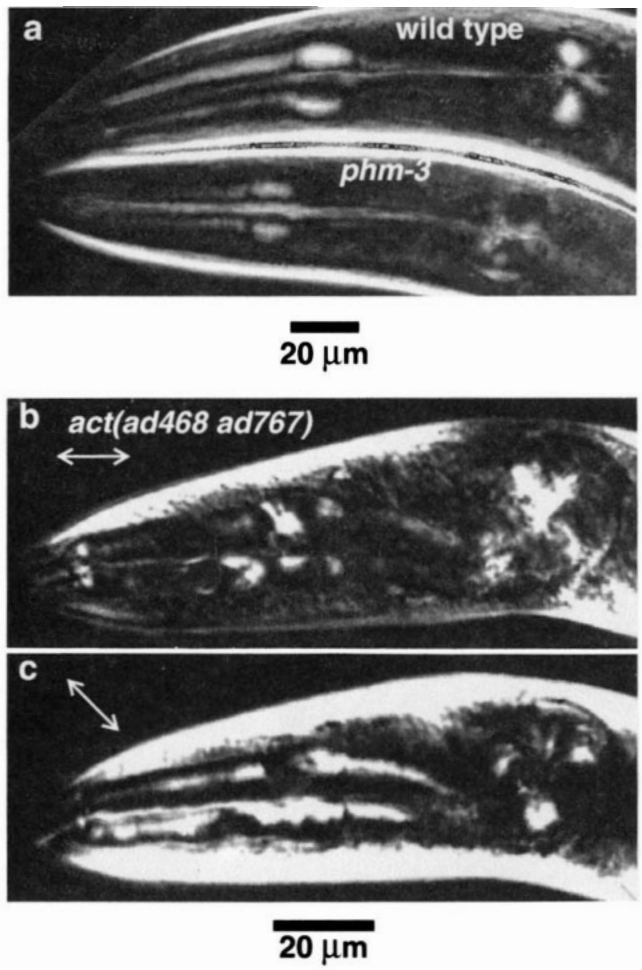

FIGURE 5.—Muscle birefringence in wild-type and Phm mutant pharynxes. Panel a shows a wild-type and a *phm-3* mutant pharynx. The anesthetized worms were arranged side-by-side and photographed together so that the intensity of the birefringence can be directly compared. Anterior is to the left. The disorganization of the (posterior) terminal bulb is most obvious, but the birefringence of the corpus (anterior) is also clearly decreased. Panels b and c show the patchy pharyngeal muscle birefringence of *act(ad468 ad767)* homozygotes. The only difference between the two panels is the plain of polarization, indicated approximately by the arrows. Comparison shows that most of the muscle is birefringent, although the birefringent axes vary from patch to patch.

function much more strongly than they did the function of body wall muscles, but they were not absolutely specific. *unc-89* mutants, although their body muscle functions normally, have disorganized body muscle birefringence (WATERSTON, THOMSON and BRENNER 1980). *phm(n2251)* was originally isolated by E. JORGENSEN because it caused weak defecation muscle contractions (personal communication). And *phm(ad598)* mutants have a progressive muscle Unc phenotype: young larvae moved normally but adults became increasingly sluggish as they aged. Mutations in *unc-78* also cause a progressive muscle Unc phenotype with abnormal pharyngeal muscle, and *unc-78* maps in the same region of the *X* chromosome (WATERSTON, THOMSON and BRENNER 1980). However, *ad598* complements *unc-78(e1217)* (D. RAIZEN, personal communication). Given the similarities in location and phenotype, intragenic complementation must be considered a possible explanation. *phm(ad699)* and *phm-3* had no obvious effect on the contraction of non-pharyngeal muscles, but I did not check body muscle birefringence in either.

phm-2 I: In *phm-2* worms the grinder was unable to come to its full forward position. The muscle fibers of the terminal bulb appeared shorter than normal, and the space posterior to the grinder was always expanded and often had lobes extending diagonally posterior (Figure 6). The timing of pharyngeal muscle contractions was normal.

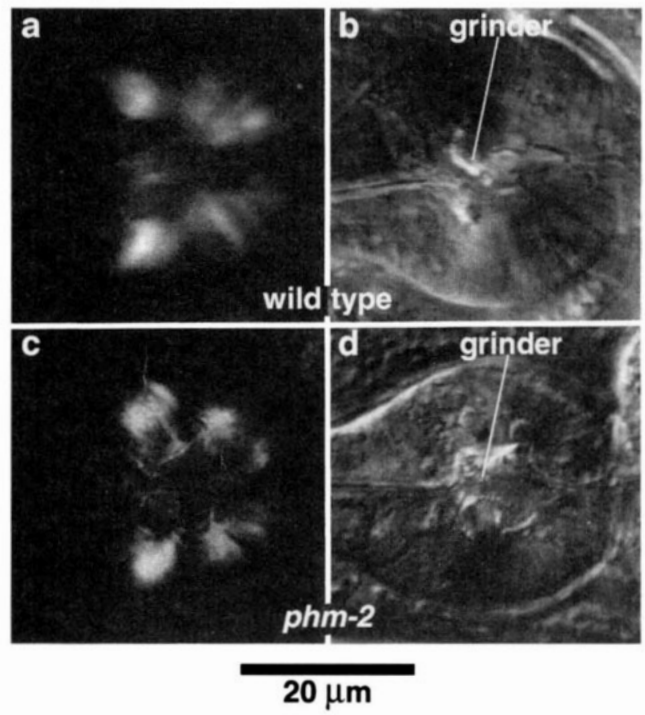

FIGURE 6.—Wild-type and *phm-2* terminal bulbs. Relaxed wild-type (a, b) and *phm-2* (c, d) terminal bulbs viewed by polarized light (a, c) or Nomarski differential interference contrast optics (b, d). Anterior is to the left. Compare the forward position of the wild-type grinder (b) to the half-inverted position of the *phm-2* grinder (d). Note also how the muscles in the *phm-2* mutant hold a broad space open posterior to the grinder, while the relatively more extended muscles in the wild-type terminal bulb allow the lumen to close.

CONCLUSION

Summary: Feeding in *C. elegans* requires first the proper development of the pharynx, then its proper function. Fast muscle contractions in nematodes are controlled by fast membrane depolarizations, *i.e.*, action potentials (DEBELL 1965; BYERLY and MASUDA 1979), as in other animals. Proper pharyngeal function therefore requires that pharyngeal muscles be capable of generating rhythmic action potentials, that the contractile apparatus respond to depolarization by producing force, that the pharyngeal nervous system send the correct signals to the pharyngeal muscles, and that the muscle respond to these signals by modulating membrane potential or force production. By screening for viable, fertile mutants with defects in feeding behavior, I have identified genes likely to be involved in each of these processes. Mutations in two *pha* genes affect pharyngeal morphogenesis. Mutations in seven *phm* genes cause abnormal pharyngeal muscle contraction. And mutations in 26 *eat* genes affect the motions of pharyngeal muscles: these are likely to affect pharyngeal nervous system function or muscle functions that regulate contraction.

The genetics of muscle excitability in *C. elegans*: Behavioral mutants are easy to isolate and handle in *C. elegans*, probably because its nervous system is largely dispensable in the laboratory (AVERY and HORVITZ 1989). Genes identified by screens for such mutants number in the hundreds. They affect locomotion (BRENNER 1974), chemotaxis (DUSENBERY, SHERIDAN and RUSSELL 1975), osmotic avoidance (CULOTTI and RUSSELL 1978), avoidance of light touch (CHALFIE and SULSTON 1981), male mating (HODGKIN 1983), egg-laying (TRENT, TSUNG and HORVITZ 1983), defecation (THOMAS 1990), and now feeding. Several of these behaviors are executed in part or entirely by muscles not used for other behaviors. For instance, egg-laying requires vulval and uterine muscles, male mating requires several types of male-specific muscles in the tail, defecation requires anal and intestinal muscles, feeding depends on pharyngeal muscle, and locomotion requires body-wall muscle [for overview, see WHITE (1988)]. (Body wall muscles are the least specific, being involved in defecation and behaviors such as male mating, chemotaxis, and avoidance that depend on locomotion.) One would therefore expect to isolate muscle-defective as well as nervous system-defective mutants in screens for worms unable to execute one or more of these behaviors normally. This expectation is fulfilled for egg-laying (TRENT, TSUNG and HORVITZ 1983), locomotion (WATERSTON, THOMSON and BRENNER 1980), and

feeding (RESULTS AND DISCUSSION) at least.

A muscle may be defective because its contractile apparatus does not work, or because its properties as an excitable cell are impaired. If the muscles are large enough (*e.g.*, body wall or pharyngeal muscle), mutants with defective contractile apparatus can often be recognized by abnormalities in muscle birefringence. They may also produce a common, easily recognized gross phenotype, such as flaccid paralysis (body wall muscle), or small, feeble pharyngeal motions (pharyngeal muscle).

Mutants that affect the electrical properties of muscle cells are particularly interesting to scientists who study the function of excitable cells. They are expected to identify genes that encode ion channels and proteins that regulate them, *e.g.*, receptors and the components of second messenger systems. In fact, these genes may be even more interesting than those that affect nervous system function. The reasons are as follows. First, nematode neurons are not generally electrically excitable (DAVIS and STRETTON 1989), whereas muscles are (JARMAN 1959). Second, muscle may be as important as nervous system in processing information and generating certain behaviors (DEBELL, DEL CASTILLO and SANCHEZ 1963; DEBELL 1965; AVERY and HORVITZ 1989). Third, muscle cells are larger and therefore potentially more accessible to electrophysiological recording than neurons, which are very small in *C. elegans* (typical cell body diameter 5 μm). Pharyngeal muscle is the most promising *C. elegans* muscle type in this last regard, since the pharynx can be easily dissected out of the body, and dissected pharynxes will function *in vitro* (D. RAIZEN and L. AVERY, unpublished).

There are at least two ways muscle excitability mutants can be recognized: by pharmacology or by phenotype. The pharmacological method is to show that mutant muscles respond differently to a drug than do wild-type. This approach has been most extensively used in the study of egg-laying (TRENT, TSUNG and HORVITZ 1983). It has also been valuable in identifying genes that control a nicotinic receptor active in body muscle (LEWIS *et al.* 1980, 1987). The phenotypic method is to show that mutant muscle behavior is very different from anything that can be produced by a nervous system defect. (In practice, this usually means that the phenotype is different from any produced by laser killing of neurons.) Application of this method requires that one know which neurons control muscle action and what their effects are. In addition, the ability to distinguish the site of gene action varies directly with the ability to observe the details of muscle motions. Feeding has a decisive advantage over other behaviors, because the motions of individual pharyngeal muscles can be followed on millisecond time scales, and the neurons that control them belong to a well-defined set, the pharyngeal nervous system.

eat-5, *eat-6*, *eat-11* and possibly *eat-12* are genes likely to affect muscle excitability. *eat-11* is included based on pharmacological criteria: mutants are much more sensitive to the acetylcholine agonist arecoline than wild-type worms. *eat-5*, *eat-6* and *eat-12* are included based on phenotypic criteria. In *eat-5* mutants corpus and terminal bulb contractions are unsynchronized (Figure 3). In wild type, the corpus and terminal bulb remain synchronized even when the entire pharyngeal nervous system is destroyed. In *eat-12* and *eat-6* mutants the terminal bulb or the entire pharynx, respectively, may stay contracted for seconds, whereas the pharyngeal neurons known to control pump length vary it only within a range from about 150–200 msec (AVERY 1993).

At present, the data in favor of a muscle site of action for these four genes are only suggestive. It is difficult to be certain a nervous system defect cannot produce the mutant muscle behavior. (For instance, while laser studies can eliminate the possibility that the phenotype is due to loss of neuron function, some of the mutations might cause neuronal hyperactivity.) Fortunately, there is a definitive test that can eliminate this possibility: kill the pharyngeal nervous system in mutant worms, and compare muscle motions to those of wild-type worms whose pharyngeal nervous systems have been killed. If mutant is still different from wild type, the mutation must act in a site other than pharyngeal nervous system. I intend to apply this test to *eat-5*, *eat-6*, *eat-11* and *eat-12*.

In summary, the pharynx offers unique advantages for the genetic study of muscle excitability. The clear delimitation of a small set of neurons that act on the muscles and the visibility of the muscle motions makes recognition of such mutants possible. The fact that the pharynx can function without the pharyngeal nervous system allows a definitive elimination of the pharyngeal nervous system as the sole site of gene action.

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